Process Automation for Analytical Measurements Providing High Precise Sample Preparation in Life Science Applications

Dissertation

zur Erlangung des akademischen Grades

Doktor-Ingenieur (Dr.-Ing.)

der Fakultät für Informatik und Elektrotechnik

der Universität Rostock

Ellen Vorberg

eingereicht am 25.02.2015 in Rostock

verteidigt am 08.09.2015 in Rostock

urn:nbn:de:gbv:28-diss2015-0183-8

Supervisors

1. Prof. Dr.-Ing. habil. Kerstin Thurow, University of Rostock, Center for Life Science Automation

2. Prof. Dr.-Ing. Norbert Stoll, University of Rostock, Institute of Automation

3. Prof. Robin A. Felder, Ph.D., University of Virginia, School of Medicine, Department of Pathology, Charlottesville, VA

Acknowledgment

First of all, I would like to thank Prof. Dr. Kerstin Thurow for her continuous support throughout my PhD studies and dissertation work. I am truly grateful for her advices and suggestions and, moreover, for the excellent laboratory equipment.

Furthermore, I would like to extend my thanks to all the staff members of celisca and the Institute of Automation. Especially, I would like to thank Sybille Horn, Dr. Heidi Fleischer, Dr. Thomas Roddelkopf, Lars Woinar, Heiko Engelhardt, Dr. Hui Liu, Dr. Sebastian Neubert, and Dr. Steffen Junginger for technical support.

Most importantly, I thank my beloved husband, Mike Vorberg, for his love, support, and encouragement.

Table of Contents

Table of Contents

Table of Contents

List of Figures

Sources of Figures

i. http://www.tecan.com/platform/content/element/6593/efficient_troughput.gif (28.11.2012)
ii. http://www.hamiltonrobotics.com/fileadmin/user_upload/prodb/images/proteomics/STARlet.jpg (06.01.2015)
iii. https://www.beckmancoulter.com/ucm/idc/groups/public/documents/webasset/glb_bci_152831.jpg (21.11.2012)
iv. https://www.chem.agilent.com/en-US/products-services/Instruments-Systems/Automation-Solutions/Bravo-Automated-Liquid-Handling-Platform/PublishingImages/bravo_automated_liquid_handling_platform.png (28.11.2012)
v. http://www.gerstel.de/pdf/MPS_Prospekt_de.pdf (28.11.2012)
vi. http://www.palsystem.com/typo3temp/pics/ac8b5525c2.jpg (05.12.2012)
vii. http://www.palsystem.com/typo3temp/pics/1e51c7655e.jpg (05.12.2012)
viii. http://www.palsystem.com/typo3temp/pics/e89196bfa0.jpg (05.12.2012)
ix. http://www.palsystem.com/typo3temp/pics/148d5c04df.jpg (05.12.2012)
x. https://www.chem.agilent.com/en-US/products-services/Instruments-Systems/Automation-Solutions/BioCel-Systems/PublishingImages/biocel_system_configurations.png (28.11.2012)
xi. http://icosym-nt.cvut.cz/odl/partners/fuh/course_main/img353.gif (16.05.2013)
xii. http://www.roboex.com/images/orca/orca.jpg (16.05.2013)
xiii. https://www.chem.agilent.com/en-US/products-services/Instruments-Systems/Automation-Solutions/BenchBot-Robot/PublishingImages/benchbot_robot.png (28.11.2012)
xiv. https://www.beckmancoulter.com/ucm/idc/groups/public/documents/webasset/glb_bci_052381.jpg (16.05.2013)
xv. http://www.nationalscientific.com/products/images/C4011-2WCV-1CV.jpg (28.10.2014)

List of Tables

List of Abbreviations

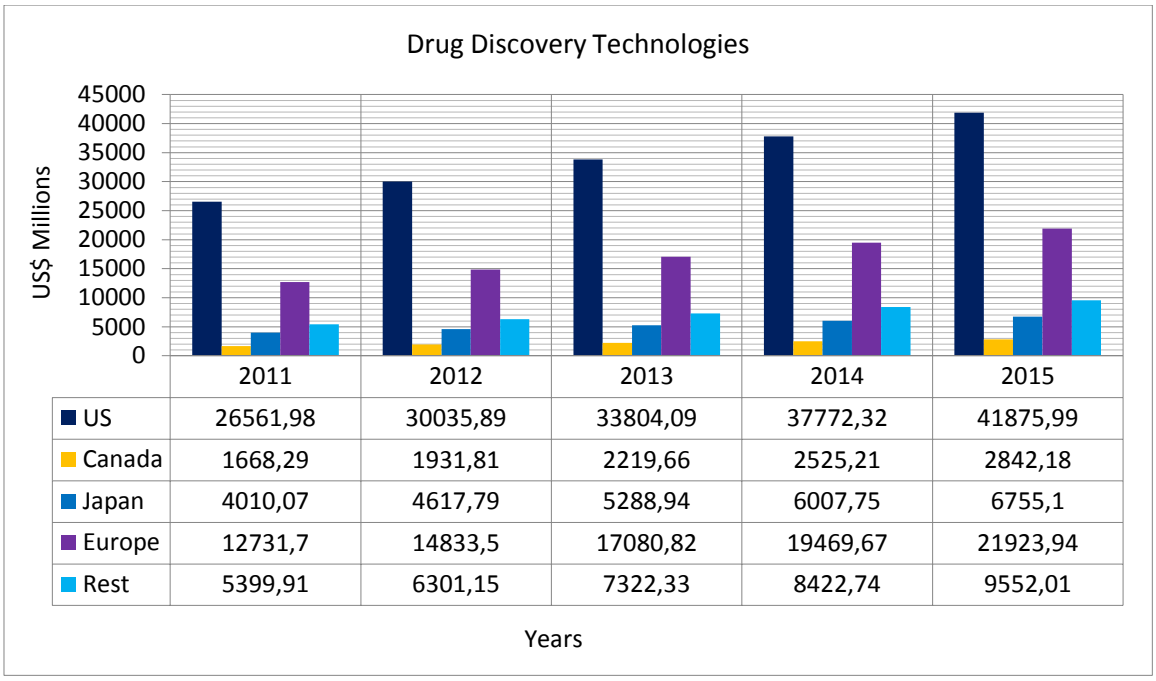
AAS	Atomic Absorption Spectroscopy
AC	Alternating Current
AFS	Atomic Fluorescence Spectroscopy
ALP	Automated Labware Positioners
CSV	Comma-Separated Value
CV [%]	Coefficient of Variation
CV-AAS	Cold Vapor Atomic Absorption Spectroscopy
DC	Direct Current
DMEM	Dulbecco's Modified Eagle's Medium
DHS	Dynamic Headspace
DPX	Disposable Pipette Extraction
EIC	Extracted Ion Current
EM	Electron Multiplier
F-AAS	Flame Atomic Absorption Spectroscopy
FA-LAS	Fully Automated Laboratory Robotic System
FCS	Fetal Calf Serum
Fig	Figure
F-OES	Flame Optical Emission Spectroscopy
GC	Gas Chromatography
GF-AAS	Graphite Furnace Atomic Absorption Spectroscopy
HPLC	High Performance Liquid Chromatography
HTS	High Throughput Screening
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IRS	Infrared Spectroscopy
ISTD	Internal Standard
ITEX	In-Tube Extraction
LC	Liquid Chromatography
LLE	Liquid-Liquid Extraction
MHE	Multiple Headspace Extraction

MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MTP	Multi-Titer-Plate
mVAP	Multi-Position Evaporation
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance Spectroscopy
OES	Optical Emission Spectroscopy
ORCA®	Optimized Robot for Chemical Analysis
PCR	Polymerase Chain Reaction
PMS@LSA	Process Management System at Life Sciences Automation
PPT	Protein Precipitation Techniques
RIA	Robotic Industries Association
RSPP	Robotic Sample Preparation Program
RTC	Robotic Tool Change
SLH	Septum-Less Head
SOP	Standard Operation Procedures
SPE	Solid-Phase Extraction
STD	Standard Deviation
STR	Short Tandem Repeat
TIC	Total Ion Current
XRF	X-Ray Fluorescence Spectroscopy

1. Introduction

Life sciences comprise the interdisciplinary field of sciences that involve the scientific study of living organisms. Therefore, cognate disciplines, such as medicine, biomedicine, biotechnology, biophysics, and bioinformatics, are included. Allowing for applied and basic research application, all of these areas require specific sample pretreatment steps, such as stirring or shaking, aliquoting, diluting, protein precipitation and sample extraction techniques, while providing the increase of the analyte´s concentration and converting the analyte into an appropriate form for the detection systems.

Life sciences place emphasis on market-orientated approaches. Thus, automation processes have been established in all associated scientific areas because of the increased needs for higher capacities, quality, and throughput [1]. Accordingly, dominated by the United States and Europe [2], the world market for life science automation is predicted to reach a compound annual growth rate of 9.8% up to 2015 [3]. Major markets are the drug discovery and biotechnology sectors followed by the clinical research sector [4]. Here, especially enantiomers are of increasing interest due to an aging population and the associated demands for new drugs and therapies [5], [6]. Fig. 1 exemplifies the world long-term projection for the drug discovery technologies in US$ millions for the years 2011 through 2015. Besides, a new and rapidly growing market is represented by the forensic technology sector [7]. In addition automation is increasingly common in the agricultural and food sector [8].



	2011	2012	2013	2014	2015
US	26561,98	30035,89	33804,09	37772,32	41875,99
Canada	1668,29	1931,81	2219,66	2525,21	2842,18
Japan	4010,07	4617,79	5288,94	6007,75	6755,1
Europe	12731,7	14833,5	17080,82	19469,67	21923,94
Rest	5399,91	6301,15	7322,33	8422,74	9552,01

Fig 1: World long-term projection for the drug discovery technologies in US$ millions for the years 2011 through 2015 [2]

1. Introduction

Regarding these market and research developments, laboratories are constantly faced with the demands for improved quality and better economical results, furthermore, for improved health and safety conditions, and shorter sample turnaround times [9]. By automating *sample preparation*, laboratories will gain on all of these demands [10] since sample preparation techniques are the rate-limiting step in many testing processes [11], [12], [13].

In detail, automated sample preparation techniques free up researchers from repetitive, tedious tasks [8] and ensure the safety of the analysts [14] by assigning risk-involving procedures, such as handling of highly active substances [15] or potentially infectious biomaterial [16]. Moreover, due to the operator-unattended sample pretreatment [17], automated systems eliminate the training time for the analyst and personal error in sample preparation [18], [19]. Thereby, they reduce the variability in sample preparation [20], [21], [22], [23], improve the accuracy of the experiments, and allow for rapid analysis. Thus, for improving sample analysis´ efficiency, robustness, and reliability [24], it is necessary to eliminate unessential human intervention [25], [26].

Integrating different functional devices that are capable of conducting most of the routine experiments [15], laboratory robotic systems streamline the analytical tasks [11], [27]. Accordingly, automated solutions help to overcome the labor intensive steps [28] and to monitor and manage the raw data produced [8]. Due to the fact that they offer the advantage of increasing sample throughput [29], [30], [31], [32], [33] and greatly reduced costs [34], automated laboratories are now becoming the laboratories of choice for those companies that require large numbers of samples to be analyzed on a 24/7 basis (on similar sample types using the same sample preparation methods and analytical procedures) [18]. Thus, more and more laboratories both large and small are starting to use automated solutions to enhance their workflows [35]. Nevertheless, smaller companies need to consider the availability of core engineering resources and capital costs as key issues [36]. Consequently, the trend in laboratory automation has moved from total automation to a modular approach, from a hardware-driven system to process control [37]. Hence, the technology design is based on the required functionality: Using loosely integrated automated workstations provides flexible solutions for throughput improvements.

Commercially available automated workstations are usually configured for handling the standardized multi-titer-plate (MTP)-format. Accomplishing DNA and RNA assays and in addition the analysis of (therapeutic) proteins [38], biological applications use especially this format and represent, therefore, the prime candidates for laboratory automation [39]. Moreover, using this format, automated biological sample preparation contributes to an increased throughput [40], [41], [42], [43] and enables

simultaneous screening of many excipients and experimental conditions, such as storage temperatures, mechanical stresses, buffers, salts, and surfactants [44]. Thereby, automated biological applications have prompted the development of new drug discovery programs [21], [45], [46], [47]. In addition forensic applications [48], clinical sample analysis [49], virus load measurements [50], Next-Generation Sequencing [51], bioprocess developments [52], and investigations of novel pathways [53] have been discussed. All of these techniques are very similar concerning the applied procedures. In detail, biological sample preparation techniques include simply diluting [54], adding of the internal standard [55], stirring, aliquoting [11], and protein precipitation techniques (PPT) [56], [57]. Furthermore, labor-intensive methods, such as solid-phase extraction (SPE) [34], [58], [59], [60] and liquid-liquid extraction (LLE) [61], [62], [63], [64], which are required for the quantitative analysis of drug concentrations in plasma or serum samples, have been described.

Nevertheless, commercially available automated platforms are not suitable for common analytical sample preparation processes. Allowing for the analysis of small molecules and mixtures of molecules, analytical requirements differ significantly from biological applications. In detail, due to the fact that environmental and industrial solid samples usually provide non-homogeneous consistency, analytical sample preparation calls for higher volume ranges [65] in order to dissolve higher quantities of the solid samples. Moreover, the utilization of highly active aqueous solutions, such as concentrated acids [67], or organic solvents with different viscosities has to be considered and requires the utilization of inert materials. However, demanding lower volume ranges [66], biological sample preparation and measurements simply involve the analysis of food [68], [69] and body fluids [60], expression of cell metabolism products [70], application of bio-molecules [71], culture media [72], and washing solutions [73]. Accordingly, all of these biological tasks provide just nonhazardous pH values and call for standard laboratory conditions, which ensure the utilization of the MTPs [74]. In contrast, while performing high volume, non-standard temperature and pressure procedures [67], the use of specialized vessels for multistep analytical sample preparation has remained indispensable.

Beside the variety of required vessels, automating analytical sample preparation processes calls for various workstations to enable different task and functions. Facilitating these pretreatment steps, instruments used for repetitive analytical measurements, such as chromatographs and atomic absorption spectrometers, are frequently equipped with automatic samplers and automatic sample processors, respectively [75], [76]. These plant components provide convenient pipetting and dilution steps. However, they are not capable of handling the entire range of vessels required for multistep analytical sample pretreatment. Moreover, they automate only a few steps of a full analysis scheme and handle just one single vessel at any time. In contrast, the utilization of the MTP-format provides

simultaneous performance of up to 384 reactions. However, the majority of recent reports about robotic sample pretreatment originate from large, mainly pharmaceutical companies [77].

Due to the fact that existing systems either handle only a few steps of a complex analysis scheme or that they offer a fixed solution for a single application, upgradable automation processes and flexible analytical sample pretreatment are still an unsolved issue.

Consequently, the most important challenge and, therefore, the purpose of this dissertation is the design, the realization, and evaluation of an automated system that ensures an optimal balance between automating the most important steps (regarding the variety of analytical sample preparation processes) and providing system adaption and high performance flexibility. Moreover, facilitating analytical sample pretreatment, the developed system has to be capable of dealing with the wide range of required vessels and has to supply extensive analytical applications. Moreover, the suitable system has to allow for simultaneous handling of vessels and has to enable less cost- and time-consuming steps.

Chapter 2 gives a wide review of laboratory automation including the state of the art concerning biological and analytical measurement processes. Furthermore, standardized systems and components, which are required for automated sample pretreatment, are described.

Chapter 3 represents the scope of work. Therefore, common process steps and the resulting requirements of biological and analytical sample pretreatment and measurement processes are incorporated.

In chapter 4 the automation concept is described comprising the system parts as follows: system integrator, liquid handling, sample handling (such as barcode reading and sample storage, (de-) capping and crimping, ionizing and weighing), sample treatment (such as extraction and derivatization), analytical devices, integration with external stations, and software integration.

Chapter 5 depicts the statistical methods including the validation parameters and further definitions concerning the acceptability of the results.

Chapter 6 describes the validation of the automated system using *element*-specific measurements. Moreover, the original process description, process adaption, and the results of the automated and the manual sample pretreatment are incorporated.

Chapter 7 describes the validation of the automated system using *structure*-specific measurements. Moreover, the original process description, process adaption, and the results of the automated and the manual sample pretreatment are incorporated.

Summarizing the most important facts, chapter 8 is the last part of this dissertation including a outlook for further investigations.

2. State of the Art: Laboratory Automation

Although it is very difficult to develop a fully automated system without human participation, highly reliable robotic systems have been developed and implemented in various fields of industry [17]. Pioneered by Unimation Inc., the world´s first robotic company, the development of robotics for use in manufacturing environment started in 1956. The first laboratory robots were developed in 1981 by the Zymark Corporation. Using laboratory automation, the results were comparable in accuracy and precision with those obtained by the manual methods [78], [79], [80]. Hence, excellent agreement and small uncertainties, equal to those expected of a talented analyst, were observed [17]. Moreover, even if it requires a lot of work to set them up, automated solutions have drastically reduced human resources, budgets, and time frames [15].

2.1 Automated Biological Sample Pretreatment Depending on Measurement Processes

Due to their complex matrices, such as blood [81], plasma [82], [83], and urine [84], [85], biological applications require a series of sample clean-up steps [11] in order to ensure the increase of the analyte´s concentration or to provide the increase of the method´s sensitivity [86]. Moreover, converting the analyte molecule into an appropriate form for the detection and separation systems [87], sample pretreatment is necessary.

In detail, sample pretreatment includes sample filtration with filter material of various porosity (paper, glass fibre, membrane filter) [88] or ultra-filtration through a membrane with specific molecular mass cut-off. In addition centrifugation, dilution, evaporation, sonication, precipitation, and extraction are employed before the analysis. Regarding biological processes, all of these pretreatment steps can be automated in order to streamline biological applications.

2.1.1 Automated Biological Processes Using Assay Detection Readers

Providing comfortable analysis approaches, biological applications perform various assays and require assay detection readers. Simplifying heteroduplex analysis, a modified Biomek® NX robot with an onboard spectrophotometer was used for DNA extraction, quantification, dilution, and mixing steps. By performing these steps on a robot, hands-on time was decreased and sample tracking errors were reduced [89].

Furthermore, an EVOlution®-Extraction System was developed performing automated DNA extraction [90]. The system consists of a Tecan Freedom EVO® 150 robot and a graphical user interface designed for the use with Freedom EVO® software as well as the instrument´s hardware. The DNA

quality and quantity obtained were comparable to that observed with the corresponding manual extraction protocol. Purified DNA was free of inhibitors and ready for automated downstream applications, such as real-time quantitative Polymerase Chain Reaction (PCR) and PCR for short tandem repeat (STR) analysis. Hence, performing assay detection, PCR was used to evaluate the automated DNA extraction.

Being twice as fast as the manual process and, therefore, more cost effective, a high-throughput antibody staining and washing system for large-scale routine indirect immunofluorescence testing was developed using a Tecan robotic sample processor. Throughput and quality of the antibody-antigen reactions were compared to the manual sample preparation. No difference between quality of antibody-antigen reactions and readability of preparations under a microscope could be detected. Using the Tecan robotic sample processor, 288 samples per day can be processed as compared to 144 samples per day using the manual process. Therefore, the Tecan robotic sample processor was found to be useful for high-throughput fluorescent antibody staining and washing in large-scale, routine indirect immunofluorescence testing [91].

2.1.2 Automated Biological Processes Using Mass Spectrometers

Performing automated biological sample preparation, the analysis of drug levels in plasma samples within a drug discovery environment was achieved through the redesign of a protein precipitation assay to the 96-well format and the detection by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The application of robotic liquid handling systems allowed for the automated performance of all transfer and pipetting steps [40].

Regarding further protein precipitation techniques, Ma *et al.* [92] described the development and validation of a robotic system that fully integrates all peripheral devices required for the automated sample pretreatment of plasma samples. The liquid handling system consists of a Tecan Freedom EVO® 200 liquid handling platform equipped with an 8-channel liquid handling arm, two robotic plate-handling arms, and two plate shakers. Additional components integrated into the platform are a robotic temperature-controlled centrifuge, a plate sealer, and a plate seal piercing station. Likewise, in order to shorten the sample preparation time and to increase the method´s precision, an automated multi-channel liquid handler was used to perform high-throughput protein precipitation and all other liquid transfers for the analyses of dog plasma while using LC-MS/MS [46].

More intricate sample preparation methods are based on extraction [93]. Thus, minimizing matrix interferences by the performance of purification and extraction protocols, several robotic laboratory systems are commercially available [94].

2. State of the Art: Laboratory Automation

Using separation and detection systems, such as chromatographs and mass spectrometers, biological sample extraction protocols comprises both, automation of direct (on-line) [95], [96], [93], [97] and off-line extraction procedures [98], [99], [100], [101], [102], [103], [104]. On-line extraction comprises aliquoting of the biological samples into the MTPs, adding of the internal standard, and centrifuging before starting the direct injection from the 96-well plates. Consequently, performing automated liquid handling provides all required pipetting steps [11].

However, performing off-line extraction protocols, the automation of plasma sample preparation for pharmacokinetic studies on VLA-4 (Integrin alpha4beta1 (Very Late Antigen-4) antagonists has been achieved by the 96-well formatted SPE, which was performed by the Beckman Coulter Biomek® 2000 liquid handling system. The Biomek® 2000 was used to perform fully automated plasma sample preparation tasks that include serial dilution of standard solutions, pipetting the plasma samples, adding of the standard solutions, and performing the SPE on Waters Oasis® 96-well plates [41].

Further biological sample preparation processes including off-line extraction and desorption were automated using commercially available automated liquid handling systems [49]. Therefore, facilitating the determination of drug compound levels in human plasma, liquid transfer, SPME extraction, and desorption processes were fully automated using a Tomtec Quadra 96 workstation and a MultiPROBE II liquid handling system.

In addition Apostolou *et al.* [42] developed an automated high-throughput (LC-MS/MS) method for quantitative determination of donepezil in human plasma. Donepezil and loratadine were off-line extracted by LLE while using common liquid handling workstations for all liquid transfer steps.

Bladergroen *et al.* [60] described two platforms for automated SPE-based sample preparation and subsequent MS-measurements enabling peptide- and protein profiling of body fluids, which are namely (I) the Hamilton® liquid handling workstation that allows for magnetic bead-based SPE of peptides and proteins from body fluids and (II) the Spark Symbiosis™ system providing cartridge-based SPE.

2.2 Approaches for Automated Analytical Sample Pretreatment and Measurement Processes

Jiang *et al.* [26] developed a fully automated cold fiber device by modifying the existing semi-automated unit and coupling it to a GERSTEL® MPS 2 autosampler and a septumless head (SLH) injector. The automated cold fiber device represents a platform for headspace analysis with improved throughput and sensitivity for a large number of volatile and semi-volatile samples from aqueous and solid matrices. The device was thoroughly evaluated for its extraction performance, robustness, reproducibility, and reliability by gas chromatograph/mass spectrometer (GC/MS). The entire automated setup has been capable of analyzing over 200 samples without any GC injector leakages by using a septumless head injector. Therefore, the device creates a platform for high throughput headspace GC analysis, which was evaluated using 14 compounds with varying volatilities and polarities in aqueous medium, such as ethyl butanoate, heptanone, octanal, and nonanol, followed by an extraction of spiked samples, such as naphthalene and fluorine, in silica gel (representing the solid matrix).

A highly automated aqueous equilibrium solubility shake-flask technique was described and validated on a set of 15 marketed drugs (without biological matrix) [28]. Aqueous equilibrium solubility is the solubility that is observed once equilibrium has been achieved between the solution and the solid material. Furthermore, requiring analytical sample preparation, it is an early indication of the drug's ability to dissolve in aqueous media. However, the assay used a Tecan Freedom Evo® 200 liquid handling robot with integrated appliances for transportation, (de-) capping, and centrifugation of the sample tubes. Nevertheless, the (de-) capper unit Abgene ALTO-8™ was merely able to (de-) cap a column of eight 1-mL polypropylene twist-lock tubes in a 96-well format. Thus, the rate-limiting step in this automated sample preparation procedure was the (de-) capping process.

Saitoh and Yoshimori [15] developed a fully automated laboratory robotic system (FA-LAS). The system comprises a volume adjuster using volumetric flasks, a decrimper capable of removing rubber stoppers, a multivalve high-throughput autosampler for high performance liquid chromatography (HPLC) analyses, and a powder dispenser. The newly developed powder dispenser offered highly accurate and precise performance with almost all types of powders including sticky, clumped, or ultra-fine powders. The FA-LAS was designed to save time for the analysts by automating experimental preparations and to enhance analytical task efficiencies by handling routine experiments. However, vessels had to be handled individually. Hence, this automated solution represents a very cost-intensive and time-consuming procedure.

2.3 Comparison and Conclusion of Automated Sample Pretreatment

Biological sample preparation mainly comprises the treatment of DNA, RNA, and especially of (therapeutic) proteins [38], whereas analytical sample preparation has to enable the analysis of small complex molecules and in addition mixtures of molecules.

However, specific sample pretreatment is required for both common biological and common analytical sample preparation procedures in order to allow for suitable analysis while converting the analyte molecules into an appropriate form for the detection and separation systems. Nevertheless, representing the most important difference, biological applications require moderate conditions, such as 37°C and standard pressure, whereas analytical sample pretreatment usually calls for specific conditions, such as non-standard temperatures and pressures. However, no special conditions were needed for the simply automated analytical processes described in literature.

Moreover, automated analytical sample preparation processes have to provide liquid handling options enabling automated pipetting of both small and higher volume ranges. Nevertheless, fitted with the 1ml polypropylene twist-lock tubes for example, automated processes described in literature solely dealt with small sample volumes, whereas common analytical sample preparation also calls for higher volume ranges. In addition the utilization of chemically active substances, which is usually necessary in order to perform analytical sample pretreatment, has not been considered during the performance of the automated processes described in literature.

Accordingly, automating non-standard temperature and pressure procedures using higher liquid levels is still an unsolved challenge. Besides, automated solutions for analytical sample preparation still represent a very cost-intensive and time-consuming procedure due to the wide range of specific vessels and the resulting individual vial treatment required for analytical sample pretreatment.

2.4 Automation Systems and Compounds

2.4.1 Workstations

The term workstation is regarded as an independent system that is highly specialized to perform a single function or task as efficiently as possible. Furthermore, it is the human operator who moves the plates (labware) between different workstations. Providing different biological assays, workstations can be combined with various liquid handling tools that allow for multiple pipetting options, such as transferring and mixing [48].

2. State of the Art: Laboratory Automation

Liquid handling equipment constitutes the largest segment of the laboratory automation market [4]. Moreover, the open architecture of commercially available workstations allows for the integration of a wide range of modules and options, such as readers, washers, incubators, thermocyclers, SPE, and magnetic bead separation [105].

These commercially available automated platforms are offered by different companies, such as Tecan, Hamilton, Beckman Coulter, Agilent, Gerstel, CTC, and others. Performing automated sample preparation, the utilization of these liquid handling systems increases the sample throughput and minimizes errors while maintaining the required accuracy and precision.

The Tecan Freedom EVO® series offers four different worktable capacities (75, 100, 150, and 200 cm). Each platform can be combined with various liquid handling tools. Application options are powered by straightforward software and a wide choice of robotic arms.

Particularly, the multi-channel arm module with the 96- or 384-channel pipetting head brings higher productivity to almost any automated liquid handling process. Furthermore, it works in parallel with other arms of the Freedom EVO® series, is compatible with all Freedom EVO® options, and able to pipette with just 8- or 12-channels if necessary. The optional gripper supplies transport of plates and tip-boxes. Moreover, an optional robotic manipulator arm, which is also able to reach positions outside the worktable, enhances the systems to provide a complete process automation platform.

The upgradeable Freedom EVO® series platforms support biological applications, such as DNA extraction, amplification set-up, normalization, and especially assay development. The worldwide interest in the automation of ELISA processes is increasing. Therefore, the Tecan's Freedom EVOlyzer® offers a validated solution for the automation of microplate-based chromogenic ELISAs. Fast delivery of results and the increased productivity are ensured by high speed processing and parallel co-ordination of all the devices placed on the platform.

Another automated solution is the FE500pro™ (Fig. 2). The FE500pro™, Tecan's front-end, pre-analytical laboratory automation solution, combines pre-analytical functions, such as pre-sorting, centrifugation, volume check, clot detection, de-capping, secondary tube labeling, aliquoting, and destination sorting into analyzer racks, on a small instrument footprint.

Fig 2: Tecan FE500pro™. Source: [i]

However, the need for increased throughput concerning molecular techniques and DNA/RNA purification methods has also led to a number of purification protocols on automated platforms. These automated methods must generate high-quality results in order to be used by downstream applications, such as sequencing, PCR, and transfection.

Therefore, the Hamilton® STAR™ line can serve as a simple pipettor (Fig. 3) facilitating serial dilutions, or as a center of a large system with multiple workstations and third party devices, such incubators, cell counters, and centrifuges, allowing for nucleic acid purification, PCR setup, sequencing, microarray sample preparation, protein precipitation, ELISA processing, and cell culture maintenance. The Hamilton® STAR™ line workstations can be configured with multiple arms. Moreover, each arm can be configured with various pipetting and labware manipulation tools, such as the 96 and 384 probe head, grippers, and lid tools, moving independently of each other.

Fig 3: Hamilton® STARlet workstation. Source: [ii]

Supporting pipetting with disposable tips or steel needles, the standard 1000µL independent channels are based on the air displacement pipetting technology. In conjunction with the needle wash station, reusable steel needles are available in the following sizes: 10µl, 300µl, and 1000µL.

Moreover, allowing for full implementation of cell culture procedures, Hamilton® offers de- and re-capping capabilities. Hence, tubes with a diameter range from 15 up to 38mm can be handled using diverse (de)capping modules. Nevertheless, limiting the available diameter range, solely two (de)capping modules can be placed on the Microlab® STAR™ deck simultaneously. Moreover, the minimum vial height is 50mm excluding the handling of common gas chromatography (GC)-vials.

The MICROLAB® NIMBUS 96 is the Hamilton Company's newest automated multi-channel pipetting workstation and offers a high density deck layout in a compact footprint while providing a dynamic pipetting range from 1.0µl up to 1000µl using the 96-channel CO-RE head. Therefore, the NIMBUS 96 is ideal for PCR sample preparation, DNA/RNA isolation, and MALDI target spotting. In addition several options and devices, such as the rotating labware gripper, the barcode reader, the heater/shaking device, and the vacuum station, can enhance the utility of the MICROLAB® NIMBUS workstation.

The Beckman Coulter Biomek® Assay Workstation offers a wide range of biological applications for different capacities, throughputs, and lab space requirements. The Biomek® Assay Workstation can be configured with either the Biomek® FX or the Biomek® NX liquid handler providing multiple pipetting and configuration options.

The Span-8 pipetting head allows for transferring of liquids to and from unique wells. Accordingly, the 96-or 384-channel pipetting heads are ideal for whole-plate transfers. Therefore, it is possible to replicate plates with the 96- or 384-well head and then add reagents unique to each well with the Span-8 pod using tubes, plates, or reservoirs as sources. Besides, the variety of Automated Labware Positioners (ALPs), such as shakers, stirrers, and others, increases the efficiency of the assay. With the ability to rotate a full 360°, the optional gripper enables the access from the left, from the right, or from integrated devices on the back of the instrument. It turns to pick up plates in different orientations, enhances on-the-fly barcode reading, and saves deck space by moving MTP-lids while the Span-8 is pipetting.

With its Biomek® System Software and the interchangeable pipetting tools (Eight-Channel Pipettor, Wash Tool), tool racks, and gripper tool, the Biomek® 3000 Laboratory Automation Workstation is designed to automate a variety of applications including nucleic acid sample preparation, reaction setup for capillary electrophoresis, genomic DNA purification, PCR/sequencing reaction setup, automated detection assays, reaction cleanup, protein purification, MALDI-TOF spotting, and much more.

The Biomek® 4000 Workstation (Fig. 4) also provides powerful liquid handling that adapts to changing situations. From its easy-to-use-icon-driven software and available application methods to its enhanced work surface with interchangeable tools, the Biomek® 4000 workstation is able to automate and streamline the laboratory workflow. As precise and robust as the Biomek® 3000, the Biomek® 4000 offers single- and 8-channeled pipetting from 1 up to 1000µL. Heating, cooling, and shaking accessories are available.

Fig 4: Beckman Coulter Biomek® 4000 Laboratory Automation Workstation. Source: [iii]

Agilent offers three levels of life science automation equipments: standalone, workstation, and containment based work cell (integrated system). The automated product line extends to the wide range of simple but time consuming tasks, such as labeling, centrifugation, and sealing. Moreover, the automated product line allows for complex processes, such as compound replication, HTS, ADME/Tox assays, PCR clean-up, cell maintenance, and other biological applications.

For simple system integration as well as for standalone use, the special open design of the Bravo Automated Liquid Handling Platform (Fig. 5) permits access from all sides. Nine microplate-positions and numerous plate-pad options are available in order to enable a wide range of assays. The liquid handling platform uses proven high accuracy pipette heads for dispensing from 100nl up to 200µl in 96- and 384-well microplates with either disposable or fixed tips for specific applications. The pipette heads can be changed in minutes.

Fig 5: Agilent Bravo Automated Liquid Handling Platform. Source: [iv]

The AssayMAP Bravo Platform is the state-of-the-art Bravo liquid handler enhanced with a Bravo AM head containing precision flow syringes. These syringes are specifically designed for use with the AssayMAP Sample Preparation cartridges, which incorporate a 5µL packed bed of resin (supported by membranes that are molded into the polypropylene cartridge). The syringes enable bidirectional flow and true high throughput chromatography. Moreover, available either as a standalone unit or

integrated into a larger robotic platform, the Vertical Pipetting Station will significantly reduce cycle time for the most required pipetting protocols. The Agilent Vertical Pipetting Station delivers industry-leading speed and unparalleled performance for sample handling and liquid-transfer applications. The two-axis positioning stage provides access to all quadrants of the 96-, 384-, and 1536-well MTPs.

Moreover, the Agilent's Encore Multispan System offers a wide range of applications, such as genomics, proteomics, cell biology, screening, ADME/Tox, and more. The system combines flexible multispan pipetting abilities including independent X and Y axis motions with the off-deck reach of the built-in robotic arm. This robotic arm reaches up to 53cm (21in) off deck making it easy to automate entire workflows both up and downstream. The used software enables researchers to visualize and optimize their protocols prior to any instrument movement. A combination of 32 deck positions (24 pipette-accessible and 8 for labware storage) enables complex multistep tasks.

2.4.2 Workstations with Embedded Autosamplers

The GERSTEL MultiPurpose Sampler (MPS) is both an autosampler and a sample preparation robot. Therefore, it offers a wide range of capabilities in one robotic system including semiconductors and electronics, chemicals and polymers, pharmaceuticals and environmental analysis. Furthermore, the MPS is compatible with all standard LC (LC/MS) and GC (GC/MS) systems and allows for highly efficient, automated sample introduction for GC/MS and LC/MS operations. For volume injection up to 1000µl the MPS performs liquid sample introduction in a highly reliable and efficient manner. Samples can be introduced from a variety of different sample vial types including micro- and deep-well plates and in addition crimp cap and screw cap vials providing the following sizes: 0.7ml, 1ml, 2ml, 10ml, and 20ml. Moreover, the MPS is able to process 10 and 20ml standard headspace vials as well as blood samples directly from Monovettes® or Vacutainers® minimizing the risk of contamination and infection. While using the MPS as a bench-top standalone version, the MPS provides sample preparation for multiple techniques and enables the automation of all liquid handling steps. In detail, using the variety of sample vial types, the MPS allows for weighing and filtration, dilution and extraction, cooling and heating, mixing and centrifugation, reading and processing barcode information.

Besides, maximizing the analytical possibilities, the MPS Dual Head version (Fig. 6) and the MPS DualRail PrepStation are available for GC (GC/MS), LC (LC/MS), or standalone operations. The additional tower enables the simultaneous use of two different syringes. In addition disposable pipette extraction (DPX), multi-position evaporation (mVAP), centrifugation, and automated SPE

techniques are available. Moreover, in combination with other GERSTEL modules the MPS offers further automated solutions for GC (GC/MS) sample preparation, such as Dynamic Headspace (DHS) and automated thermal desorption.

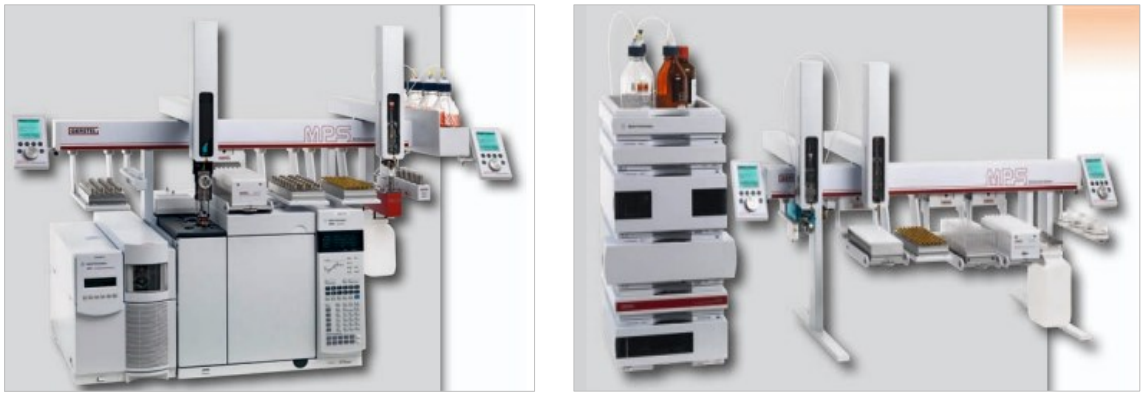


Fig 6: MPS Dual Head for automated sample introduction to a GC/MS and an HPLC system. Source: [v]

The MPS enables sample preparation for multiple techniques and automation of all liquid handling steps while using the GERSTEL MAESTRO software. In more detail, the Freedom EVOlution® software enables an easy setup of sophisticated pipetting procedures for pre-analytical sample distribution, assay preparation, and fully automated ELISAs. Exemplifying these procedures, Jiang *et al.* [55] developed and validated a Microsoft Excel based robotic sample preparation program (RSPP) that automatically transforms Watson worklist sample information, such as identification, sequence, and dilution factor, into comma-separated value (CSV) files. The Freedom EVO® liquid handler software imports and transforms the CSV files to executable worklists (.gwl files) allowing the robot to perform sample dilutions at variable dilution factors. The whole process including pipetting samples, diluting samples, and adding of the internal standards is accomplished within 1h for two racks of samples (96samples/rack). The developed platform also supports online sample extraction, LLE, SPE, and protein precipitation using the 96 multichannel arms. Besides, the RSPP saved more than 50% of the time for sample pipetting and diluting, and reduced human errors. The generated bioanalytical data are accurate and precise. Therefore, the automated sample preparation process has been applied to several drug development programs.

The CTC HPLC-xt PAL product line provides precise and accurate sample loading for high throughput environment and flexible analysis requirements. The open and modular architecture makes it an adaptable autosampler for almost every LC (LC/MS) operation. Samples can be introduced from a variety of different sample vial types including micro- and deepwell plates, various types of test tubes, crimp cap and screw cap vials of the following sizes: 1ml, 2ml, 10ml, and 20ml. Furthermore,

the DLW option offers near zero carryover and up to 4 times faster cleaning cycles compared to the standard fast washing process. Sample storage options from 4°C up to 70°C are available.

The PAL *HTC*-xt (Fig. 7) features the smallest footprint (50cm length) in the industry standard range of PAL-xt autosamplers. However, it still supplies major sample capacities. Therefore, the PAL HTC-xt enables precise and accurate sample loading in spite of the limited bench space situation. Injection volumes from 100nl up to 5ml enhance the flexibility. The open architecture provides easy access to samples, valves, and syringes.

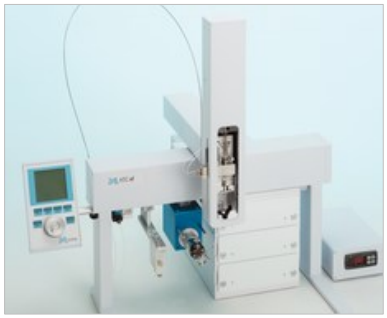


Fig 7: PAL HTC-xt. Source: [vi]

Upgradable with the PAL Dilutor Option, the PAL *HTS*-xt platform (80cm length) is designed to meet the requirements of the chromatography front end automation in terms of speed, capacity, and precision. Moreover, the 4-valve operation offers parallel or staggered sample analysis. Providing protection of thermo labile samples, various tray- and micro-plate cooling options are available.

The ultra-high throughput system PAL *HTX*-xt features an extended x-axis (120cm length). Built for unattended 24hours/day MS-analysis, the HTX-xt fits into the chain of the combinatorial chemistry / HTS screening / preclinical research market where large numbers of samples have to be characterized in a short period of time. Throughput is extremely high since recovery time between injections is reduced to a minimum of less than 30 seconds. Various options and accessories including the PAL Dilutor Option and the 4-valve operation are also available.

The PAL GC-xt product line provides powerful working capabilities, interfaces with all major GC (GC/MS) systems and dual injection port mode. The dual injection port mode allows for injections from samples placed in the same or in different vials in a single GC-run. This ensures high sample throughput, dual column and detector confirmation. Samples can be introduced from a variety of different sample vial types including micro- and deep-well plates, crimp cap and screw cap vials of the following sizes: 1ml, 2ml, 10ml, and 20ml. Sample storage options from 4°C up to 70°C are available.

For maximizing the performance, the PAL *GC*-xt (Fig. 8) is capable of handling up to six different syringe sizes, which cover an injection volume range of 0.1µl - 5000µl. The capability to inject larger volume samples eliminates the need of evaporation resulting in essential time savings. For lower volume samples, the fast injection speed minimizes needle discrimination and reduces background interferences.

Different injection modes include the traditional, the hot empty needle, the sandwich, or the internal addition technique. The sandwich mode prevents the effects of boiling point discrimination in low volume applications, whereas the internal standard addition is used for quantitative calculations, retention index-studies, or matrix spiking. Every single injection step is individually controlled through the PAL GC-xt's advanced software package.

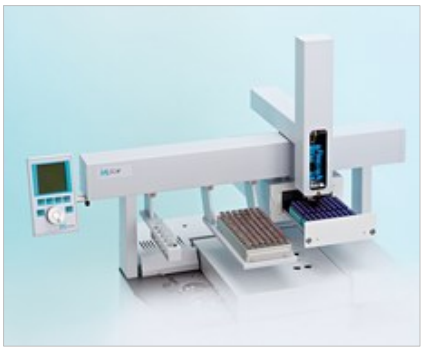


Fig 8: PAL GC-xt. Source: [vii]

The PAL COMBI-xt Extended (Fig. 9) is designed to meet the requirements of a large sample capacity. The extended version can load up to 686 2ml and 224 10/20ml vials (in comparison: the COMBI-xt version can load up to 294 2ml and 96 10/20ml vials). For both the PAL COMBI-xt and the PAL COMBI-xt Extended version SPME-, in-tube extraction (ITEX)-, direct thermal desorption-, multiple headspace extraction (MHE) -, and static headspace options are available. Headspace eliminates dead volume and adsorption effects.

Fig 9: PAL COMBI-xt. Source: [viii]

Controlled by the PAL Sample Control software, the PAL RTC (Robotic Tool Change) (Fig. 10) is the evolution of the PAL-xt product line. The additional versatility in combination with the increased volume range offers significant benefits and allows for the definition of flexible, tailor-made automation processes. Using the RTC enables utilization of up to six different syringe types during one single performance cycle. Thereby, the PAL RTC increases the productivity and widens the application range. Automatic sample preparation steps provided by the RTC are sequential dilution, calibration dilution, standard addition, and derivatization. The RTC changes between three different injection tools. In detail, liquid injection, headspace, and SPME methods change within one sample list and without need of manual operation in less than 30 seconds.

Further modules, such as the optional vortex mixer, the barcode reader, the valve drive, or the tray holder, are available. Faster GC injection times down to 100ms reduce discrimination in split and splitless injections modes. Moreover, the vial bottom sensing allows for reliable aspirations of small volumes even out of a few micro-liter samples. However, depending on sample preparation and clean-up efforts, matrix components, such as salts, non-precipitated small molecules, and particulate matter, can remain in the sample having a detrimental effect on the performance of the autosampler - especially if the sample manipulation mechanism is syringe-based. Using a Teflon tubing loop eliminates the contact of samples and potentially abrasive and corrosive matrix components with both the syringe plunger and the barrel [106].

Fig 10: PAL RTC. Source: [ix]

2.4.3 Comparison of Fully Integrated Systems and Traditional Workstations

A fully integrated system has been defined as a collection of instruments, such as those workstations described above and further single devices. Moreover, the fully integrated system is served by a system integrator moving labware, such as plates and tip-boxes, between the peripheral devices (workstations).

Checking the most important features, the traditional workstation and the fully integrated robotic system are compared in the following Table 1.

Table 1: Comparison of the traditional workstation and fully integrated systems [105]

Feature	Traditional Workstation	Fully Integrated Robotic System
System size	Typically benchtop	Dedicated room and services, fully enclosed robotic cell
Number of tasks performed	One, or specialised part of a process	Multiple and diverse, fully automated process
Walk away plate processing capability	<100 plates	<1,000 plates
Plate movement	From manually loaded stacker	Fully articulated robot arm, usually on a track
Scheduling software	None	Essential
Task/application complexity	Specialized component	Less specialized, but greater diversity
Staffing resource	Minimal, supervisor	Several dedicated
Staff training needed	Moderate	Extensive
Source of components	Single supplier	Multiple/made to customer order by third party integrator
Main area of use	Batch processing, typically plate reading or sample preparation	High-throughput Screening (HTS) and ultra HTS
Ease of implementation	Simple/immediate	1-6 months to agree specification 6-12 months from placing order

Including multiple third party devices, such as incubators, cell counters, centrifuges, and several kinds of readers (supplying various screening options), the Hamilton® STAR™ line provides a fully integrated system using an external gripper. However, due to the scalability of the STAR™ line instruments, the required range of throughput can be accommodated using additional instruments, such as pipetting channels or 96/384-probe heads, and an integrated robotic arm that can be fitted to the existing configurations.

The Biomek® Cell Workstation (Fig. 11) is based upon the Beckman Coulter Biomek® liquid handler and was developed in cooperation between celisca and Beckman Coulter. The Cell Workstation supplies modular integration of various components for fully automated cell culture while using MTPs and tissue culture flasks. Moreover, the Biomek® Cell Workstation allows for fully automated cell seeding, cell harvesting, growth rate monitoring, and quality control.

Representing a collection of instruments, the commercially available, highly flexible Agilent BioCel System (Fig. 11) is served by a system integrator (Direct Drive Robot). This system automates any MTP-based protocol providing a wide range of biological applications. To create a fully contained and contaminant-free environment, various enclosure and environmental control options can be added.

Fig 11: Beckman Coulter Biomek® Cell Workstation (left) and Agilent BioCel System. Source: [x]

2.4.3.1 System Integrators

The Robotic Industries Association (RIA) defines a robot as follows:

“A robot is a reprogrammable, multifunctional manipulator designed to move material, parts, tools, or specialized devices through variable, programmed motions for the performance of a variety of tasks.” [14].

Accordingly, system integrators are capable of moving labware, such as MTPs, between various peripheral devices (workstations).

Applied in the industrial areas, system integrators also allow for sample handling options, such as welding and lasing, whereas the scope of operation is limited to transport options for common laboratory environments. Providing an integrated system, several laboratory system integrators are commercially available. Described in the current section, high degrees of freedom, grip forces, and adequate carrying capacity are the main eligibility criteria.

Representing the first choice for system integrators, the ORCA® (Optimized Robot for Chemical Analysis) facilitates easy access to a broad range of peripheral devices and instruments. With six axes of movement, this robotic arm provides a broad and flexible range. The articulated robot is usually mounted on a linear rail enhancing the accessible workspace. The ORCA® moves with dexterity manipulating various labware, such as MTPs, vials, and tubes, throughout the integrated system.

Capable of being floor, ceiling, or wall mounted, the compact Motoman HP3JC robot features a large work envelope with an 804mm vertical reach and 532mm horizontal reach. The compact Motoman is a small, high-speed robot providing high flexibility and six axes of movement. Offering superior performance in small part handling and in addition easy access to a broad range of peripheral devices, the HP3JC facilitates lab automation.

The Agilent BenchBot Robot is designed for the integration of a wide range of third-party instruments. Therefore, the robot can be installed on standard laboratory benches, within enclosed hoods, or on portable docking tables. Using the BenchBot Robot offers a variety of MTP-handlings. Therefore, the available applications include genomic workflows, such as Next-Generation Sequencing, microarray sample preparation, cell-based assays, MTP-based cell maintenance, high-throughput HPLC sample management, and enzyme assays.

The Agilent BenchCel Microplate Handler is a compact, MTP-storage and -handling system designed for the integration of a variety of laboratory devices. The BenchCel Microplate Handler features a high-speed robot. Its modular design that includes the 2-, 4-, or 6-rack options for a maximum of 360 standard MTPs and de-lidding functions provides the flexibility and scalability required to meet the needs of the most diverse laboratory applications.

The Agilent Direct Drive Robot (DDR) is fast, precise, and designed with safety in mind. State-of-the-art direct drive technology reduces the number of moving parts resulting in a robotic arm with increased speed and reliability. The DDR can grip in portrait or landscape orientation to minimize or eliminate re-gripping. The DDR can be used either as a standalone robot or as the center of the Agilent BioCel System.

For choosing the type that is best suited to the application needs, understanding of the configuration feasibilities is essential. Moreover, the accessible workspace and the workspace geometry have to be considered. Common robotic configurations are shown in the following Table 2 providing at least three degrees of freedom and, therefore, a further criterion for the robot´s definition.

Table 2: Description of common robotic configurations

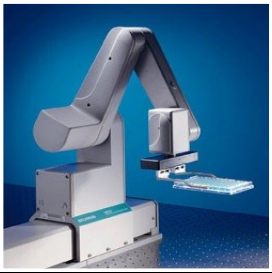
Configuration	Description	Figure
Cylindrical	▪ Two orthogonal prismatic axes of movement (horizontal and vertical direction) ▪ One revolute axis ▪ Cylindrical coordinate system ▪ Basic pick and place device ▪ Central system integrator	
Articulated	▪ Two or more revolute joints ▪ Usually mounted on a linear rail to enhance the workspace ▪ High degrees of freedom (5-6) ▪ First choice for system integrator	
SCARA	▪ Selective Compliance Assembly Robot Arm ▪ Two/more revolute joints ▪ One prismatic joint ▪ Fast pick and place devices ▪ Less complex system integrator	
Cartesian	▪ X, Y, and Z axes of movement ▪ Prismatic joints ▪ Linear configuration ▪ Largest surface area requirement ▪ Basis for automated liquid handling workstations	
Gantry	▪ Cartesian robot, whose X and Y axes have been elevated	

Fig 12: Robot RT3300, ORCA®, Agilent BenchBot Robot, Biomek® FXP. Source: [xi] - [xiv]

2.5 Analytical Techniques of Measurement

Performing analytical techniques of measurement allows for different approaches that provide element- and structure-specific detection modes. Element-specific measurement facilitates the determination of the identity and the concentration of certain elements, whereas structure-specific measurement supplies structure determination and quantification of chemical compounds and substances.

2.5.1 Element-Specific Measurements

Element-specific spectroscopy, such as atomic absorption (AAS), optical emission (OES), atomic fluorescence (AFS), X-ray fluorescence spectroscopy (XRF), and inductively coupled plasma mass spectrometry (ICP-MS), allows for the determination of elements.

AAS is an analytical procedure providing the qualitative and quantitative determination of chemical elements using the *absorption* of optical radiation by free atoms in the gaseous state [107], [108]. Using different kinds of atomizer, AAS is divided into further subclasses, such as flame, graphite furnace, and cold vapor (CV) AAS. However, the valence electrons of the atoms in the atomizer can be excited by absorbing a defined quantity of energy (radiation of a given wavelength), which is specific to a particular electron transition in a particular element providing elemental selectivity.

Dividing OES [107], [108] into further subclasses, such as flame or inductively coupled plasma, OES provides different kinds of atomizers, such as flames, plasma, arcs, or sparks. However, excited by the atomizers, electron transmission of the valence electrons causes *emission* that allows for the determination of elements. In general, the wavelength of the atomic spectral line defines the identity of the element while the intensity of the emitted light is proportional to the number of atoms of the element. However, OES usually does not include fluorescence. Fluorescence of the samples is determined using ultraviolet light for the excitation of the valence electrons (AFS) [107], [108].

Regarding the binding energy of the excited electrons, absorption and emission are defined using the following equation (2-1):

$$\Delta E = E_b - E_a = h\,v = h\,\frac{c}{\lambda} \qquad (2\text{-}1)$$

XRF spectroscopy [108], [109] provides bombarding with high-energy X-rays or gamma rays resulting in excitation of the *inner* electrons. Electron transmission causes fluorescent X-rays that correspond to the differences in binding energy of the excited electrons, which is described using the following equation (2-2):

$$\Delta E = \frac{2\pi\, m_e\, e_0^4\, (Z-\sigma)}{h^2}\left(\frac{1}{n_1^2}-\frac{1}{n_2^2}\right) = h\,\nu \qquad (2\text{-}2)$$

m_e… Rest mass of the electron

e_o… Elementary charge

σ… Screening constant

Z… Atomic number

$n_{1/2}$… Principal quantum number

Using the results of the previous equation allows for the determination of the corresponding wavelength (2-3):

$$\lambda = \frac{1239.6}{E} \qquad (2\text{-}3)$$

Moreover, considering the Moseley-Law, wavelength of the emitted X-rays depends on the atomic number (2-4):

$$\frac{1}{\lambda} = const\,(Z-\sigma)^2 \qquad (2\text{-}4)$$

However, routine element analysis is currently carried out by the ICP-MS, which is of increasing importance due to its high sensitivity, good interference control, analysis speed, and possibility for multi-element analysis [67]. Moreover, ICP-MS is the most powerful multi-element analytical technique available today. Nevertheless, the number of determined elements varied, so the term multi-element is used for studies involving 5 to 15 elements.

The very low detection limits of ICP-MS makes it an attractive option in the wide range of environmental [67], medical [110], biological [111], industrial [112], and archaeological applications [113]. Since publication of the first ICP-MS mass spectra almost 35 years ago [114], the number of commercial ICP-MS instruments sold worldwide has drastically risen [113].

ICP-MS instrument consists of several distinct parts, which are:

1. Sample introduction: Produced by passing the liquid sample through a simple pneumatic nebulizer, the sample aerosol is introduced into the inductively coupled plasma. Here, larger aerosol droplets are removed from the gas stream (by the spray chamber), whereas the remaining smaller droplets are swept into the central channel of the argon plasma.

2. Ion generation: The plasma is generated in a stream of argon contained in a quartz tube or a so-called torch. This torch is located in the center of a cooled copper coil through which a high power, high frequency electric current (produced by radio frequency generator) is passed. Created by the electric current, an intense electric field causes collisions between free electrons and argon atoms producing ions and more electrons - until a stable, high temperature plasma is formed. Due to the fact that RF frequency of 27.12 MHz results in high plasma temperatures, aerosol droplets are dried, decomposed, vaporized, atomized, and ionized.

3. Interface: The positively charged ions are extracted into the vacuum system - via a pair of interface cones. These cones are metal plates with central orifices through which the ions pass. To maintain the high vacuum in the mass spectrometer region, small orifices are used (1mm diameter or less).

4. Ion focusing: The ions are focused in a compact ion beam by electrostatic lenses as they pass through the vacuum system (to the final chamber where the MS and the detector are housed). Decreasing the random background noise, the lenses perform a second essential function by separating the ions from the photons and residual neutral material.

5. Ion separation: The most common mass analyzer used in ICP-MS is the quadrupole. Furthermore, magnetic sector and time-of-flight analyzers have been applied. Employing a combination of direct current (DC) and alternating current (AC) electrical fields (fixed ratio), the quadrupole separates the ions based on their mass to charge ratio (m/z). In more detail, the m/z is equal to the masses of the ions due to the fact that the plasma produces almost exclusively singly-charged ions. The voltage settings can be changed. For a given voltage setting only one m/z is stable. Passing each mass of interest sequentially to the electron multiplier (EM) detector, the quadrupole scans rapidly across the mass range 2 - 260 amu.

6. Ion detection: The electron multiplier detects each ion as it exits the quadrupole. Creating a mass spectrum, the detector electronics count and store the total signal for each mass (m/z). Therefore, the spectrum provides a simple and accurate qualitative representation of the sample. By comparing signal intensities to those generated by the calibration standards, the quantitative results are produced.

2.5.2 Structure-Specific Measurements

Structure-specific methods allow for the identity and structure determination of compounds using precedent extraction and isolation steps. Therefore, structure-specific analytical measurement comprises various chromatography techniques, such as GC and high performance liquid chromatography (HPLC), as well as spectrometric techniques, such as infrared spectroscopy (IRS), X-ray chrystallography, and mass spectrometry (MS).

IRS capitalizes the fact that molecules absorb specific frequencies (resonant frequencies) that are characteristic of their structure [115], [116]. The frequency of the absorbed radiation matches with the transition energy of the bond that vibrates allowing for the determination of the atomic masses and the associated vibronic, coupling energies. Correlation between the irradiated and the passing light is described in the following equation (2-5) regarding the attenuation of light by absorption I_A, reflection I_R, and scattering I_S.

$$I_0 = I + I_A + I_R + I_S \tag{2-5}$$

X-ray chrystallography can be used for materials forming a crystal, such as salts, metals, and minerals, due to the fact that crystalline atoms cause a beam of incident X-rays to diffract into many specific directions [117]. X-ray scattering is determined by the density of the electrons within the crystal allowing for the identification of the atomic and molecular structure including the mean positions of atoms, their chemical bonds and disorder. Thus, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal while performing measurement of the angles and the intensities of the diffracted beams, which is considered in the following equation (2-6) for one particle with mass *m* and the charge *q*.

$$I_0 = I_e \left(\frac{q^4}{m^2c^4}\right)\frac{1+cos^2 2\theta}{2} = I_e 7.94.10^{-26}\frac{1+cos^2 2\theta}{2} = I_e f \tag{2-6}$$

MS is an analytical technique that allows for element- and structure-specific measurements by ensuring the ionization of chemical compounds in order to generate charged molecules or molecule fragments. Finally, MS provides the measurement of the mass-to-charge (m/z) ratios [107], [108], [109].

The most common mass analyzer is the quadrupole. However, magnetic sector and time-of-flight analyzers provide common application techniques. The quadrupole separates the ions based on their mass to charge ratio (m/z) using a combination of DC and AC electrical fields (fixed ratio). The common field equation (2-7) describes the correlation between the potential of the electric field φ *(x, y, z, t)* and the co-ordinates *x, y, z.*

$$\varphi = f(t) = (\alpha x^2 + \beta y^2 + \gamma z^2) \quad (2\text{-}7)$$

However, the m/z is equal to the masses of the ions due to the fact that the plasma produces almost singly-charged ions. The voltage settings can be changed. For a given voltage setting only one m/z is stable. Passing each mass of interest sequentially to the electron multiplier (EM) detector, the quadrupole scans rapidly across the mass range 2 - 260 amu.

Facilitating chromatography techniques [118], the gas chromatograph utilizes a capillary column heated by an oven and retaining the molecules of the sample. The molecules elute from the column at their specific retention times, which depends on the column´s dimensions (length, diameter, film thickness) as well as the phases´ properties. Therefore, the difference in chemical properties between the different molecules (of the sample) and their relative affinity for the stationary phase of the column promotes separation of the molecules, while an inert carrier gas carries the solutes through the column. Therefore, separation occurs as a result of a unique equilibrium established between the solutes and the stationary phase.

HPLC is a chromatography technique [118] that is used to separate the components in a mixture and to identify and to quantify each component. Containing the sample mixture, a pressurized liquid solvent passes through a column filled with solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material of the column, which causes different flow rates for the different components and leads to the separation of the components as described in the following Van-Deemter equation (2-8).

$$H.E.T.P. = A + \frac{B}{v} + C\ v \quad (2\text{-}8)$$

- H… Height equivalent to a theoretical plate
- A… Eddy diffusion
- B… Longitudinal diffusion coefficient
- C… Mass transfer coefficient
- v… Linear velocity

3. Scope of Work

In order to define the scope of work, automation requirements of analytical sample pretreatment have to be considered.

However, due to the fact that automating biological sample pretreatment is already established using current existing workstations, these processes have to be regarded first. Subsequently, common analytical processes have to be discussed and compared with the defined requirements of biological applications.

3.1 Common Biological Processes

Using a flowchart, the following Fig. 13 represents the sequence of common biological processes.

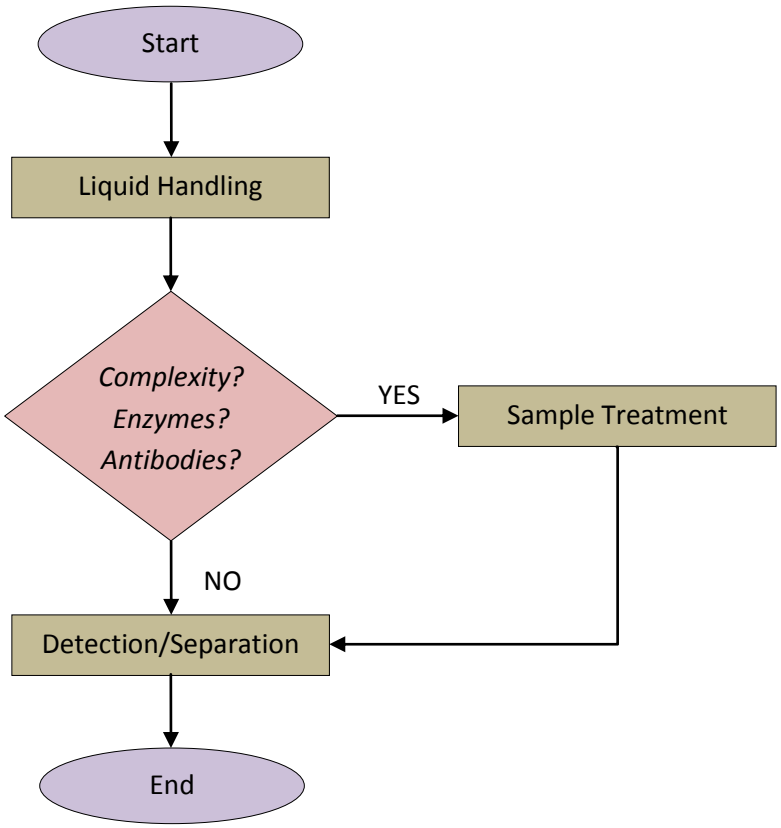


Fig 13: Flowchart – Sequence of common biological processes including liquid handling, sample treatment (such as incubation steps, vortex mixing, protein precipitation, and extraction), and detection while using different detection modes (such as absorbance, fluorescence, and luminescence) for the required MTP-assays or off- and online separation (using chromatography and mass spectrometry)

3. Scope of Work

Buffers are extensively used in the wide range of biochemical assays due to the fact that buffers offer an attractive choice for sustaining biological molecules in their native state [119]. Pipetting buffers supplies stable and nonhazardous pH values (mostly pH = 7.4). Moreover, pipetting media and washing solutions is included. Performing biological applications, liquid handling comprises liquid transfer, liquid aspiration and dispension [120], transfer to waste, and serial dilution [121] while handling small volume ranges (≤ 1ml) [122].

Considering temperature (mostly 37°C), humidity, and atmosphere (CO_2 = 5%), incubation steps are required if the solutions used in the liquid handling steps contain cell metabolism products, such as enzymes and growth factors and, moreover, bio-molecules, such as proteins, nucleic acids, and nucleotides [123]. Due to their complex matrices, evaporation, sonication, vortex mixing, protein precipitation, and extraction of the samples is necessary. Thereby, sample treatment provides an appropriate form of the analyte for the detection and separation systems.

For the final analysis step assay detection readers are required that are mostly designed for the MTP-format. As assays detection technologies have been advanced by the MTP-format, space-saving and user-friendly multimode (or multi-detection) readers have been developed [124], [125] enabling researchers to perform multiple assay types in one instrument [126]. Nevertheless, for some biological measurements chromatographs and mass spectrometers, such HPLC/MS [127], are needed requiring the injection into the separation/detection system. In contrast, assay detection readers facilitate easy online measurements without any injection step. Common detection modes for MTP-assays are absorbance, fluorescence, and luminescence.

The MTP-development replaced the use of test tubes and allows, therefore, for easy automation. Hence, laboratory robots are mostly designed for especially this format. Due to the nonhazardous conditions and the small volumes that occur during biological applications, MTPs can be used during the whole application process: liquid handling – sample treatment – detection/separation. Automating biological applications, commercially available liquid handlers usually support on-deck integration of additional components, such as washers, heaters, shakers, thermocyclers, incubators, centrifuges, SPE, and magnetic bead separation. Using these additional components, liquid handlers enable liquid handling *and* sample treatment in one process step.

3.2 Common Analytical Processes – Comparison and Conclusion

Using a flowchart, the following Fig. 14 represents the sequence of common analytical processes.

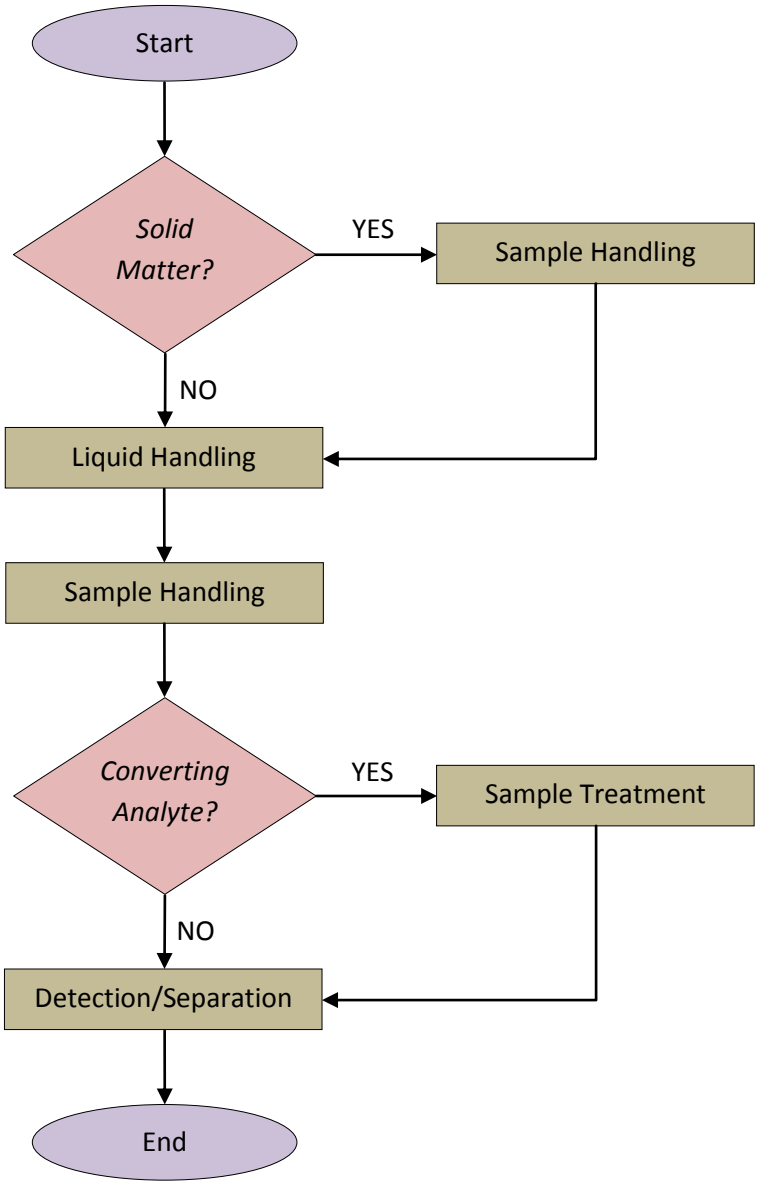


Fig 14: Flowchart – Sequence of common analytical processes including liquid handling, sample handling (such as weighing and capping), sample treatment (such as derivatization and microwave digestion), detection and separation (such as element- and structure-specific measurements using chromatography and mass spectrometry modes)

Depending on the sample properties, samples have to be dosed by weighing (sample handling) or pipetting (liquid handling) first. For weighing and pipetting the utilization of highly active substances, such as concentrated acids or organic solvents with different viscosities, has to be considered demanding the application of inert materials. Furthermore, comparing to biological applications, analytical sample pretreatment calls for higher volume ranges (in some cases up to liters [65]) that are required in order to dissolve higher quantities of the solid samples with non-homogeneous consistency. Moreover, sample handling, such as individual capping, is required in order to ensure the concentration stability while handling volatile solvents and components, such as hexane, acetone, and ethanol. In detail, crimp caps with septa have to be chosen due to the very good seal that is allowed by this combination [128].

In order to ensure analytical measurements, converting the analyte molecules into an appropriate form for the detection and separation systems is required and can be accomplished by derivatization, separation, and microwave digestion. Performing these sample treatment steps, special conditions, such as non-standard temperatures and pressures, are required. Moreover, the required vessel types have to be chemically inert, temperature and pressure resistant, and capable of handling higher volume ranges. Thus, analytical sample pretreatment calls for a wide range of individual vials and tubes that fulfill the requirements mentioned above.

For the final analysis step, detection and separation systems, such as chromatographs and spectrometers [129], [130], are required in order to allow for the determination of the identity and the concentration of a certain, chosen element. Furthermore, selective structure-specific determination and precise quantification of molecules and mixtures of molecules are ensured using these systems. In contrast, biological assay detection supplies easy cell component analysis while simply reacting (binding, adsorbing, activating signal-pathway) with the target substances (as defined by the chosen assay type). However, the main differences between common biological and analytical processes are depicted in the following table.

Table 3: Comparison of common biological and analytical processes

Process Step	Biological Process	Analytical Process
Liquid Handling	▪ Handling smaller volume ranges: 1µl up to 1ml ▪ Pipetting buffers, media, and washing solutions ▪ Non-hazardous pH values	▪ Depends on samples´ consistency ▪ Liquids: equal to biological processes ▪ For solid matters: 1ml up to 1l ▪ Highly active solvents/substances
Sample Handling	▪ No individual capping necessary due to easy liquid handling	▪ Volatile substances ▪ Individual capping necessary to ensure concentration stability
Sample Treatment	▪ Mostly incubation steps ▪ Temperature 37°C ▪ Atmosphere CO_2 = 5%	▪ Derivatization and microwave digestion steps ▪ Wide range of temperatures and pressures
Detection	▪ Mostly target based assays ▪ Cell component detection	▪ Chromatography, spectrometry ▪ Selective analysis of certain elements, molecules, or mixtures of molecules

Consequently, allowing for the analysis of single elements, small molecules, and mixtures of molecules, the requirements of element- and structure-specific analyses differ significantly from biological applications. Therefore, commercially available automated systems are not suitable for analytical sample pretreatment. Furthermore, existing systems either handle only a few steps (mostly at the end) of a complex analytical scheme or they offer a single solution for a fixed process. Therefore, the purpose of this dissertation is the design, the realization, and evaluation of a flexible, automated system that will be capable of

1. Processing extensive analytical applications while considering the specific process requirements, such as the handling of higher volume ranges and highly active substances, non-standard temperature and pressure conditions, and the selective analysis of single elements, molecules, or mixtures of molecules.

2. Processing upgradeable automation steps and flexible analytical sample pretreatment while dealing with the wide range of required vessels simultaneously in order to provide less cost- and time-consuming steps.

4. System Concept and Design

4.1 General Conspectus

Supplying flexible and precise sample transport between the automated sample preparation system and further external stations, such as the detection and separation systems, a mobile robot (H20, Dr. Robot Inc., Markham, Canada) system (1) has been developed (Chapter 4.8).

Nevertheless, the central elements of the fully automated system (as shown in Fig. 15/16) are two classical laboratory robots (ORCAs®, Beckman Coulter, Brea, CA) (2) that act as system integrators and transport systems. The ORCAs® are mounted on two orthogonal (90°) 2m linear rails enhancing the accessible workspace. Thus, both ORCAs® facilitate easy access to a broad range of peripheral devices and instruments. The extended envelope feature enables the robot arms to operate on both sides of the rails, respectively, effectively doubling the useable workspace. In addition the designed re-grip station (3) allows for sample transfer between both ORCAs® (Chapter 4.3).

Liquid delivery and dilution steps can be performed by the integrated Biomek® 2000 (Beckman Coulter) (4) supplying volumens up to 1ml and, moreover, by an in-house designed diluting station. The in-house designed liquid handler provides volumes up to 10ml (Chapter 4.4) using a Hamilton® dispenser (Hamilton®, Bonaduz, Switzerland) (5) with an incorporated 10ml syringe.

Furthermore, the Digitus HQ Webcam USB 2.0 (ASSMANN, Luedenscheid, Germany) (6) has been implemented into the automated system ensuring sample identification while using 2D-barcode processing and reading (Chapter 4.5.1).

Samples are stored in a flexible sample-hotel (7) that provides 196 shelves varying in height and allowing for the storage of different kinds of MTPs – until they are processed within the system (Chapter 4.5.1).

Moreover, the SCARA robot (TS60, Stäubli®, Bayreuth, Germany) (8) assembly – including a crimping die, which is part of the Zymark® Crimp Capping Station (Zymark®, Hopkinton, MA), – offers individual gripping, placing, and crimping of various vial types (Chapter 4.5.3).

Furthermore, the 2m solid shuttle transport system (9) has been integrated supplying linear transport to the SCARA robot assembly (Chapter 4.5.3).

Due to safety reasons, the system has been partially covered with a housing (10) that can be exhausted in addition (Chapter 4.5.4).

The weighing station (analytical balance BP211D, Sartorius, Goettingen, Germany) (11) is loaded by the high precise SCARA robot that ensures individual vial handling (Chapter 4.5.5).

Moreover, the ionizer ANTISTAT 2000 (CEM, Kamp-Lintfort, Germany) (12) avoids electrostatic charge of the samples during the weighing steps and enables fast and precise sample handling (Chapter 4.5.5).

The heating and shaking device MHL 23 (HLC BioTech, Bovenden, Germany) (13) has been integrated allowing for derivatization and homogenization of the samples. Moreover, the designed Positive Pressure Unit can be simply assembled on the Biomek® liquid handler and provides positive pressure SPE applications. In addition, automated analytical sample pretreatment includes microwave digestion steps. However, due to safety reasons, these steps have to be performed under a separated hood (Chapter 4.6).

A specific integration module has been developed for every custom integrated device using the SILAS software (Beckman Coulter) developer kit (Chapter 4.7).

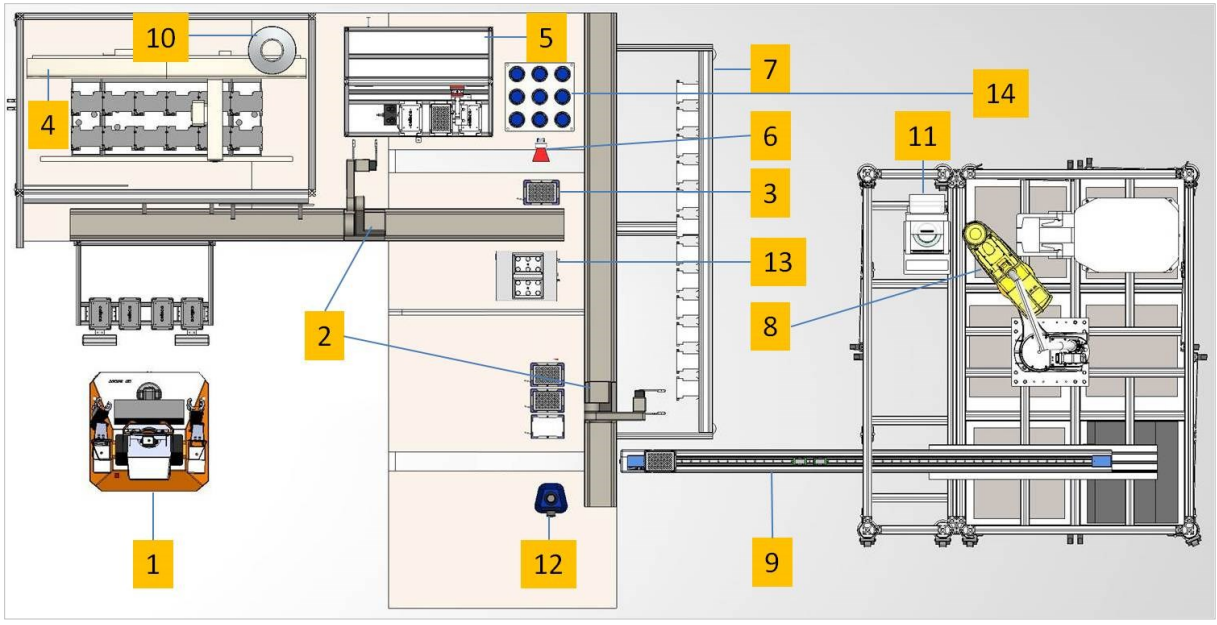


Fig 15: Top view CAD design of the automated system: (1) mobile robot, (2) two ORCA® robots, (3) designed regrip station, (4) Biomek® 2000, (5) diluting station, (6) Digitus HQ webcam, (7) flexible sample hotel, (8) SCARA robot Stäubli® TS60, (9) shuttle, (10) housing of the Biomek® 2000 including an exhausting system, (11) analytical balance BP211, (12) ionizer Antistat 2000, (13) MHL 23 heating and shaking device, (14) solvent reservoirs

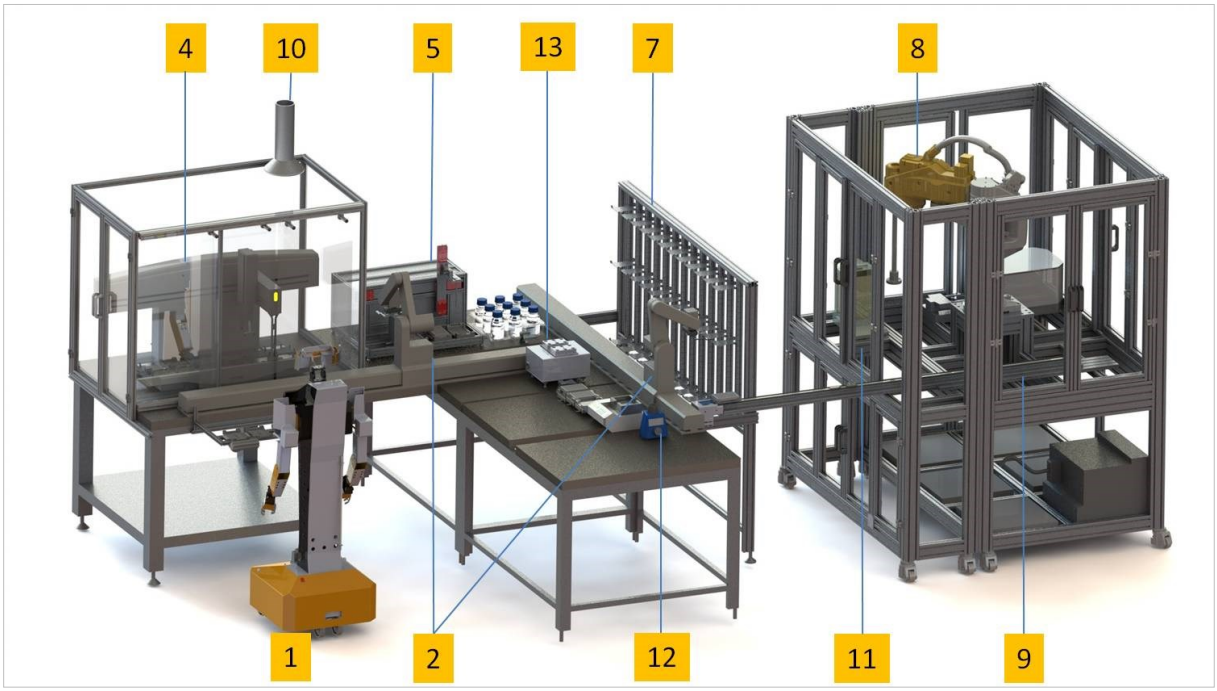


Fig 16: 3D CAD design of the automated system: (1) mobile robot, (2) two ORCA® robots, (3) designed regrip station (not shown), (4) Biomek® 2000, (5) diluting station, (6) Digitus HQ webcam (not shown), (7) flexible sample hotel, (8) SCARA robot Stäubli® TS60, (9) shuttle, (10) housing of the Biomek® 2000 including an exhausting system, (11) analytical balance BP211, (12) ionizer Antistat 2000, (13) MHL 23 heating and shaking device

4.2 Concept of Standardized Labware Design

Commercially available automated workstations are usually configured for handling the standardized MTP-format. Nevertheless, reaction chambers of this labware format are not sufficient to fulfill multistep analytical sample pretreatment due to the higher volume ranges and the specific conditions needed for performing analytical procedures (as described in the previous chapters). Therefore, the use of specialized vessels has remained indispensable.

Considering the wide range of analytical labware requirements, even for one specific application several labware types are required, such as the analysis and calibration vessels, special treatment vessels (such as the microwave digestion vessels) and, moreover, several reservoirs for organic and inorganic solvents. Exemplifying this wide range, the following Table 4 depicts the applicable vessel types required for a common microwave digestion procedure.

Table 4: Description of specific vessels required for common microwave digestion procedures

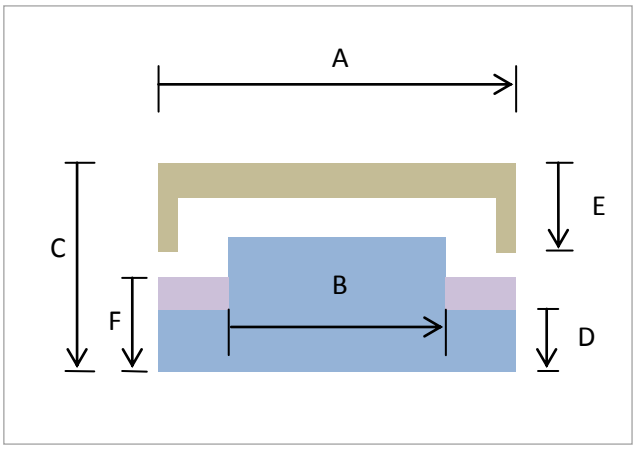
Function	Kind of vessels	Volume	Material
Analysis vessel	Tube	50ml	PP
Calibration vessel	Tube	15ml	PP
Microwave vessel	MARS Xpress vessel	20ml	PFA
Reservoir (water)	Narrow mouth bottle	125ml	LDPE
Reservoir (acid)	Beaker	100ml	PFA

However, embedding all of these vessels into the standardized MTP-footprint represents a suitable solution that ensures the availability of existing workstations for multistep analytical sample pretreatment while using system adaption. Nevertheless, to ensure the entire task performance of the adapted workstations, the embedded vessels have to meet the specifications of the controller software. Thus, using the Biomek® 2000, the labware parameters are predetermined by the Biomek® Editor software (Bioworks®). These parameters are based on the MTP-contours including a maximum length of 130mm and a maximum width of 120mm.

Nevertheless, by way of derogation from the standard MTP-contours, Bioworks® enables a maximum height of 115mm (top height parameter). Thus, with simultaneous consideration of the maximum diameter of 120mm (regarding the maximum length and width), the embedded vessels have to be smaller than the top height value.

Consequently, due to the deviation of the top height parameter from the standard MTP-contours, embedding the applicable vessels into the standardized MTP-footprint is ensured and provides the availability of existing workstations for analytical processes. Moreover, the concept idea allows for simultaneous handling of up to 24 vessels and supplies, thereby, less cost- and time-consuming steps and high-throughput options. Due to the fact that the concept idea is applicable for a wide range of vessels, the performance of a wide range of analytical applications is ensured. Exemplifying the presented concept idea, a specific embedded vessel type is shown in Fig. 17. All of the predetermined parameters of the controller software are depicted in Fig. 18.

Fig 17: Specific vessel type embedded into the MTP-footprint exemplifying the concept idea of system adaption



Lid length	≤ 130mm
(A) Lid width	≤ 100mm
(B) Top width	≤ 120mm
(C) Lid on height	≤ 120mm
(D) Lip height	≤ 60mm
(E) Lid height	≤ 60mm
(F) Lifter height	≤ 60mm
Top height	≤ 115mm

Fig 18: Scheme of the Biomek® 2000 Editor Software (Bioworks®) including the predetermined labware parameters

4.3 System Integrator

Working as system integrators, two laboratory robots (ORCA®) were implemented into the automated system providing six axes of movement. The ORCAs® are mounted on two orthogonal (90°) 2m rails enhancing the accessible workspace and facilitating easy access to a broad range of peripheral devices and instruments (Fig. 19). The extended envelope feature enables the robot arms to operate on both sides of the rail, respectively, effectively doubling the useable workspace.

With high degrees of freedoms and four revolute joints these articulated robots provide high flexibility and move with dexterity manipulating various labware, such as MTPs, vials, and tubes, throughout the integrated system [131]. Hence, based on the MTP-footprint, the ORCA® robots are able to deal with the designed labware types. Moreover, the additional re-grip station supplies transfer of the designed labware types between both robot arms.

Due to the utilization of the standardized MTP-formatted footprint, the presented concept idea enables the ORCA® robots to place every kind of designed labware type on any existing labware position throughout the integrated system. Thus, using the concept idea provides more flexibility during all transportation steps and enables the utilization of the system for various kinds of applications without any changing of labware positions. Moreover, handling several vessels (up to 24 depending on the conceived tray) simultaneously, the designed labware types facilitates less cost- and time-consuming transportation steps.

Fig 19: 3D CAD design of the automated system with two ORCAs® supplying system integration and sample transport

- Arm articulated, rail-mounted
- Six degrees of freedom
- Reach: ±54cm
- Height: 78cm
- Weight: 8.0kg
- Precision: ±0.25mm
- Maximum speed: 75cm/s
- Payload: 0.5kg continuous, 2.5kg transient (with restrictions)
- Dwell time: 50ms typical (for moves within a motion)
- Power requirements 100V, 120V, 220V, or 240V (+5%, -10%), 350VA, 47.5 to 66Hz
- Teach pendant: Joy stick with emergency stop

4.4 Liquid Handling

In order to fulfill pipetting options for automated analytical sample preparation, the Biomek® 2000 has been chosen performing liquid handling in the range of 1µl up to 1,000µl. However, pipetting precision depends on the required volume and the chosen pipetting tool, which is depicted in the following Table 5 using the Coefficient of Variation (CV).

Table 5: Specifications of the different pipetting tools as certified by Beckman Coulter

Pipetting Tools	Volume Range	CV [%]
P20 Tip Tool	1 - 20 µL	1µl < 5%
P200L Tip Tool	5 - 200 µL	5µl < 5%
P1000L Tip Tool	50 - 1000µL	50µl < 2%

The Biomek® 2000 (Fig. 20) has been chosen due to the following advantages:

- By way of derogation from the standard MTP-contour: Maximum height of 115mm is ensured allowing for every kind of pipetting while using the designed labware types
- High precision pipetting in the range of 1µl up to 1,000µl
- Chemically inert and disposable tip support
- Integrated labware transport by the Biomek® gripper tool
- Proven technology

Concerning the consumables, pipetting tips for the Biomek® 2000 liquid handler need an additional barrier to avoid contaminations of the Biomek® 2000 pipetting tools that can occur during the utilization of evaporating fluids, such as dichloromethane and high concentrated acids.

For handling higher volume ranges, a further liquid handler has been designed and implemented into the automated system providing volumes up to 10ml in one single performance step. The liquid handler is equipped with a Cartesian robotic configuration and two additional labware holders requiring the MTP-footprint. Using a Hamilton® dispenser (Microlab® 511C) with an incorporated 10ml Gastight® syringe, inaccuracy (CV) of liquid handling is merely ≤ 1%.

The Microlab® Gastight® syringe consist of borosilicate glass, a PTFE Luer Lock termination, and a PTFE tipped plunger supplying inert materials. Teflon® FEP tubing creates the fluid path connecting the reservoir with the Hamilton® dispenser, and the Hamilton® dispenser with the Cartesian robotic configuration. Automated dispensing is accomplished by the performance of two process steps:

1. Fill the syringe with the programmed amount of reagent from the reservoir.

2. Dispense the programmed amount into the test tubes, which are embedded into the MTP-footprint, in order to complete the dispense cycle.

The designed liquid handler is capable of performing high precise dispensing in order to provide the required accuracy for higher volume ranges.

The additional, designed diluting station (Fig. 20) fulfills the following requirements:

- Certified accuracy within ± 1%
- Certified precision within + 0.2% traceable to NIST (National Institute of Standards and Technology)
- High volume bulk dispensing
- Pipetting volumes up to 10ml in one single performance step
- Simplified sample preparation steps
- Syringe speeds from 1 up to 250 seconds per stroke
- RS232 and TTL communication interfaces

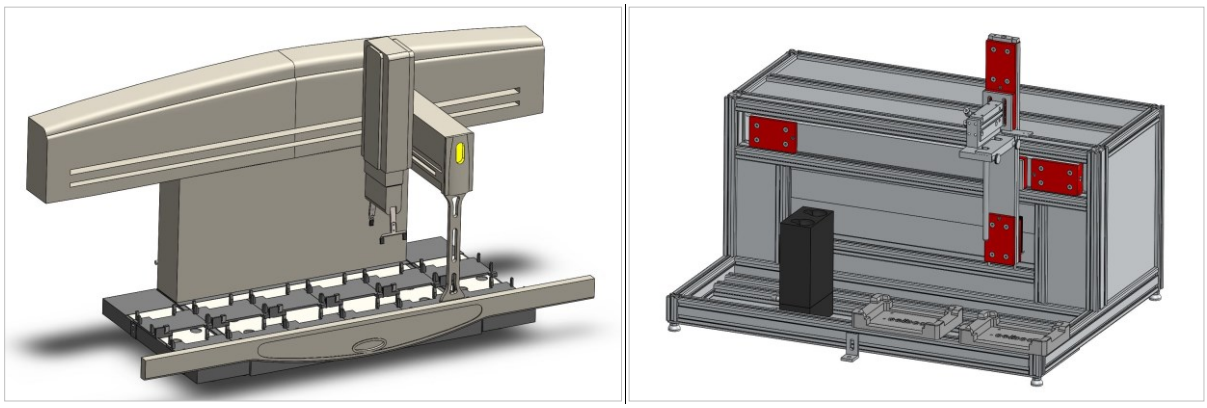


Fig 20: 3D CAD designs of the Biomek® 2000 (left) and the diluting station (Hamilton® Dispenser not shown)

Using the presented MTP-formatted labware design, both workstations are capable of performing a variety of analytical applications. Moreover, the liquid handlers ensure the handling of the wide range of required vessels and provide the required accuracy for the wide volume ranges. Furthermore, the workstations are capable of handling several vessels simultaneously in order to provide less cost- and time-consuming steps. Therefore, using the designed labware types represents the first stage of developing fully automated analytical sample pretreatment.

4.5 Sample Handling

4.5.1 Barcode Reading and Sample Storage

A large percentage of laboratory errors are especially related to errors in sample identification [132]. Hence, for certification or product registration under the ISO 9000 series of standards, specific improvements made in the areas of measurement traceability and data audit trails are essential, which have become an important part of manufacturing quality systems documentation [133]. Thus, in order to ensure sample identification, the Digitus HQ Webcam USB 2.0 has been implemented into the automated system supplying 2D barcode reading.

Besides, the Digitus HQ Webcam provides the following advantages:

- Resolution: 1600x1200 Megapixel
- Frame-rate: up to 30 frames per second
- Driverless installation
- Supports Windows 8, 7, Vista and XP
- Cost-efficient
- USB 2.0 interface

Moreover, barcode processing and reading are necessary due to the high number of samples that have to be analyzed. To be capable of handling all of these samples, sample storage options have to be offered by the automated system.

The flexible sample-hotel provides 196 shelves calling for the MTP-footprint – but allowing for various heights, which ensures the storage of the conceived trays with the embedded analytical vessels. Moreover, the sample-hotel has been constructed using diverse aluminum profiles supplying adaption to changing requirements and calling for labware loading that is facilitated by the central ORCA® robots.

4.5.2 (De-) Capping and MTP-Handling

Allowing for sample handling options, sealing in analytical sample preparation comprises crimping, capping, and screwing. Crimping and screwing supply a very tight seal if caps with septa have been chosen. Nevertheless, crimping and screwing the vessels is an underestimated challenge, respectively, due to the fact that the vessels have to be transferred to a crimp/screw-tightening robot one by one. In addition screw heads are solely suitable for a low range of port diameters. However, to provide a wide range of various diameters, the screw heads have to be replaced

automatically. Furthermore, screwing calls for force measurement and straight alignment of the screw caps allowing for tight sealing. Hence, in order to avoid these time-consuming, cost-intensive, and defective steps, capping was chosen.

The presented concept comprises the idea of capping the whole MTP-footprint including the implemented vessels with just one lid. Irrespective of the port diameter of the vessels, the Biomek® gripper tool is capable of gripping this lid in one single step and allows for simultaneous opening and covering of up to 24 vessels. Regarding the specifications of the liquid handler software, the conceived trays (including the lid) have to be smaller than 120mm (parameter lid on height) to provide reliable gripping of the lid. For gripping the whole tray, a gripper-lip is necessary. The highest feasible level of this lip amounts to 60mm (parameter lifter height). However, ensuring especially these levels is necessary to avoid collisions between the vessels and the Biomek® gripper tool (in vertical direction). Moreover, a second lip has been attached providing the tight fit of the vessels. A CAD design is shown in the following Fig. 21 representing the footprints and the contours of the conceived trays (including an indicated gripper position).

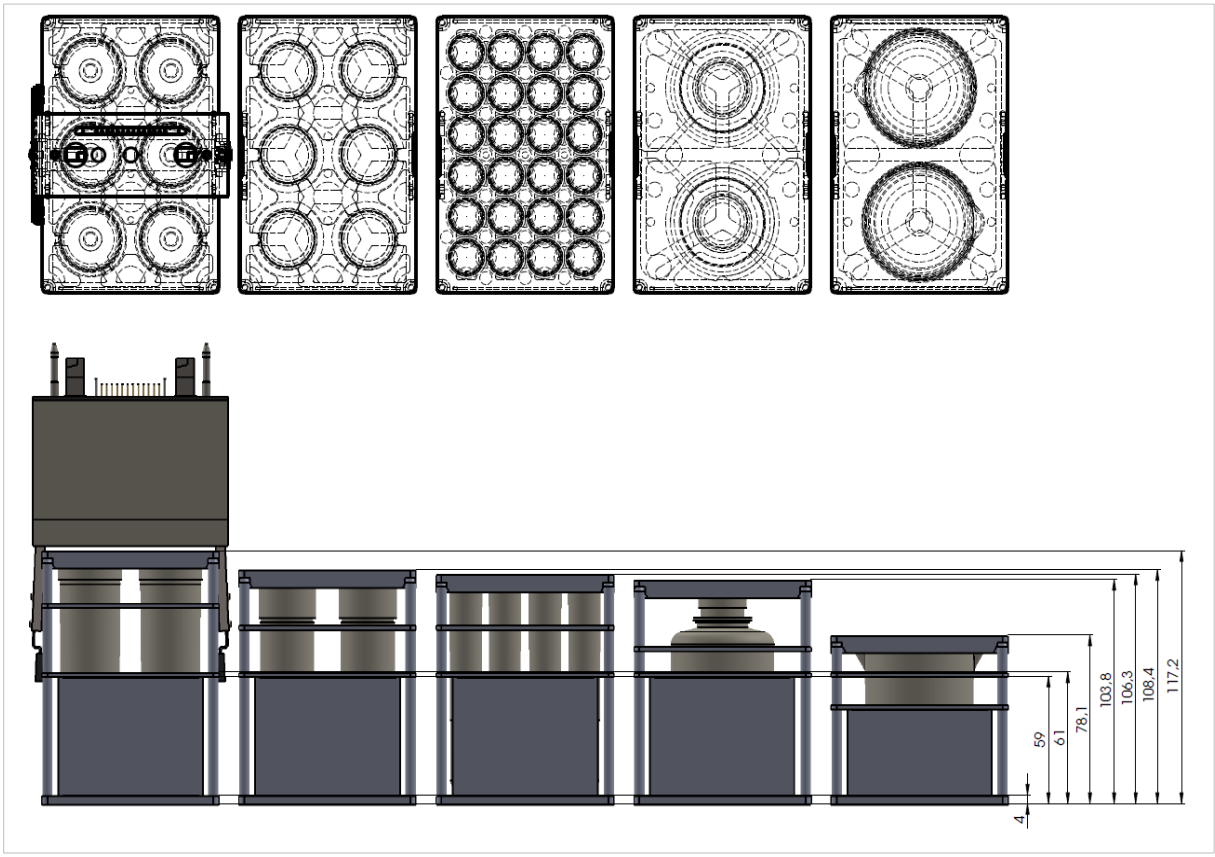


Fig 21: CAD design of the footprints and contours of the designed labware types including an indicated gripper position

The designed labware types can be moved on the adapted workstations if a gripper tool is available. The Biomek® gripper tool is capable of moving the whole tray or – if required – the designed lid of a specific tray. Therefore, the operator has to specify the movement procedure.

However, the Biomek® 2000 always assumes an automatic move (reliability: 99.9%) if the labware and the selected labware position are compatible. Due to the fact that the designed labware types and the conceived lids meet the specifications of the liquid handler software (Bioworks®), the gripper tool supplies automated (de-)capping as sure as certified by the manufacturer for all automatic moves. Hence, the Biomek® 2000 accomplishes (de-)capping without any fail (n = 100).

For automating the sealing process, a further impetus was that the manual sealing puts physical stress on the human hands that may contribute to occupational injuries, such as tendonitis and carpal tunnel syndrome [11]. Moreover, this task is potentially hazardous as it may expose the analyst to the samples via accidental spillage or breakage [11]. In the following Fig. 22 the concept idea for automated (de-) capping is represented.

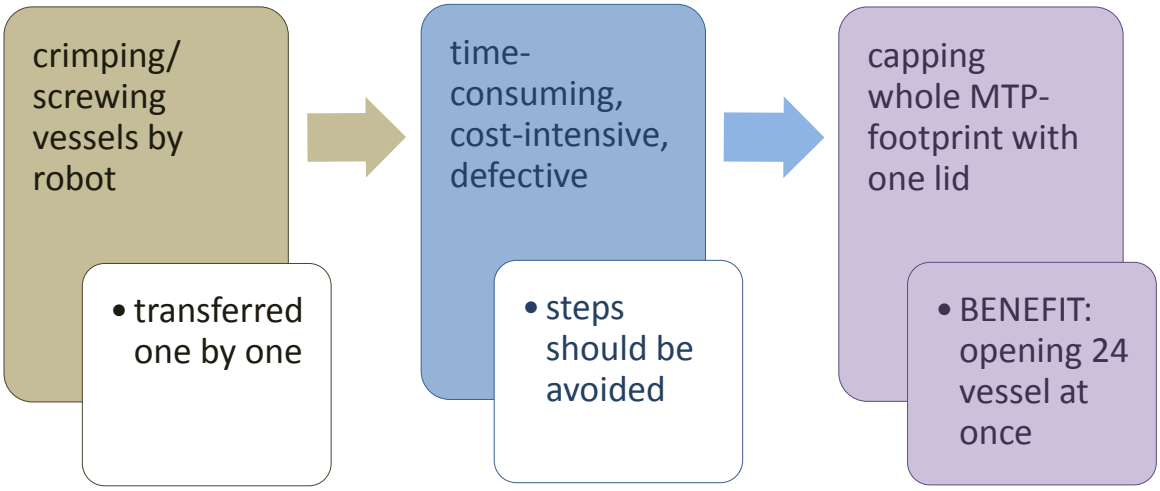


Fig 22: Flowchart – Concept idea for (de-) capping

4.5.3 Crimping and Individual Sample Handling

Even if capping the whole MTP-footprint with just one lid represents a suitable solution providing less cost- and time-consuming automation steps, individual capping – including a very tight seal – is still required in order to ensure concentration stability while handling volatile components. Consequently, crimp caps with septa have to be chosen. The septa consist of two layers, silicone and Teflon®. The thick silicone layer provides a very tight seal and is soft enough for the sample needle of the injection system (needed during the measurement process) to penetrate without being damaged. Based on the fact that Teflon® is chemically inert, any possible reaction or contamination between the sample and the septa is eliminated (minimized).

However, individual sealing, such as crimping, requires individual sample handling due to the fact that the caps and vessels have to be transferred to a crimp-tightening robot one by one. Therefore, individual sample handling is enabled using the Stäubli® TS60 SCARA robot, which provides high speed and high precise sample handling. Using a pneumatic system including a suitable end-effector (SMC, Japan) and the developed, flexible finger design (consisting of three individual fingers) the TS60 robot is capable of gripping and placing the individual vials and caps (Fig. 23).

Fig 23: Stäubli® TS60 SCARA robot (left), pneumatic end effector with flexible finger design (middle), one individual finger (right)

The TS60 SCARA robot assembly includes a crimping die, which is part of the Zymark® Crimp Capping Station. The crimping die is mounted on special aluminum profiles that can be ordered pre-configured. Furthermore, the crimping construction comprises of two additional linear pistons (SMC) providing x- and z-axis of movement. The pistons, the crimping die, and the TS60 are connected via a pneumatic system (SMC), which is also used to control the end-effector.

The whole crimping process is performed as follows: The TS60 is capable of gripping both the vials and the crimp caps, which are embedded into the MTP-formatted, conceived trays. After gripping, the TS60 place them both into the crimping adapter as shown in Fig. 24. Performing x- and z-axis of movements, the pneumatically controlled pistons provide the final crimping position of the crimping adapter (including the crimp vial and the crimp cap). Crimping is pneumatically accomplished using the crimping die. Subsequently, the pistons back up to their initial position. Finally, the TS60 robot place the crimped vials back into the conceived trays using its flexible finger design.

Once taught, gripping and placing is accomplished without any fail due to the high repeatability (±0.01mm) of the TS60 SCARA robot. Due to the fact that the success of crimping depends on the right positions of the caps placed by the TS60, crimping is accomplished without any fail (n = 100).

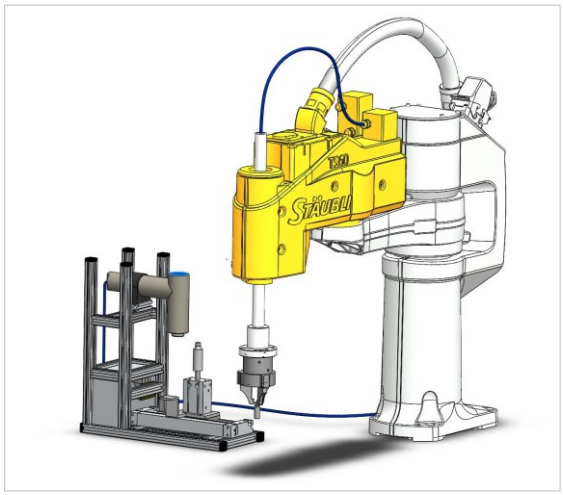


Fig 24: CAD design of the SCARA robot assembly including the Zymark® crimping die mounted on aluminum profiles and two additional pistons enabling x- and z-axis of movements

The specifications of the TS60 are as follows:

- High speed robot: up to 100picks/min.
- High payload capacity
- Large work envelope: 400mm stroke version
- Great operating distance: 600mm
- Repeatability: ±0.01mm
- Degrees of freedom: 4
- Maximum payload capacity: 8kg
- Nominal payload capacity: 2kg
- Solid protection: IP54-rated angled connector plate design and bellows provide optimum protection against dust and liquids.

Due to the limitation of the existing workbench (including the liquid handling systems), the increased space requirements of the TS60 and its performance vibrations during rotation, the SCARA robot calls for a separated workbench. This workbench has to be integrated using a solid shuttle system. Supplying 2m linear guidance while using an engine-driven toothed belt, the designed shuttle system has been constructed using aluminum profiles. Providing a MTP-formatted labware holder, the shuttle system requires labware loading by the central ORCA® robots.

4.5.4 Housing

Due to safety reasons depending on the high speed robot and its operating distance, the SCARA robot assembly has been covered with a housing (Fig. 25). Aluminum profiles have been used for constructing the housing. At the front side and at the lateral sides there are two doors, respectively, providing fast and easy operator access for service, cleaning, and placing labware. The dimensions of the housing are as follows: 1,600mm x 1,441mm x 1,085mm.

In addition the Biomek® system has been covered with a housing (Fig. 25). This housing can be exhausted allowing for dealing with evaporating fluids and samples. For constructing the housing, acrylic glass panes and aluminum profiles have been used supplying the following dimensions: 1,310mm x 835mm x 966mm.

Fig 25: 3D CAD design of the housing for the TS60 SCARA robot assembly (left) and the housing for the Biomek® 2000

The Biomek® housing is designed as a removable hood. At the front side and at the lateral side there are two doors providing fast and easy operator access. The front door can be opened by the ORCA® robot using a twin track guide with rollers. On the top side the housing is connected with an exhausting system, which is capable of aspirating up to 37 cubic decimeters air per second. The aspirating rate can be adjusted manually.

4.5.5 Weighing Station and Ionizer

The implemented weighing station BP 211D offers weighing in the range of 0.01mg up to 210g. Allowing for accurate weighing processes of individual vials, the weighing station provides an internal calibration weight. Due to the fact that weighing requires individual sample treatment, the weighing station is loaded by high-precision SCARA motions as shown in Fig. 26. Therefore, no errors occurred during picking and placing the individual vials (n = 100). Furthermore, allowing for data storage and sample dosage options, weights will be logged in an Excel file.

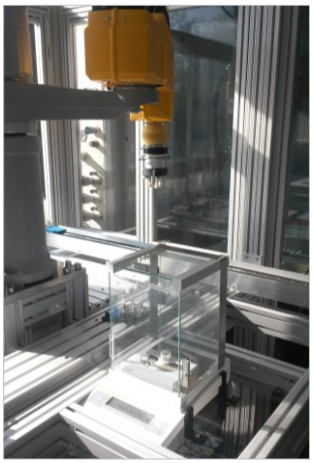


Fig 26: TS60 SCARA robot and the weighing station BP 211D

The analytical balance BP 211D provides the advantages as follows:

- Capacity: 210g
- Readability: 0.01mg
- Internal calibration weight
- Low inaccuracy of ≤ 0.05mg up to 80g
- Low inaccuracy of ≤ 0.1mg up to 210g
- RS232 communication interface

Due to the fact that electro-statically charged samples will adhere at the vessels´ wall during the weighing steps, samples have to be loosened during pipetting using rotary motions. Nevertheless, automated pipetting supplies vertical motions, which fail to dissolve the whole sample masses. However, using the ionizer ANTISTAT 2000 (Fig. 27) supplies easy and precise sample pretreatment avoiding the electrostatic charge of the samples and, thereby, the adherence of the samples at the vessels´ wall. Furthermore, the ionizer prevents the introduction of contamination particles during the weighing steps and ensures the fast and simple handling of solid samples.

Fig 27: Ionizer ANTISTAT 2000

4.6 Sample Treatment and Analytical Devices

Accomplishing derivatization reactions, the heating and shaking device MHL 23 attains temperatures up to 130°C converting the analyte molecule into an appropriate form for the detection and separation systems. Moreover, providing mixing steps, shaking options enable homogenization of the treated sample solutions, which is necessary in order to allow for completed reaction processes.

Considering the conceptual conditions, two exchangeable thermo-block adapters for six GC vials, respectively, have been added supplying the MTP-footprint (Fig. 28). The designed thermo-block adapters can be used for any kind of application using 2ml crimp, snap, or screw cap vials.

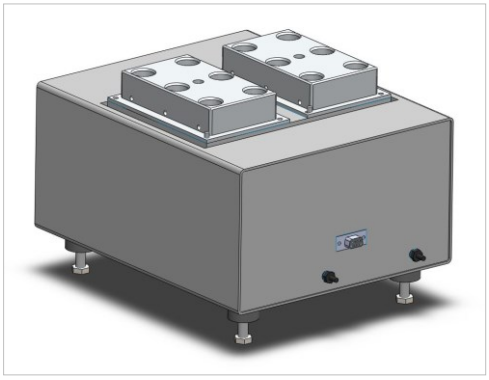


Fig 28: 3D CAD design of the heating and shaking device MHL 23 including two exchangeable thermo-block adapters

However, using the concept idea as described in the previous chapters, the applicable vials have to be embedded into a conceived tray that supplies the MTP-formatted labware design and matches with the two exchangeable thermo-blocks in order to allow for precise stacking steps. Furthermore, providing connective heat transfer, the chosen material of the two exchangeable thermo-block adapter and the conceived trays is aluminum.

The specifications of the MHL 23 are as follows:

- Working temperature: 3 - 130°C
- Range of adjustment: 0 – 135°C
- Accuracy/resolution: ±0.1°C/±0.1°C
- Mean heating rate: 5°C/min up to +40°C, 4°C/min from +41°C
- Shaking frequency: 200 up to 1300 min^{-1}, Timer, short-mix function
- Programmable temperature ramps
- Dimension: 220 x 330 x 93mm
- Voltage: 230V, 50Hz
- USB 2.0 Interface

In addition providing the effective removal of sample matrix and allowing for the pre-concentration of the target substances, SPE is a valuable application [134]. Thus, using vacuum manifold processing [135], automated SPE applications are increasingly common [136].

Vacuum manifold allows for the utilization of commercially available column plates (using the MTP-format) and for simultaneous utilization of individual columns parallelizing the SPE process. However, regarding the disadvantages, vacuum-based SPE techniques supply a maximum pressurization of 14.5psi depending on the atmospheric pressure on earth (14.5psi) and the resulting maximum of pressure differences. Moreover, performing simultaneous treatment of columns, successive discharging can occur – especially if samples with varying sample content will be processed. Due to the resulting leakages and the decrease of pressure, the rate of sample yield will differ between the simultaneous treated columns. Moreover, even if the operating-time of pressurization will be extended, the drying of the column material and, therefore, the loss of conditioning solution will affect the accuracy of the sample yields. Thus, in order to avoid differing in sample yields, SPE applications have to provide consistent pressurization for each individual column during parallel treatment. Moreover, in order to enable the handling of viscous samples, positive pressure applications have to be performed supplying pressurization up to 100psi.

An already existing solution that enables positive pressure processing is provided by Waters®. Nevertheless, the Waters® Positive Pressure-96 Processor calls for manual sample loading – due to the fact that the dimensions of the Waters® processor does not meet the specifications of current existing liquid handlers [137]. To provide fully automated SPE processes enabling high-throughput possibilities, the designed Positive Pressure SPE Unit was developed matching with the specifications of the Biomek® liquid handler.

Therefore, the Positive Pressure Unit can be simply assembled on the Biomek® workspace that provides automated loading of columns by the Span-8 pipetting tool and automated stacking of labware by the Biomek® gripper tool. Moreover, using the designed fritting-based pressure flow reduction technology, the Positive Pressure Unit avoids vacuum-manifold specific difficulties.

Due to the fact that the extraction process calls for continuous pressure *differences* between the in- and the outlet of the columns, reduction of pressure *flow* supplies a suitable solution avoiding leakages and the resulting loss of pressurization at the columns´ inlet. Pressure flow reduction is enabled right during the pressure feeding by micro porous fritting for each individual column as shown in Fig. 29. Thus, providing a constant pressure reservoir, the fritting-based technology enables continuous pressurization for each individual column. Thereby, the technology supplies single treatment of columns even in the 96-well format.

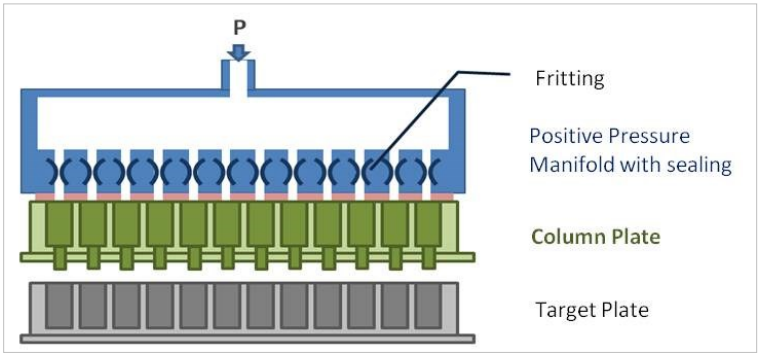


Fig 29: Concept of fritting-based pressure flow reduction technology

The fully automated Positive Pressure Unit allows for adjustable pressure controlling (SMC, Japan) (1) and supplies a designed MTP-formatted labware-slide including the applicable labware (2). Regarding the labware requirements, the stacking options allow for the utilization of commercially available filter plates and, moreover, for a designed labware adapter that ensures the utilization of 24 individual columns (using the concept idea of embedding as described in the previous chapters). The labware-slide provides the initial or – if required – the specific pressurization position while moving back or forth, respectively. Facilitating these kinds of moving steps, the Positive Pressure Unit features a pneumatically controlled linear piston (SMC, Japan) (3) providing x-axis movements.

During pressurization, the self-adjusting plate with 96 individual ports (4) allows for the accommodation to most of the 96-well plate formats, such as standard flat-well plates, deep-well plates, or plates with different kinds of bottoms (such as U or C shape), and enables tight sealing using pneumatically controlled z-axis movements (linear piston, SMC) and a designed surface sealing.

Pressurization is equally distributed among all the 96 individual ports using the pressure reservoir and the fritting-based technology. Contact pressure can be adjusted manually in the range of 14.5psi up to 100psi.

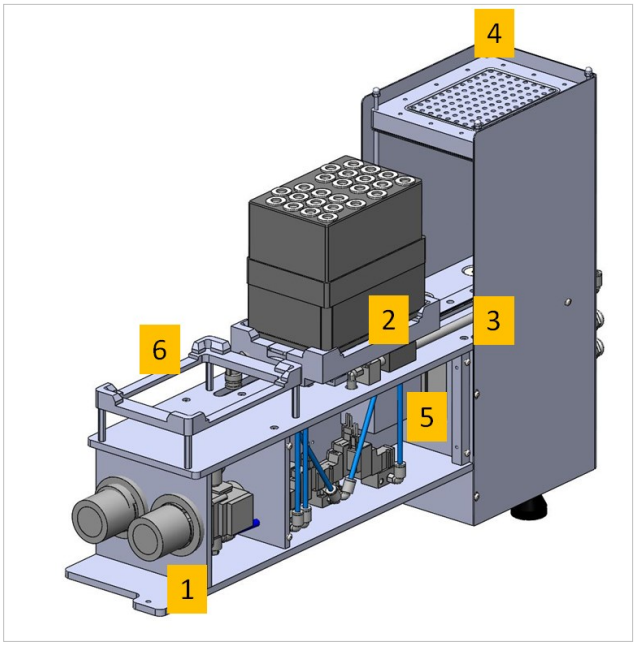


Fig 30: Setup of the automated Positive Pressure SPE Unit without covers illustrating the technical details: (1) adjustable pressure controlling, (2) designed MTP-formatted labware-slide including the applicable labware, (3) pneumatically controlled piston (x-axis movements), (4) pneumatically controlled self-adjusting plate (z-axis movements), (5) pneumatic unit and microcontroller, (6) additional labware position

During pressurization, compressed air (dried and cleaned) extrudes the samples through the columns and can be adjusted manually in the range of 14.5psi up to 100psi allowing for the handling of high viscous samples. During the rinsing steps, the extruded liquids will be collected in a refuse bin that is connected with the labware-slide using PTFE tubing. However, for the final elution step, the stacking arrangement has to be changed as shown in Fig. 31. Thereby, the rinsing adapter has to be replaced with the 96-deep-well plate collecting the target substances for the analysis process.

Further components are consequently the pneumatic unit including magnetic (electro-pneumatic) valves and pneumatic throttles (SMC, Japan) and, moreover, an additional microcontroller (5). The microcontroller allows for controlling of the pneumatic actuator and, furthermore, for communication with the liquid handler software via USB 2.0. Therefore, operating-time of pressurization can be adjusted via the liquid handler software facilitating an incubation step. However, operating-time should depend on the viscosity of the samples. The additional labware position (6) supplies the availability of further labware components, such as adapters or plates.

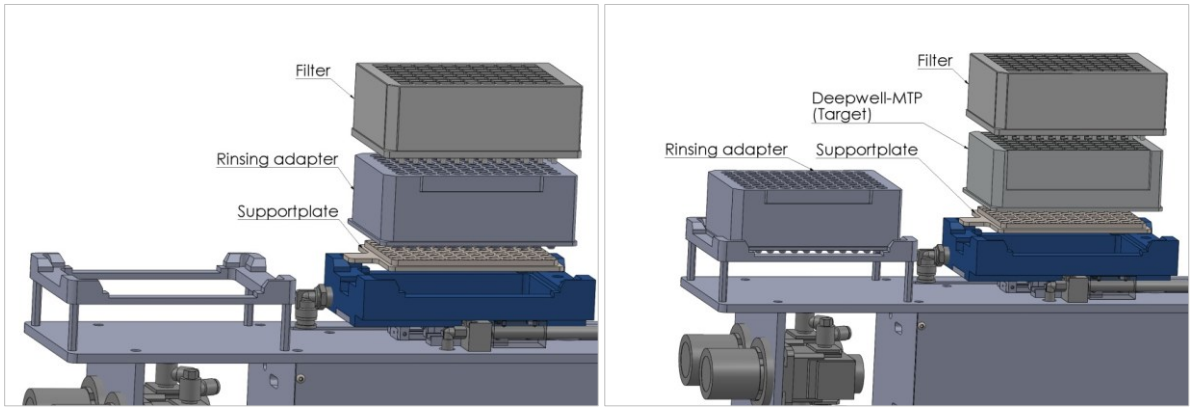


Fig 31: Initial and final stacking arrangement

The Positive Pressure SPE Unit features the following advantages:

- Simply mounted on common liquid handlers
- Fully automated positive pressure SPE processes
- Improved flow for viscous samples while using up to 100psi for pressurization
- Fritting-based pressure flow reduction technology for each individual port
- Avoides vacuum-manifold specific difficulties
- Tight sealing of columns due to self-adjusting plate and an additional surface sealing (silicon)
- Reproducible analyte recoveries

Considering the wide range of applications, automated analytical sample preparation includes, moreover, microwave digestion steps. Due to safety reasons, this steps have to be performed under a separated hood. However, performing microwave digestion while using the applied Xpress vessels is simple and fast. Process steps comprise sample dosage as provided by the automated system, insertion of the vessles, and starting of the microwave digestion programm.

After sample treatment the converted analyte molecules have to be analyzed using analytical devices, such as ICP-MS and GC/MS. Modes of operation are described in chapter 2.5. Performance reports of the analytical devices are represented in chapter 6 and 7 (bias error of the measuring instrument) using real measurement applications.

4.7 Software Integration

Allowing for easy integration of third party instruments, automation lines with open software architecture are best suited and most cost-effective for research applications where task types grow continuously. Those systems are known from different companies, such as Beckmann Coulter [138], HighRes Biosolutions [139], and Hamilton® Robotics [140], comprising five different levels of control software:

1. Firmware inside single devices
2. Device specific control software running on PC
3. Integration software
4. Complex method editing software
5. Multi process management software (highest level of software components)

Providing interface connectivity to the upper level of control software, firmware regulates the elemental functions of the devices. Almost every device used in automated processes is controlled internally by microcontrollers or microprocessors running firmware.

Running on external computers, more complex devices in lab automation, such as liquid handlers, are controlled by the device control software. Therefore, requiring their own external control software, devices with higher functionality, flexibility, and higher number of parameters are sold together with this kind of software.

For integrating 3rd party equipment, devices have to be interfaced with the upper level of control software that is called integration software. Hence, translating data and commands from upper control instances to the devices or devices´ software and vice versa, drivers are written. Laboratory robots have been programmed and executed by different software including commercially available software, such as SAMI®, and self-implemented software modules [141].

However, regarding the represented automated system, the standardized *SILAS* protocol is used supporting custom integration into the automated system, which is controlled by the SAMI® Workstation Ex software. SILAS is an integral content of the SAMI® Workstation Ex software and based on Microsoft ActiveX messaging. SAMI® Ex and its integration modules normally run on PCs with Microsoft Office.

Integration of devices running under other platforms is feasible using network communication. Hence, any device that is controllable via electronic interfaces, such as RS232, USB, Firewire, or Ethernet, can be integrated. Moreover, any device that is controlled by its own software can be integrated if the device software has a remote control interface, such as COM, ActiveX, OLE, DDE, or TCP/IP communication. Generally, there are three different kinds of devices´ integration.

1. Direct integration: Using hardware interfaces, such as RS232, CAN, Bluetooth, or Ethernet, the automation device is connected with the device integration module. Direct integration has been used for the designed dilution station, the heater and shaker, the analytical balance, the barcode reader, and the shuttle system.

2. Device software integration: Using hardware interfaces, the automation device communicates with its own proprietary host controller software. Running on the same automation controller, also the device integration module communicates with this host controller software. Device software integration has been required for the ORCA®, the Stäubli® TS60, and the Biomek® 2000. Moreover, the *whole* TS60 robot *assembly* – including the pneumatic end-effector and the crimping construction – is controlled via the TS 60 controller software (CS8C).

3. Additional controller integration: Devices are integrated exerting their own automation controller (PC, etc.). Using hardware interfaces, the device integration module communicates with the automation device software. Supporting control and data exchange with higher instances, in some cases the original device software has to be added by a remote control module. Device software integration has been required for mobile robot transport.

Therefore, communicating with the SILAS-Router and with the device/device control software, a specific executable module has been developed for every custom integrated device. Hence, every subsystem or device in the automation system has its own SILAS integration module. Facilitating custom integrations, the SILAS software developer kit contains a prebuilt module framework. Communication on the SILAS level is message based and controlled by the SILAS router (as shown in the following scheme).

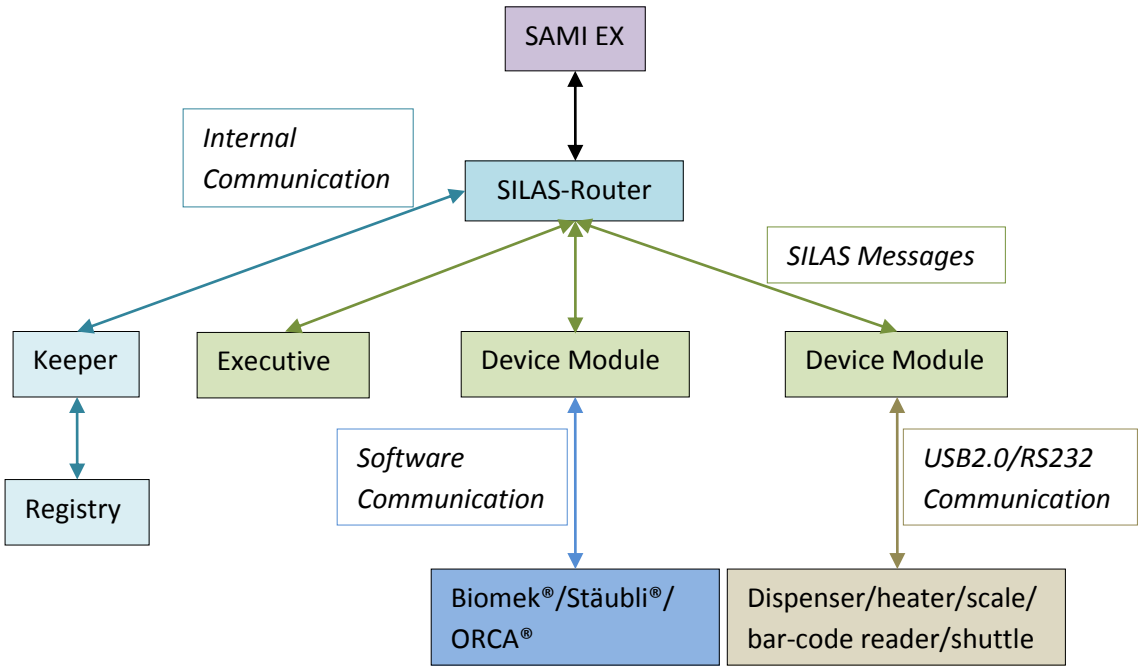


Fig 32: Scheme of the SILAS environment

One of the main parts of SILAS is the SILAS executable program, which contains the message router and the keeper to access the keeper registry database and to load and unload modules. This database is also one of the main parts and contains system information of all components of the automation system, such as module specific information, communication details, and transportation information. Furthermore, the Message Control ActiveX is one of the main parts and provides general SILAS communication features and rules and is in addition embedded into any custom integration. Moreover, it is an interface to the internal router.

Providing direct settings of operation parameters for more simple devices or a selection of predefined methods for more complex devices, such as liquid handlers, every device needs a user interface to parameterize the functions of the devices. Generally, laboratory automation software includes graphical method editing interfaces.

Using more complex applications, additional hardware platform independent method editing, scheduling, and run time control software is required that provides high throughput, time-efficient run, and cost-effective system usage. Powered by SAMI® Workstation EX software, which is used for all automation processes of the represented dissertation, the software allows for developing (via SAMI® Editor), scheduling (via SAMI® Scheduler), monitoring and running (via SAMI® Run Time) of multiple processes on the integrated system.

Therefore, SAMI® Workstation EX software incorporates optimized planning and data-driven dynamic rescheduling for pre-validated schedules and run-time flexibility when needed. Furthermore, the software provides a graphical interface with language geared specifically to the scientist, which empowers scientists and technicians to describe complex processes in a straightforward manner. Therefore, operator learning time is reduced.

SAMI® Workstation EX software features are as follows:

- Graphical intuitive method editor
- Schedule optimization
- Dynamic rescheduling
- Rigid and flexible timing of steps
- Tip tracking
- 21 CFR Part 11 Tools
- Microsoft Windows XP operating environment
- Graphical Run Time environment includes status information, live method and system view, generates labware reports

Furthermore, the management of multiple processes is supported by the custom developed process management software of the SAMI® Workstation EX software, which is a custom tool that supports planning and combining of automated methods. The process definition editor, the process management software, and the data acquisition and reporting tools are integral parts of the process management software. In detail the process definition editor plans multiple automated methods, which can be combined to one type of process. Besides, the process management software supports scheduling of the processes. Furthermore, the data acquisition and reporting tools report data and labware positions.

4.8 Integration with External Stations (Mobile Robot Transport)

Providing flexible and precise sample transport between the automated sample preparation system, the microwave hood, and other external stations, such as the detection and separation systems, a mobile robot system has been integrated.

The mobile robot system comprises several H20 robots. These robots are designed and built on the i90 robot base, featuring 12" touch screen tablet, two large arms (Hawk arms), dual-camera animated head (Hawk head), indoor GPS navigation system, and auto-docking/recharging station.

Moreover, the H20 supplies the following features:

- 12" touch screen tablet on chest, playing video (.wmv) and audio and displaying images
- Dual arms with 6 joints (DOF) + 2DOF gripper, reaching 60cm, with max. lifting weight of 800g (optional 1kg)
- 6DOF animated head with dual 640x480 color cameras
- Overall height of 1.4m
- Dimension: 43cm(L) x 38cm(W) x 140cm(H)
- Navigation and localization providing collision-free point-to-point autonomous navigation
- Vision-landmark based indoor localization (indoor GPS) sensor and landmarks together provide precise position and direction information covering every inch of the floor
- Auto-docking and recharging station
- Fully wireless networked 802.11g
- OS independent application development tools
- Navigation sensors including 5 sonar and 10 IR range sensors
- Max. speed 0.75m/sec.
- Comprehensive circuit protection
- High resolution pan-tilt-zoom (10x) camera
- Max payload of 40kg (optional 80kg) with body weight of 24kg
- Tele-operation and remote monitoring
- Extended operating time, 2 hour nominal operation time for each recharging
- Upgrade options: Laser Scanner; Power and battery systems for 4/8 hours operation time

Supplying performance data, 60 test runs on the mobile robot system were conducted on five consecutive days. Each run comprised five activities.

Therefore, the mobile robot system executed 300 activities including 120 times of grasping, 120 times of placing, and 60 times of on the robot frame.

Moreover, providing a two-directional mobile robot transport (as shown in Fig. 33) between the automated sample preparation system (reformatting system) and the element-specific detection system (ICP-MS), 30 test runs were performed in the following order: grasping at the reformatting system – placing at the reformatting system – on the robot frame – grasping at the ICP-MS – placing at the ICP-MS. However, 30 runs were performed using the opposite direction: grasping at the ICP-MS – placing at the ICP-MS – on the robot frame – grasping at the reformatting system – placing at the reformatting system.

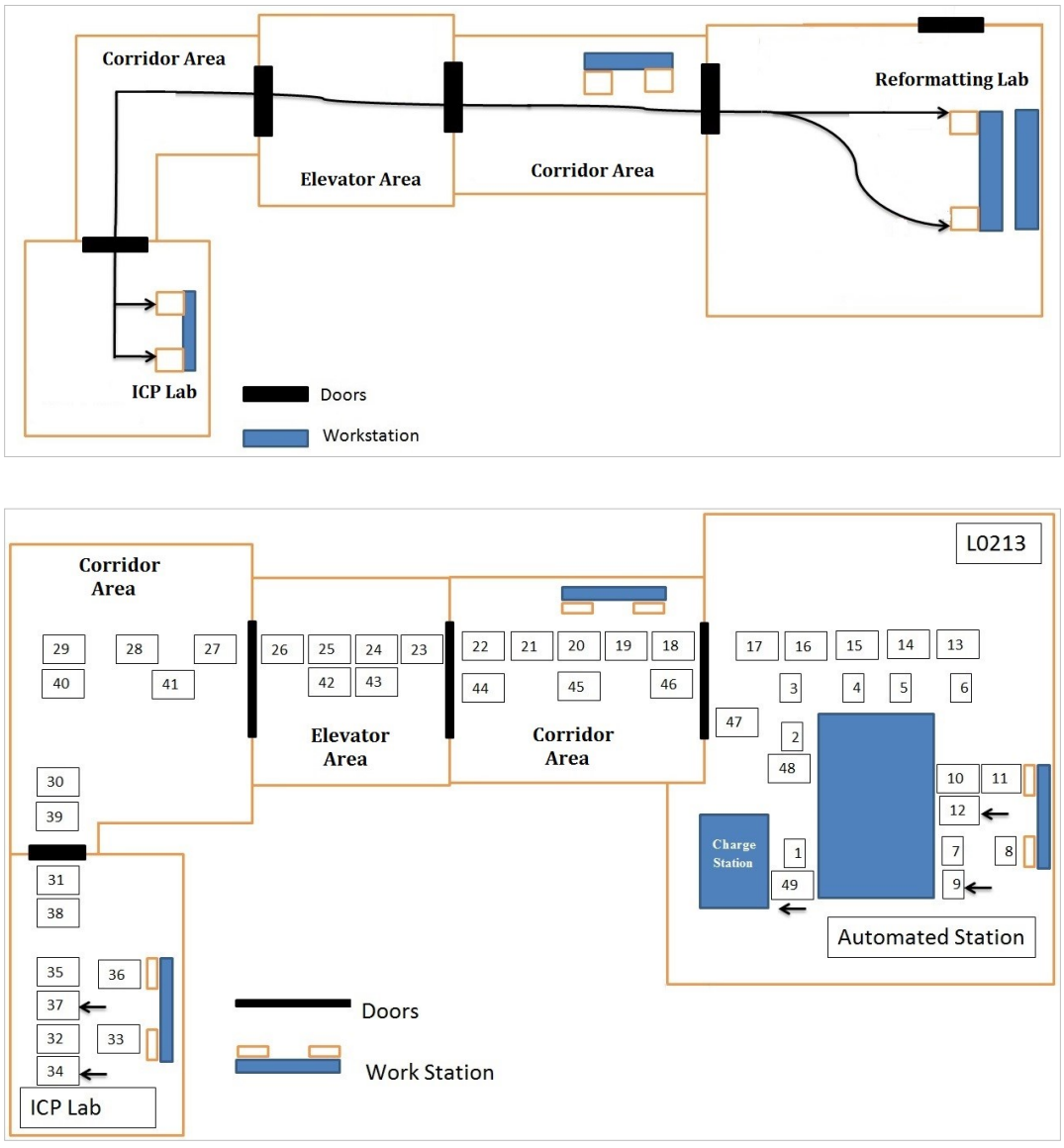


Fig 33: Floor map with automatic doors representing the distances (above) and landmarks (below) for mobile robot transport

Regarding the evaluation procedure, 14 of the 300 activities failed corresponding to a repeatability of 95.33% (as shown in Fig. 34). In detail, 9 of the 14 activities failed occurred during grasping corresponding to a repeatability of 92.5%. Moreover, 5 of the 14 activities failed occurred during placing corresponding to a repeatability of 95.83% (Fig. 35).

To be more detailed, 4 of the 9 activities failed during grasping occurred at the reformatting system corresponding to a repeatability of 93.33%, whereas 5 of the 9 activities failed during grasping occurred at the ICP-MS corresponding to a repeatability of 91.67%. Furthermore, 3 of the 5 activities failed during placing occurred at the reformatting system corresponding to a repeatability of 95.0%, whereas 2 of the 5 activities failed during placing occurred at the ICP-MS corresponding to a repeatability of 96.67%. Besides, the mobile robot system opened all of the automatic doors without any fail.

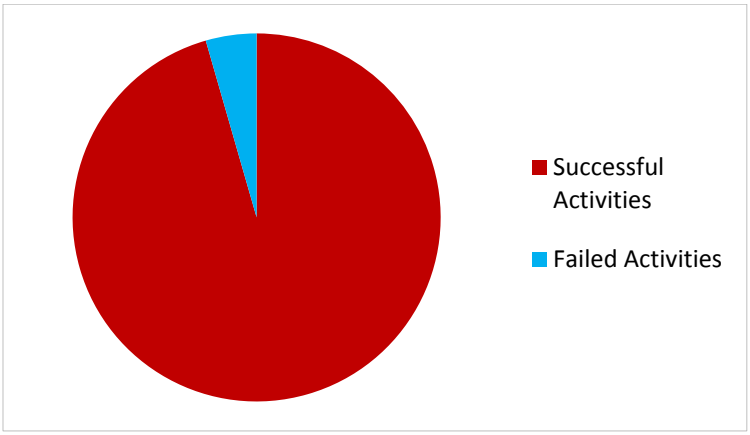


Fig 34: Percentage of successful and failed activities occurred during mobile robot transport evaluation

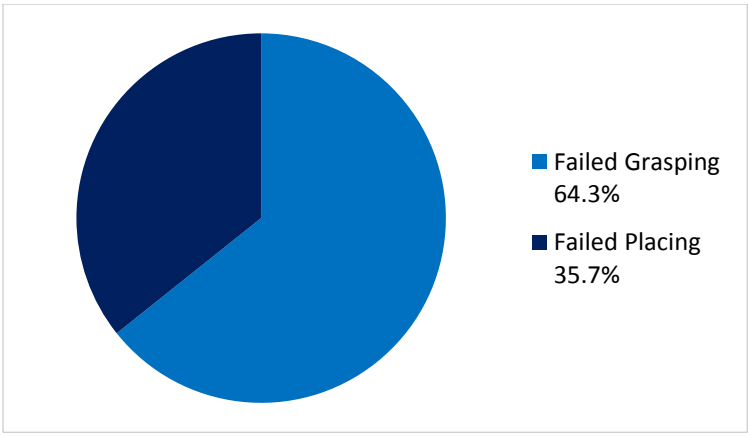


Fig 35: Percentage of failed activities divided into grasping and placing steps occurred during mobile robot transport

The mobile robot transport is provided by the Hierarchical Workflow Management System (HWMS) using the Transportation and Assistance Control System. This system controls not only the robots´ but also the humans´ resources for transportation tasks while getting queried for transportation orders. Moreover, monitoring the process environment, the Transportation and Assistance Control System allows for periodic and maintenance tasks that have to be performed on the automation islands. With respect to the HWMS´s pre-selection, all of the transportation orders are locally queued and distributed among the available transporters. Dealing with their specific requirements, the Transportation and Assistance Control System provides different communication interfaces.

Moreover, fulfilling the particular tasks, the HWMS facilitates not only labware- and substances- but also data-exchange (Fig. 36) between the automation islands (such as the automated sample preparation system, hoods, and analytical devices).

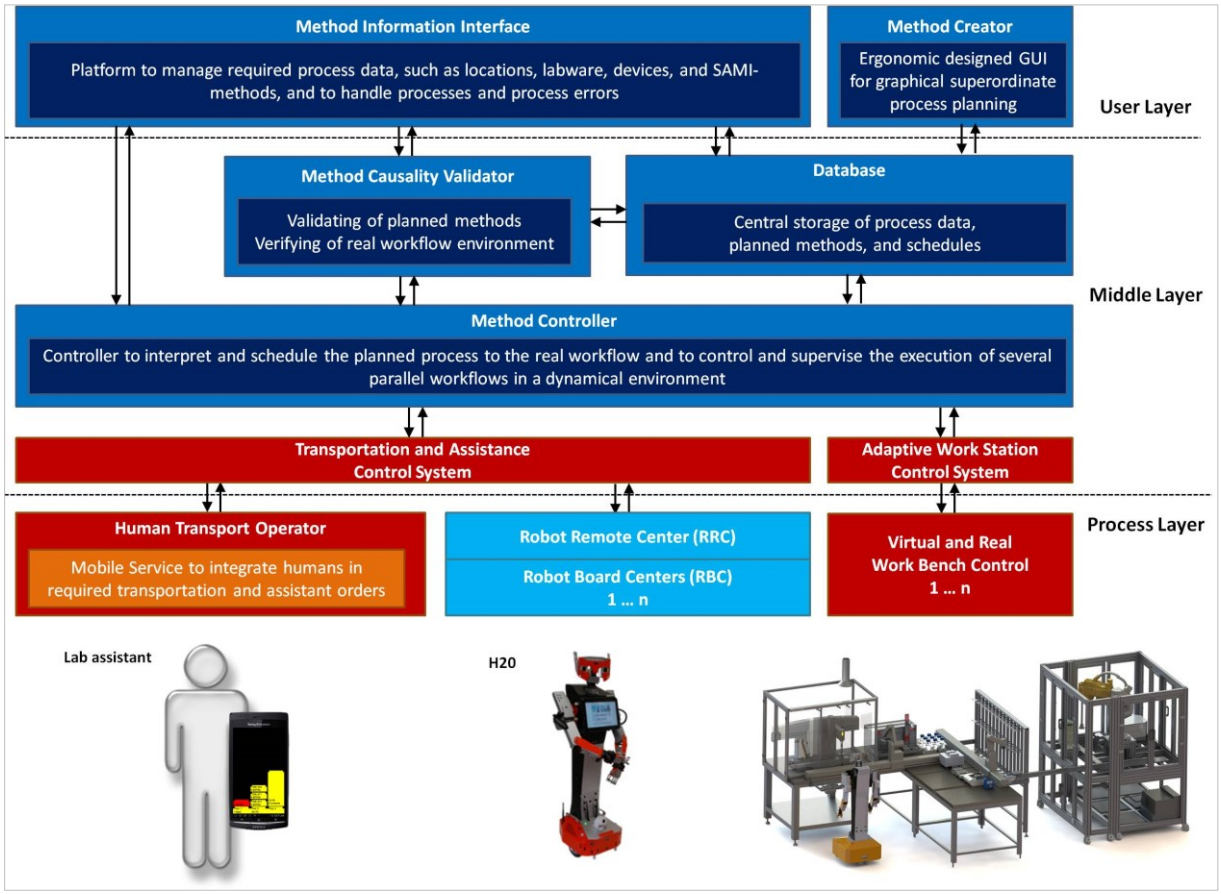


Fig 36: Flowchart – Hierarchical Workflow Management System

Thus, allowing for remote control of the automation islands, the Adaptive Workstation Control System connects different third party device software, such as SAMI® EX and MassHunter, to the HWMS. Executing analytical measurement processes, reporting the results, and allowing for status updates, the Adaptive Workstation Control System queries the analytical operations.

Consequently, the HWMS provides a complete software structure ensuring fully automation of analytical processes while including the integration of external stations (using mobile robot and human transport), automated sample preparation (using remote control of the automation islands), and post-sample preparation steps (such as detection and separation including sample analysis and evaluation processes) as shown in the following Fig. 37 using the Method Creator.

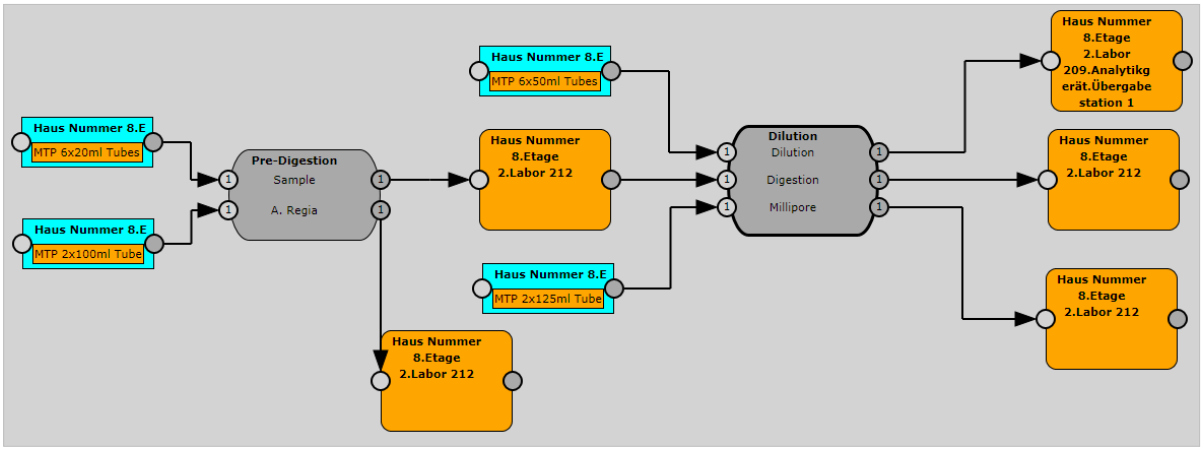


Fig 37: Method Creator (detail) – Mercury process including the environmental monitoring tasks (blue boxes), the automated sample preparation steps (grey boxes) and, moreover, the transportation orders (arrows) between the external stations (orange boxes) and the developed system

5. Statistical Methods and Definitions

5.1 Validation Parameters

For validating the manual and the automated sample preparation processes, bias error of the measuring instrument (according to DIN 1319), repeatability standard deviation (STD) (according to DIN 1319), reproducibility STD (according to DIN 1319), and discrimination threshold (according to DIN 1319, also known as limit of detection according to DIN 32645) have been calculated.

5.1.1 Bias Error of the Measuring Instrument

Bias error of the measuring instrument was calculated by measuring ten times the same sample solution. Subsequently, STD [%], which is corresponding to the Coefficient of Variation (CV), was calculated using the following equation (5-1):

$$\mathrm{CV} = \frac{\mathrm{STD}}{\bar{\mathrm{x}}}\,100\% \qquad (5\text{-}1)$$

CV… Coefficient of Variation [%]

STD… Standard Deviation

$\bar{\mathrm{x}}$… Sample mean (n = 10)

5.1.2 Repeatability Standard Deviation

Preparing at least 25 samples, repeatability STD was calculated using repeatability conditions. Hence, repeatability STD represents a measure depicting the repeatability of the pretreatment process.

5.1.3 Reproducibility Standard Deviation

Preparing at least ten samples per day on five consecutive days, reproducibility STD was calculated using reproducibility conditions. Thus, reproducibility STD represents a measure that depicts the reproducibility of the sample preparation process.

5.1.4 Discrimination Threshold

Discrimination threshold was calculated from the mean value of ten blank measurements and the threefold of the value´s STD as shown in the following equation (5-2).

$$\mathrm{D} = \bar{\mathrm{x}}_{\mathrm{Blank}} + 3\mathrm{STD} \qquad (5\text{-}2)$$

5.2 Further Definitions Considering the Acceptability of the Results

5.2.1 Horwitz Definition

Defining the acceptability of the results, the maximum CV [%] has to be calculated using the Horwitz` definition [142] and the expected final sample concentration. The Horwitz` definition is described by the following equation (5-3):

$$\frac{1}{2} * 2^{(1-0.5\log C)} \leq CV[\%] \leq \frac{2}{3} * 2^{(1-0.5\log C)} \quad \text{Horwitz' definition} \tag{5-3}$$

5.2.2 Confidence Interval using Student´s t-Distribution

The confidence interval, as shown in equation (5-4), supplies an estimated range of values that is likely to include an unknown population parameter. The estimated range is calculated from a given set of sample data.

$$df = n - 1$$
$$P = 0.95$$
$$Cl = \bar{x} \pm t(P,df) * STD/\sqrt{n} \tag{5-4}$$

- df… Degree of freedom
- P… Probability
- Cl… Confidence Interval
- t… Student´s t distribution

5.2.3 David-Test

Normal distribution of the results is verified by the David-Test as shown in equation (5-5).

$$R = x_{max} - x_{min}$$
$$R/STD = L$$
If $L_l \leq L \leq L_u$, values are normally distributed (5-5)

- R… Range
- STD… Standard deviation
- L… Limit (upper/lower)

6. Application I: Element-Specific Analytical Measurements

Facilitating element-specific measurements, the fully automated system has to provide precise and reliable sample preparation processes. Thus, the system´s functionality had to be confirmed in a validation sequence using an established analysis scheme (6.1) that has been validated using manual sample preparation first. Considering the automation requirements and the resulting system adaption (6.2), evaluation of the automated system has been performed. Finally, results of the automated and the manual validation process were compared with each other using ICP-MS detection mode (6.3).

6.1 Process Description Including Motivation and Process Steps

Entering the environment through industrial pollution [143] or municipal waste [144], high levels of mercury exposure can occur. However, toxic elements, such as mercury, can lead to adverse health effects and potentially death even in minor concentrations. Thus, by recognizing and minimizing common sources of toxic elements, dangerous exposures have to be prevented using laboratory testing, which is, therefore, an important tool for detecting and managing the mercury exposure [145]. Providing sensitive determination of mercury, a multitude of analytical methods and systems are available today including spectrophotometric [146] and spectroscopic techniques, such as CV AAS [143], ICP-OES [147], and CV ICP-OES [148]. However, due to its high sensitivity, good interference control, the analysis speed, and the possibility of multi-element analysis, especially ICP-MS is of increasing interest [67].

Allowing for mercury analysis, element-specific measurements have been performed that supply the validation sequence. Performing manual sample preparation while using an established analysis scheme, 8ml of the high concentrated acid (Aqua Regia) were added to 250mg of the sample, which was referenced wood material ERM®-CD100 from the German Federal Institute for Material Research and Testing (BAM, Berlin, Germany). Subsequently, the samples were predigested for a period of 20 minutes. After microwave digestion and cooling down, the clear sample solution was transferred to a volumetric flask (vol. 100ml) and filled with ultra-pure water. One blank sample was included at every digestion run. Finally, the results were evaluated using the ICP-MS for the detection process. The manual sample preparation steps are shown in the following Fig. 38 using a flowchart.

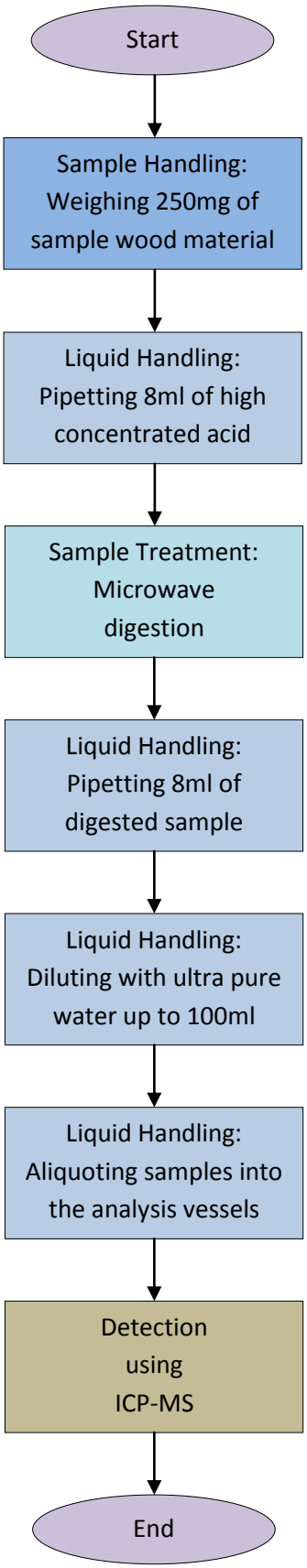


Fig 38: Flowchart – Manual sample preparation and measurement procedure for the mercury analysis

6.2 Automation Concept: Automation Requirements and Process Adaption

Based on the high costs of many reagents, the trend in laboratory automation is toward increasing miniaturization [8] and simplification [149]. Thereby, automated systems enable less consumption of solvents [19], [150] and decreased costs for waste disposal [151]. Moreover, miniaturized techniques improve the throughput possibilities and reduce the sample consumption [152]. Corresponding to the idea of miniaturization, sample preparation vessels have to be smaller than 115mm due to the specifications of the liquid handler software (as described in chapter 4.2).

The microwave vessels used in the original, manually operated sample preparation procedure did not comply with these requirements. However, using miniaturization for the mercury analysis allows for the utilization of smaller vessels, which were in agreement with the specifications of the liquid handler. The required vessels are shown in the following Table 6. Moreover, for automating the sample preparation process, the vessels were embedded into the standardized MTP-footprint using the concept idea of system adaption.

Table 6: Specific embedded vessels used for the automated mercury analysis process

Tray No.	No. of vials	Volume	Material	Function	Kind of vessels
#1	6	50ml	PP	Analysis vessel	Tube
#2	6	20ml	PFA	Microwave vessel	MARS Xpress vessel
#3	24	15ml	PP	Calibration vessel	Tube
#4	2	125ml	LDPE	Reservoir	Narrow mouth bottle
#5	2	100ml	PFA	Reservoir	Beaker

Regarding the utilization of evaporating solvents and high concentrated acids (as described in the previous section), not only the embedded vessels but also the designed MTP-formatted labware types and the required pipetting tips have to be chemically inert. Hence, surface and lids of the conceived trays consist of Teflon®. In addition pipetting tips need an additional filter to avoid contaminations of the Biomek® pipetting tools.

The conceived trays are shown in Fig. 39 representing the footprints, the lateral views, and the dimensions. Fig. 40 supplies pictures of the real trays. In Fig. 41 the miniaturized, automated sample preparation process for mercury analysis is depicted using a flowchart.

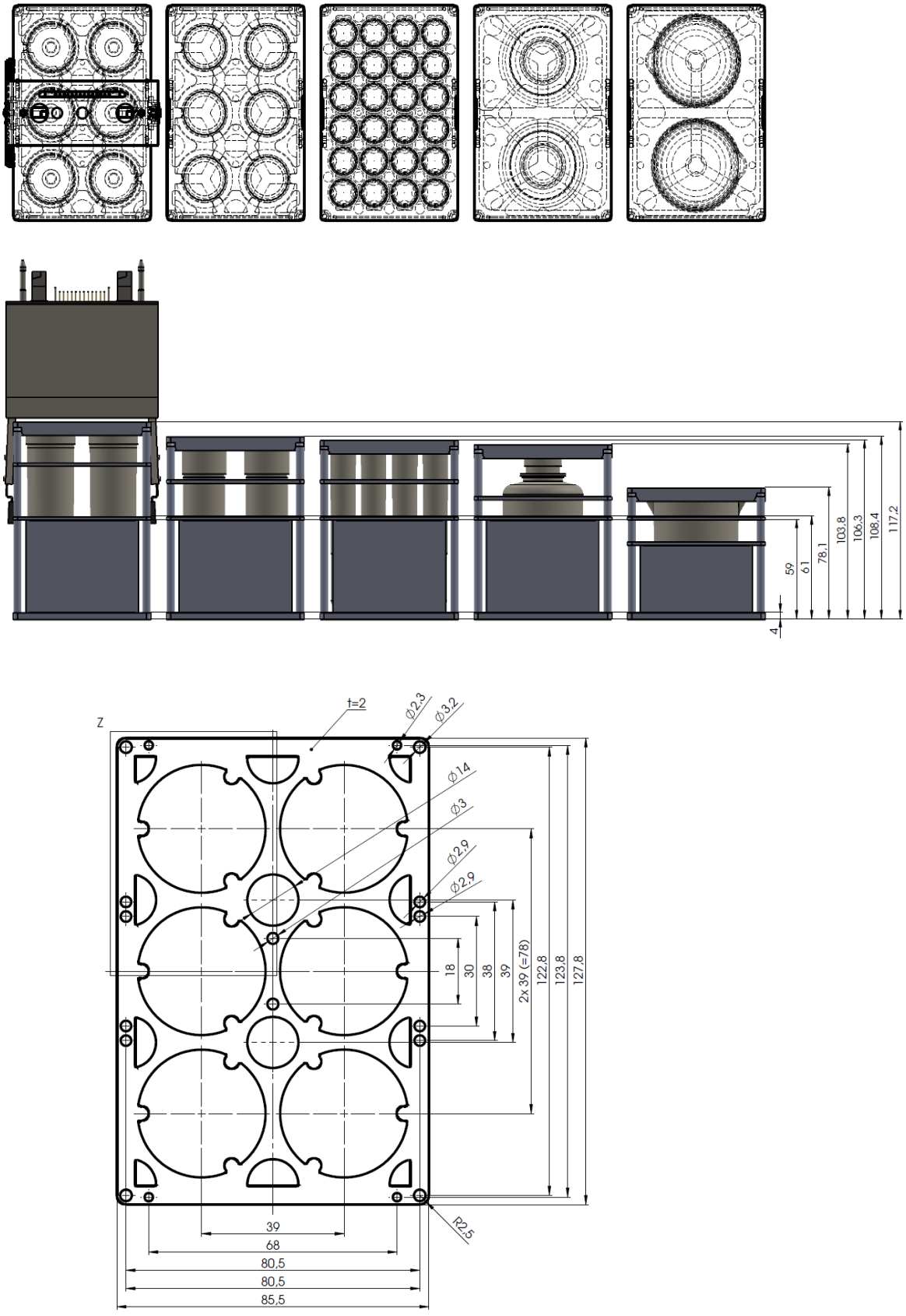


Fig 39: CAD design of the footprints, lateral views, and dimensions of the conceived trays as used for the automated mercury analysis process

Fig 40: Conceived trays with embedded vessels for the automated mercury analysis process: (1) tray with two reservoirs for high concentrated acid, (2) tray with two reservoirs for water, (3) tray with six vessels for microwave digestion treatment, (4) tray with 24 calibration vessels, (5) tray with six analysis vessels, (6) tray with six analysis vessels covered by lid

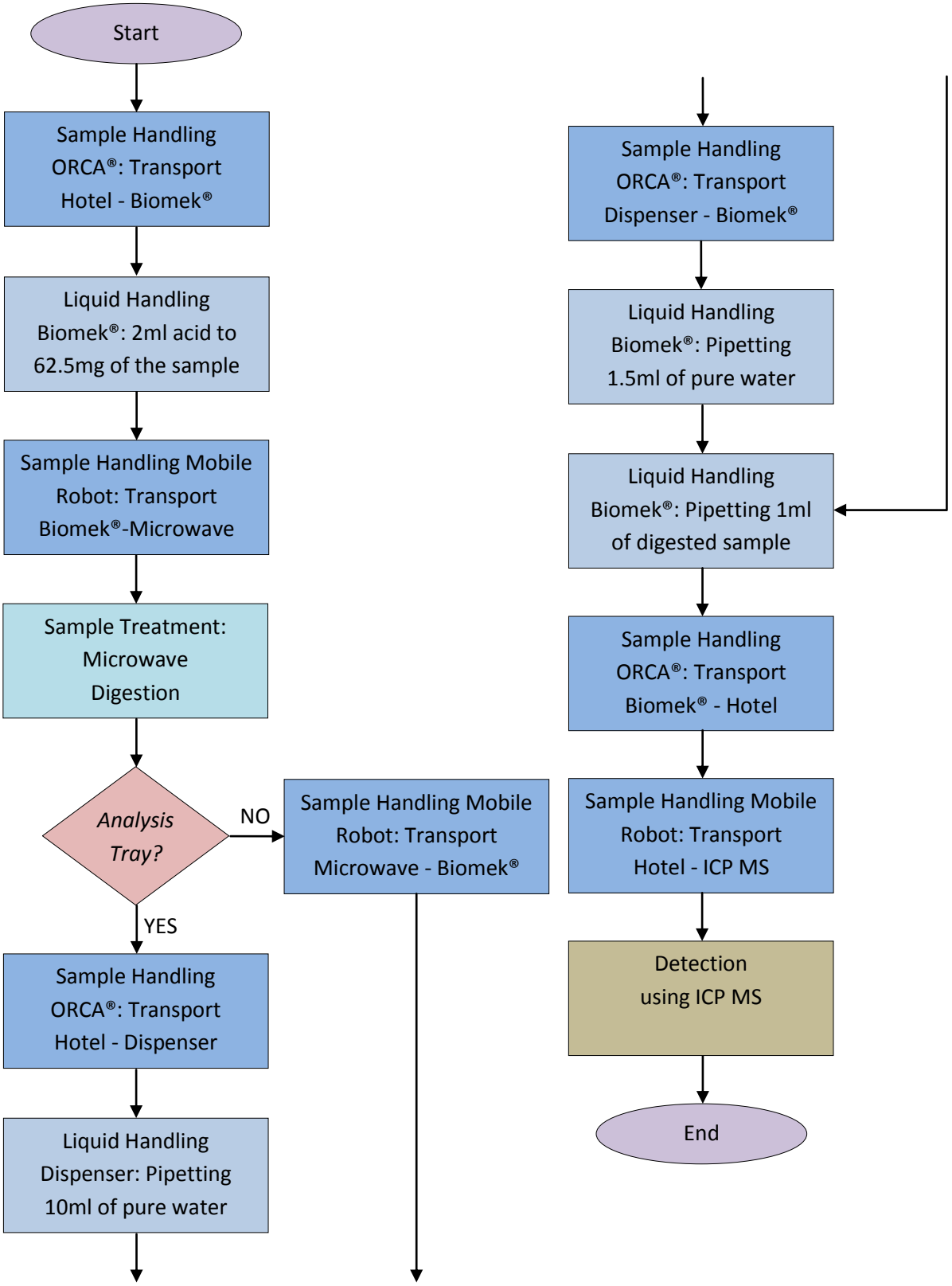


Fig 41: Flowchart – Automated sample preparation and measurement process for the mercury analysis

Changing the pretreatment process to smaller volumes, merely one quarter of the original sample mass and acid volume was used. Moreover, as shown in the previous figure, only 1ml of the digested sample solution was transferred to an analysis tube enabling the utilization of merely 11.5ml of ultra-pure water – corresponding to 12.5% of the solvent required for the original sample preparation procedure. The concept of miniaturization is depicted in Fig. 42.

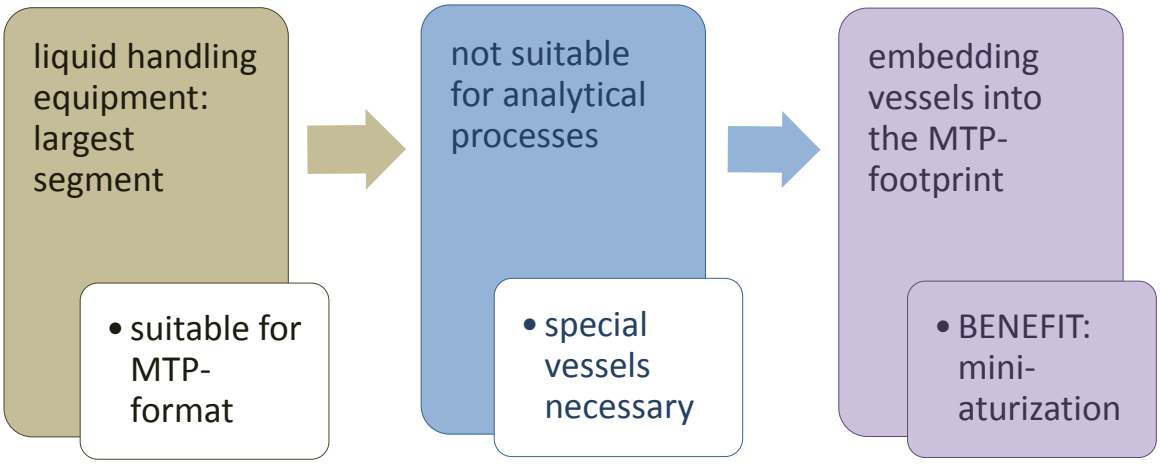


Fig 42: Flowchart – Concept idea of automation with the benefit of miniaturization

Allowing for the appraisal of potential evaporation during the microwave digestion steps, an additional internal standard (ISTD) was added to the high concentrated acid during the miniaturized pretreatment process. All benefits of miniaturization are shown in Table 7. The enhanced (miniaturized) process was used for both validation of the manual and the automated process facilitating comparability.

Table 7: Comparison of the original sample preparation procedure and the enhanced, miniaturized process

	Original Procedure	Enhanced Procedure	Ratio
Sample Mass	250mg	62.5mg	4:1
Acid Volume	8ml	2ml	4:1
Digestion Volume	8ml	1ml	8:1
Dilution Volume	Up to 100ml	11.5ml	8:1
Format	Various single vessels	MTP-format with embedded vessels	-
2nd ISTD	-	amendment of calculated $C_{Mercury}$	-

The additional ISTD, calling rhenium, provides the amendment of the calculated mercury concentration. If there is no evaporation at all, the detected rhenium concentration has to be 100ppb. Thus, the calculated mercury concentration (6-1) has to be corrected (6-3) by the utilization of the correction factor (6-2). Performance of the enhanced, automated process is shown subsequently in Fig. 43 using the SAMI® Workstation EX Editor software.

$$\text{Concentration Mercury } \left[\frac{mg}{kg}\right] calculated = \frac{Concentration \left[\frac{\mu g}{l}\right] \times Volume\ [l] \times Dilution\ Factor}{Weight\ [g]} \quad (6\text{-}1)$$

$$\text{Correction factor} = \frac{Concentration\ Rhenium\ expected}{Concentration\ Rhenium\ measured} \quad (6\text{-}2)$$

$$\text{Concentration Mercury } \left[\frac{mg}{kg}\right] corrected = Concentration\ Mercury\ calculated \times Correction\ Factor \quad (6\text{-}3)$$

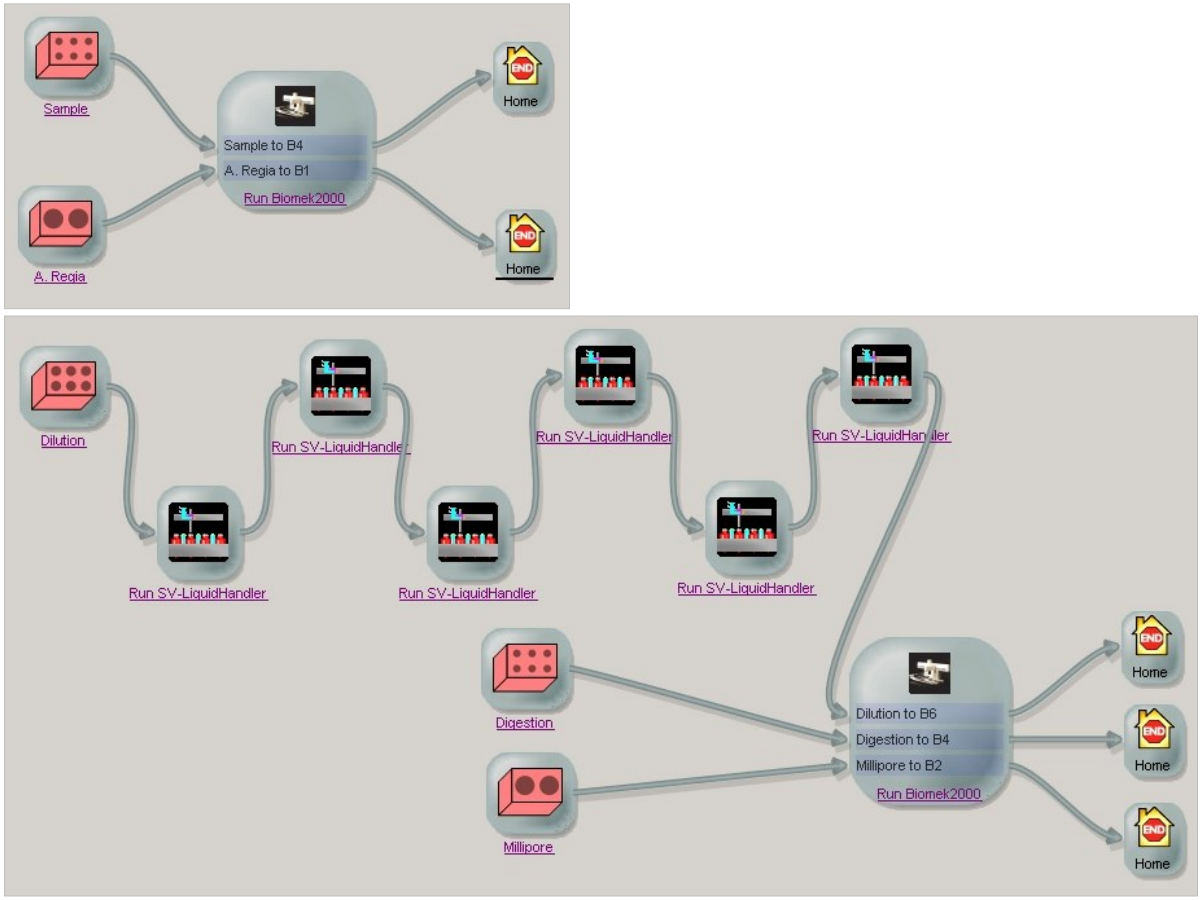


Fig 43: SAMI® Workstation EX Editor. Automated sample preparation for mercury analysis. The conceived trays – including the samples, the high concentrated acid (A. Regia), ultra-pure water (Millipore), the digested samples (Digestion), and the analysis solutions (Dilution) – are processed within the system while using the implemented devices, such as the Biomek®2000 and the in-house designed liquid handler

6.3 Results: Manual Validation Sequence – Element-Specific Measurement

6.3.1 Bias Error of the Measuring Instrument

Bias error of the measuring instrument (ICP-MS) was 0.026µg/l (STD [%] = 1.6%) and was calculated by measuring ten times a sample that was processed using the miniaturized manual sample preparation procedure. Measuring ten times a sample that was processed using the original manual pretreatment, STD [%] of the measuring instrument was 1.3%.

6.3.2 Repeatability Standard Deviation

Repeatability STD of the miniaturized manual procedure was 0.066mg/kg corresponding to a STD [%] of 11.5%. The calculated STD [%] did not exceed the maximum value according to the Horwitz` definition. Nevertheless, repeatability STD [%] of the original manual method was merely 8.35% and, therefore, 25% lower compared to the miniaturized manual procedure.

However, due to the very low final concentration of mercury, which was 1.5µg/l in the analysis solution and 0.6mg/kg in the solid matter, the maximum STD [%] is allowed to be 11.52% (according to the Horwitz` definition). Moreover, resulting in higher STD [%], homogeneity of the sample masses is much lower while performing the miniaturized procedure (compared to the original process) due to the fact that less masses of solid matter have been used.

6.3.3 Reproducibility Standard Deviation

Reproducibility STD of the miniaturized manual procedure was 0.063mg/kg, which is corresponding to a STD [%] of 10.98%. Reproducibility STD [%] of the miniaturized manual procedure was similar to the original procedure. Moreover, the calculated STD [%] did not exceed the maximum value according to the Horwitz´ definition.

6.3.4 Discrimination Threshold

Discrimination threshold of the miniaturized manual procedure was 6.1ng/l and discrimination threshold of the original method was 5.7ng/l.

6.4 Results: Automated Validation Sequence – Element-Specific Measurement

6.4.1 Bias Error of the Measuring Instrument

Bias error of the measuring instrument (ICP MS) was 1.2% and was calculated by measuring ten times a sample that was processed using the automated sample preparation process.

6.4.2 Repeatability Standard Deviation

Repeatability STD of the automated process was 0.051mg/kg corresponding to a STD [%] of 8.6%. The STD [%] did not exceed the maximum value according to the Horwitz` definition (STD [%] = 11.52%).

6.4.3 Reproducibility Standard Deviation

Reproducibility STD of the automated process was 0.065mg/kg corresponding to a STD [%] of 11.13%. The STD [%] did not exceed the maximum value according to the Horwitz´ definition.

6.4.4 Discrimination Threshold

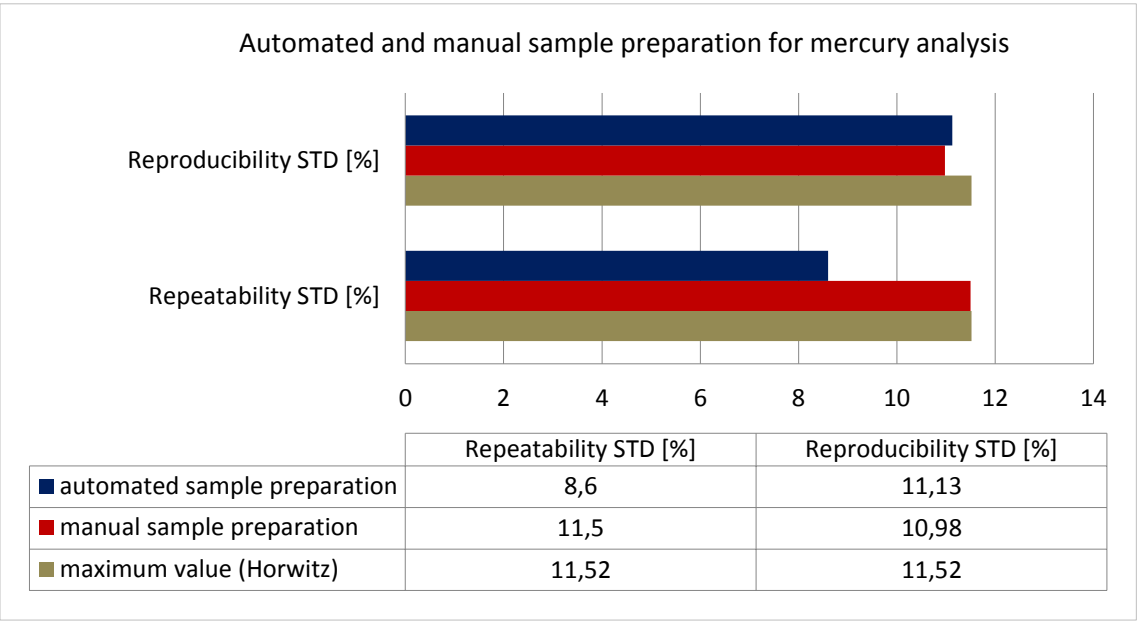
Discrimination threshold of the miniaturized automated process was 8.4ng/l.

6.5 Comparison and Conclusion of the Validation Sequences – Element-Specific Measurement

Preparing 25 samples, repeatability STD was calculated using repeatability conditions for the automated and the manual sample preparation processes. Repeatability STD [%] of the automated process was 8.6% and, therefore, 25% lower than repeatability STD [%] of the miniaturized manual procedure – even if the applied sample masses and, therefore, homogeneity of the samples were the same – and similar to the original process. Preparing ten samples per day on five consecutive days, reproducibility STD was measured using reproducibility conditions. Using the automated system, reproducibility STD [%] was similar to the reproducibility STD [%] of both manual procedures. Moreover, results did not exceed the maximum value (11.52%) according to the Horwitz` definition. Results are shown in the following table and in Fig. 44 (depicting the miniaturized processes).

Table 8: Comparison of the automated and the manual validation sequences for mercury analysis using repeatability and reproducibility testing

	Original method	Enhanced, miniaturized manual method	Enhanced, miniaturized automated method
Repeatability STD [%]	8.35	11.50	8.60
Reproducibility STD [%]	10.81	10.98	11.13



	Repeatability STD [%]	Reproducibility STD [%]
automated sample preparation	8,6	11,13
manual sample preparation	11,5	10,98
maximum value (Horwitz)	11,52	11,52

Fig 44: Comparison of the automated and the miniaturized manual validation sequence for mercury analysis using reproducibility and repeatability testing

Determining five calibration points in the range of 0.1µg/l up to 10µg/l, the coefficient of determination for both mercury calibration curves (for the automated and the manual validation sequence) was ≥ 0.9999 using blank offset and linear curve fit.

Additional information is shown in the subsequent Table 9. Moreover, calibration curves are shown in Fig. 45. Providing a general survey of the elements detected by the ICP-MS, the spectrum of elements is shown in Fig. 46.

Table 9: Further information about the mercury calibration curves (for the automated and the manual validation sequence) using blank offset and linear curve fit

	manual	automated
Ratio	202 Hg [He] : 175 Lu [He] ISTD	202 Hg [He] : 175 Lu [He] ISTD
Curve fit	linear	linear
Origin	Blank offset	Blank offset
R	≥ 0.9999	≥ 0.9999
m	0.0054	0.0055

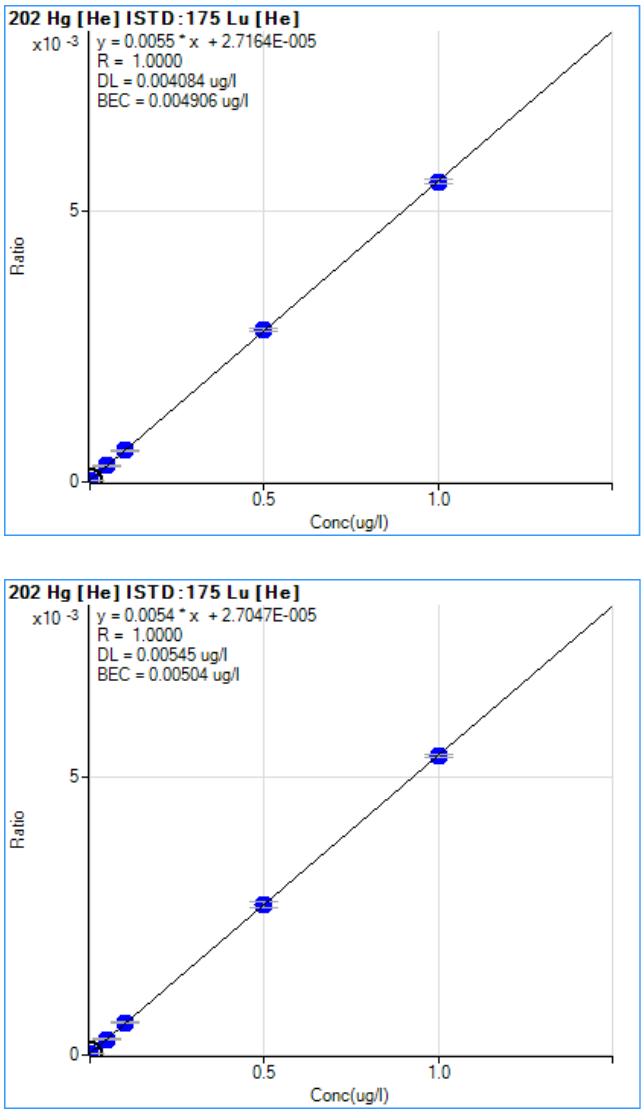


Fig 45: Calibration curves of the automated (below) and the manual mercury analysis process using blank offset and linear curve fit

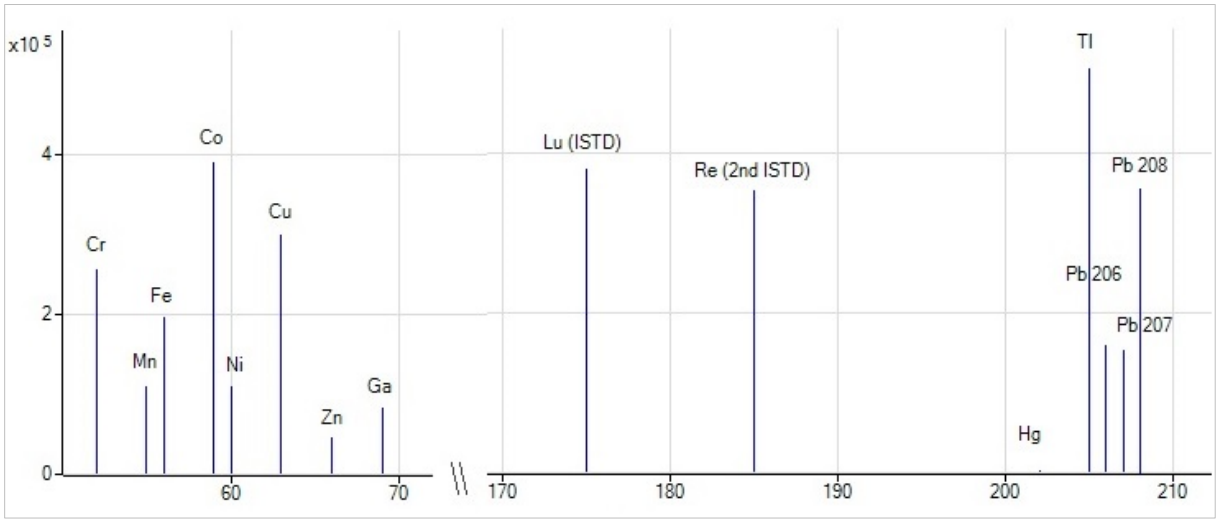


Fig 46: Spectrum of elements measured by ICP-MS for mercury detection, axis: Counts per Second vs. Mass

Therefore, the system's functionality has been confirmed in the presented validation sequences. The results are in excellent agreement with the true value using reference material as defined in the guide to the expression of uncertainty in measurement (ISO, 1993) for both the manual (0.566±0.018mg/kg; n = 50) [153] and the automated process (0.574±0.018mg/kg; n = 50) [154]. Ensuring also real sample measurements, Fig. 47 depicts the measurement of three different roof beams.

The most important features of the validated workstations are that they are not only capable of handling the wide range of required vessels but also able to handle several vessels simultaneously using the flexible labware design and miniaturization. Further workstations can be implemented easily due to the open SAMI® architecture. Using the automated sample pretreatment (with 24/7 investigations) ensures the mercury analysis for up to 480 samples per day. However, the rate limiting step is the detection step using ICP-MS.

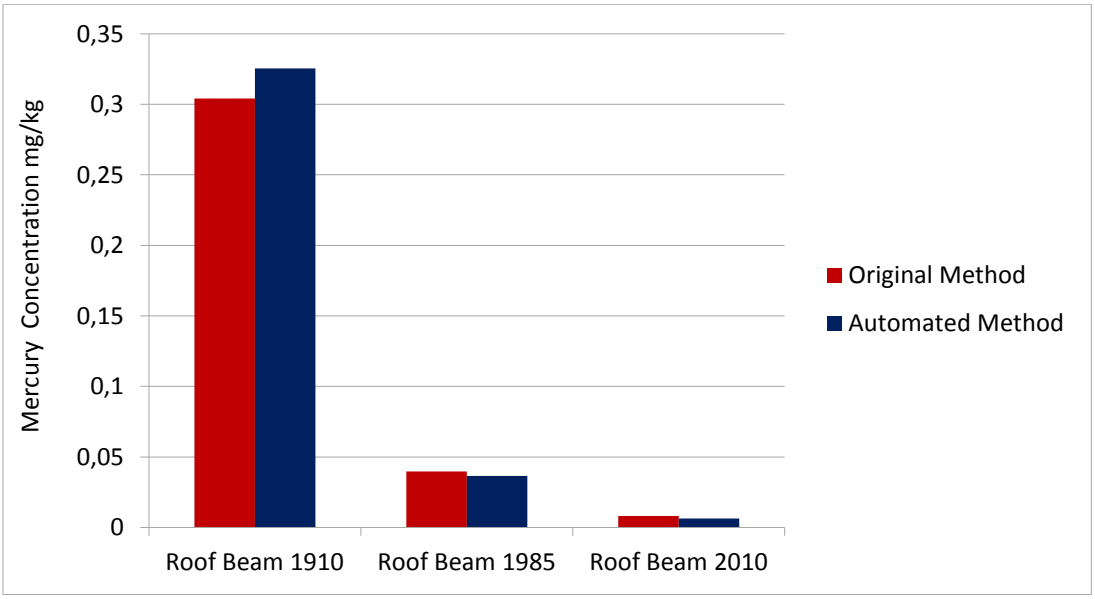


Fig 47: Comparison of the automated and the manual validation sequence for mercury analysis using three real samples

6.6 Further Element-Specific Measurements

Element-specific analysis of human bones provides information about the dietary habits [155] and the treatment of the diseases in the past [156] and allows for forensic and toxicity studies using ICP-OES [155] and IR detection modes [157]. Furthermore, carrying out specific investigations, such as the consideration of the chemically inertness of dental materials [158], further research investigate the migration of certain elements (Al, Ba, La, Sr) from the fillings placed into dental cavities to the healthy part of the teeth using ICP-MS [156].

However, due to the fact that element-specific analysis of bone material is extensively required, a precise and reliable method for the determination of total calcium and phosphor content in bone materials using ICP-MS was developed [159]. Performing miniaturization, merely one quarter of the original sample mass and acid volume was used. Therefore, 2ml of high concentrated nitric acid were added to 62.5mg of the samples, which was crashed and milled bone material. Nitric acid was prepared using the additional ICP rhenium standard. After microwave digestion, a predefined fraction of the 2ml clear sample solution was transferred to an analysis vessel and diluted using ultra-pure water and diluted nitric acid providing a final sample dilution of 1:1,000. Results were measured by ICP-MS. Miniaturization was used for both validation of the manual and the automated sample pretreatment in order to ensure comparability. The automated sample preparation process was performed using the fully automated system. The required vessels (similar to the mercury analysis process due to microwave digestion steps) were embedded into the conceived trays using the concept idea as described in the previous chapters. The automated sample preparation process for Ca/P analysis is shown in Fig. 48 using the SAMI® Ex Editor software.

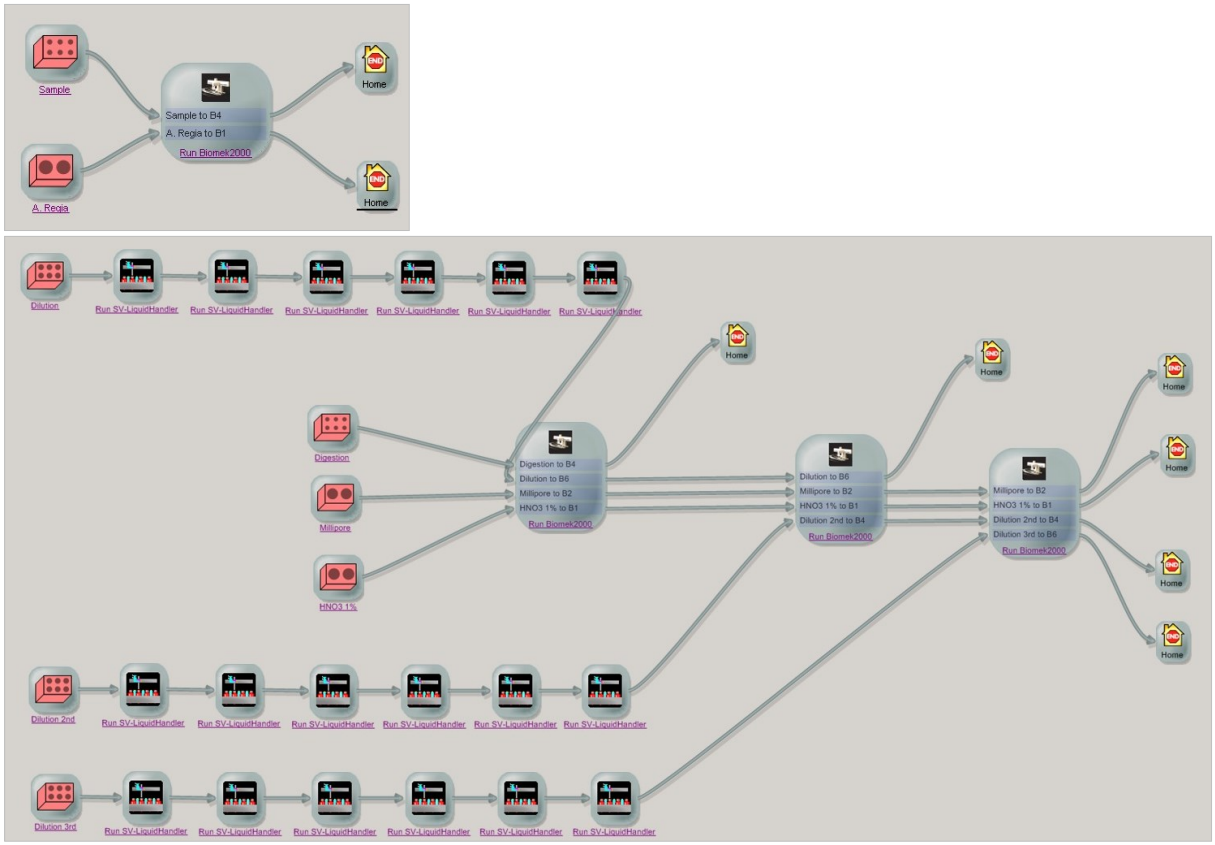


Fig 48: SAMI® Workstation EX Editor. Automated sample preparation for Ca and P analysis. The conceived trays – including the samples, the high concentrated acid (nitric acid), ultra-pure water (Millipore), the digested samples (Digestion), and various kinds of dilutions – are processed within the system using the integrated devices, such as the Biomek®2000 and the in-house designed liquid handler

Repeatability testing was conducted using repeatability conditions. Analyzing 46 samples, repeatability STD [%] was calculated. All measured values followed normal distribution, which was verified by the David´s test. STD [%] of both the miniaturized manual and the automated sample preparation process were significant lower than STD [%] of the original method. Performing the automated method provides STD [%] as follows: 2.04% for phosphor (P) analysis and 1.56% for calcium (Ca) analysis. Besides, using repeatability testing, the automated process was more precise than the enhanced manual procedure supplying STD [%] as follows: 2.5% for P analysis and 2.2% for Ca analysis. Performing the original method provides significant higher STD [%]: 3.17% for P and 2.92% for Ca analysis. Results are shown in Table 10.

Reproducibility testing was conducted by the preparation of ten samples per day on five consecutive days. STD [%] were calculated and compared with each other. Providing excellent reproducibility, STD [%] of both the enhanced manual and the automated sample preparation process for bone analysis were significant lower than STD [%] of the original sample preparation method. In detail, performing the automated method provides STD [%] as follows: 1.84% for P analysis and 1.73% for Ca analysis. Reproducibility of the miniaturized manual procedure was similar to the reproducibility of the automated process and supplies STD [%] as follows: 2.01% for P analysis and 1.50% for Ca analysis. The original method provides significant higher STD [%] as follows: 2.95% for P and 2.14% for Ca analysis.

Table 10: Comparison of the automated, the original and the miniaturized manual validation sequence for P and Ca analysis using reproducibility and repeatability testing

	Original method	Enhanced, miniaturized manual method	Enhanced, miniaturized automated method
Repeatability STD [%] for P analysis	3.17	2.50	2.04
Repeatability STD [%] for Ca analysis	2.92	2.20	1.56
Reproducibility STD [%] for P analysis	2.95	2.01	1.84
Reproducibility STD [%] for Ca analysis	2.14	1.50	1.73

The referenced material of the National Institute of Standards and Technology (NIST Gaithersburg, USA) provides certified values for P and Ca concentration allowing for accuracy testing (Table 11). Moreover, the fully automated system ensures real sample pretreatment as shown in Fig. 49/50.

Table 11: Accuracy testing with NIST samples providing certified values

	Original method	Enhanced, miniaturized manual method	Enhanced, miniaturized automated method
Recovery Range P analysis	99 – 108.4%	100.5 – 103.4%	98.9 – 110.1%
Recovery Range Ca analysis	101.3 – 105.6%	97.7 – 100.5%	90.3 – 100.6%

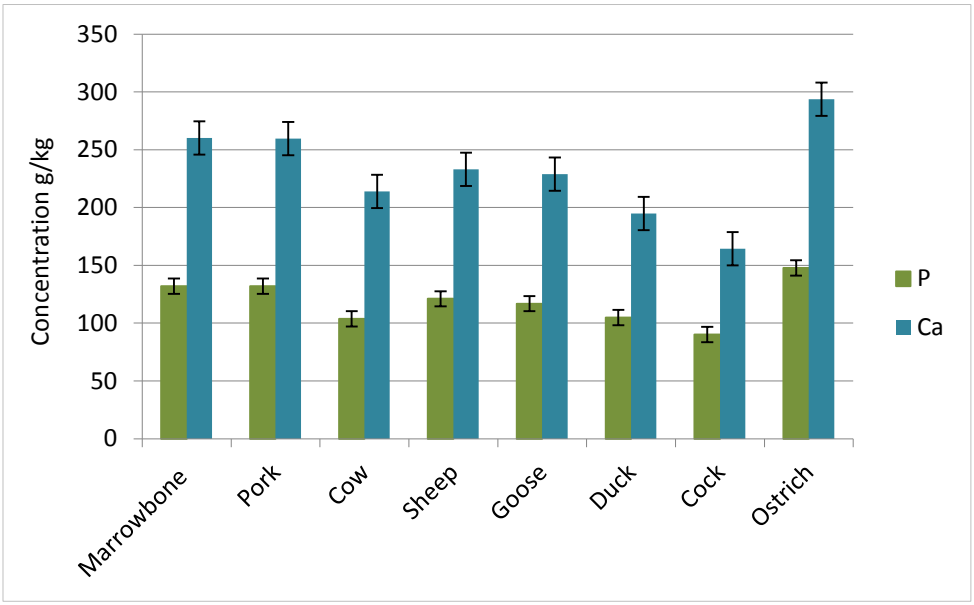


Fig 49: Results of P and Ca analysis while using automated sample preparation for several real samples

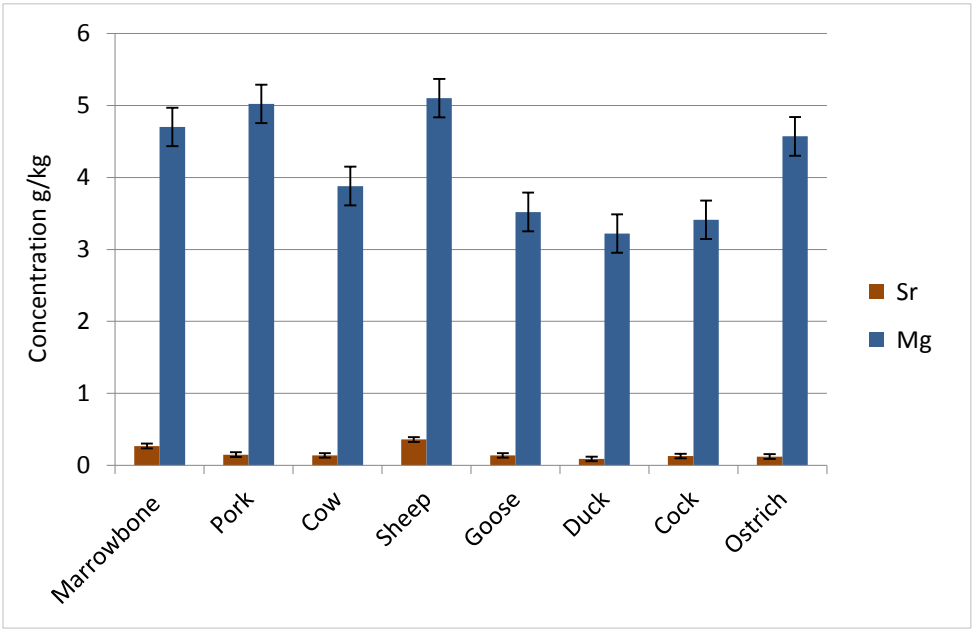


Fig 50: Results of Sr and Mg analysis while using automated sample preparation for several real samples

7. Application II: Structure-Specific Analytical Measurements

Facilitating structure-specific measurements, the fully automated system has to provide precise and reliable sample preparation. Therefore, the system´s functionality had to be confirmed in a validation sequence using an established analysis scheme (7.1) that has been validated using manual sample preparation first. Considering the automation requirements and the resulting system adaption (7.2), evaluation of the automated system has been performed. Finally, results of the automated and the manual validation process were compared with each other using GC/MS detection (7.3).

7.1 Process Description Including Motivation and Process Steps

Released into the environment, synthetic dyes are widely used in the industrial areas [160]. However, to estimate the resulting biodegradation products, such as benzoates, chromatographic analyses and mass spectrometry are required. Consequently, benzoates are widely spread in the environment [162] and laboratory testing of these organic compounds is an important tool for detecting and managing the environmental pollution of waterbodies: An *in situ* solid-phase microextraction (SPME) for simultaneous underwater sampling and extraction has been performed by conducted analysis with LC/MS detection mode [163]. Furthermore, HPLC-UV and LC-MS/MS methods were developed and validated for the quantitative analyses of benzoates in foods and beverages [164]. Moreover, identification is supported by NMR [161].

Allowing for benzoic acid analysis, structure-specific measurements have been performed that supply the validation sequence. Performing manual sample preparation while using an established analysis scheme, 25µl of the ISTD (cis-decahydronaphthalene), 132µl of the derivatisation reagent solution (trimethylsulfonium hydroxide, TMSH) and 100µl of three different benzoic acids, respectively, were added to the solvent, which was dichloromethane (CH_2Cl_2). Subsequently, the sample vials were individually crimped and heated for a period of 30 minutes (at 90°C). Allowing for tight sealing, 2ml clear crimp vials (as shown in Fig. 51) were used for the sample preparation and the analysis process.

Fig 51: Clear (left) and amber crimp vial with crimp caps. Source: [xv]

In the following Fig 52 the manual sample preparation process for benzoic acid analysis is shown using a flowchart.

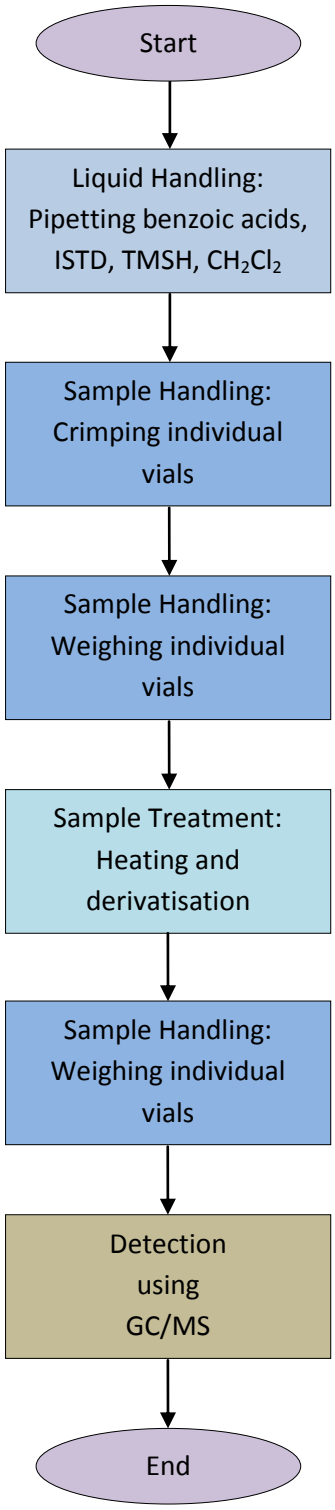


Fig 52: Flowchart – Manual sample preparation and measurement procedure for benzoic acids analysis

7.2 Automation Concept: Automation Requirements and Process Adaption

In order to automate the sample preparation steps for benzoic acid analysis, the required 2ml crimp vials have been embedded into the MTP-footprint (using the concept idea as described in the previous chapters). Supporting precise stacking steps and connective heat transfer, the tray configuration (as shown in Fig. 53), especially the rear side, matches with the two exchangeable thermo-block adapters (designed for the MHL 23 and described in chapter 4.6). Moreover, chosen material for the conceived tray has been aluminum.

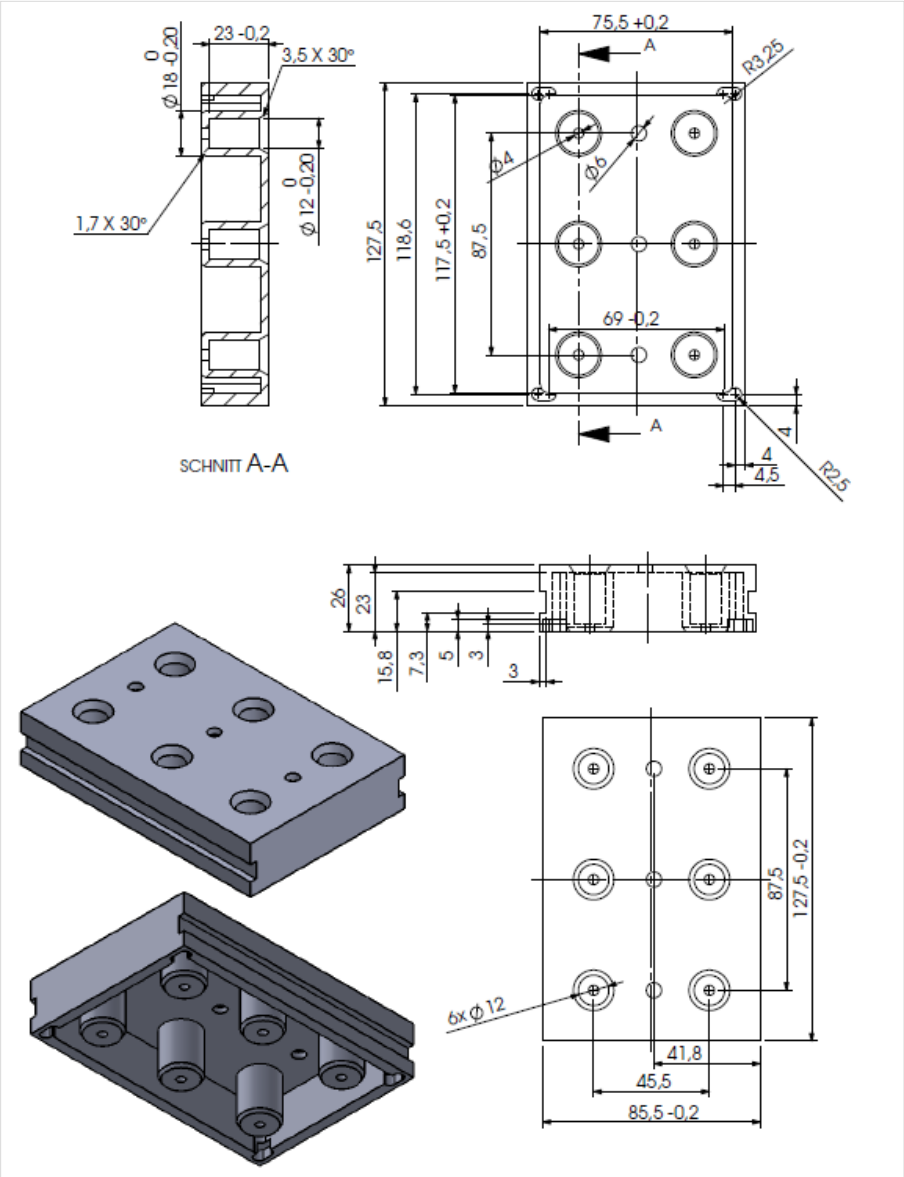


Fig 53: CAD design – Footprints, lateral views, and dimensions of the conceived trays used for the automated sample preparation procedure for benzoic acid analysis

The conceived tray is used during the whole sample preparation process. Nevertheless, the SCARA TS60 robot allows for individual sample handling, which is needed during crimping and weighing. Hence, while processing these pretreatment steps, the TS60 robot removes individual crimp vials from the tray – one by one – until finishing the current process for the current vial. The automated process for benzoic acid analysis is shown in the following Fig. 54 using a flowchart.

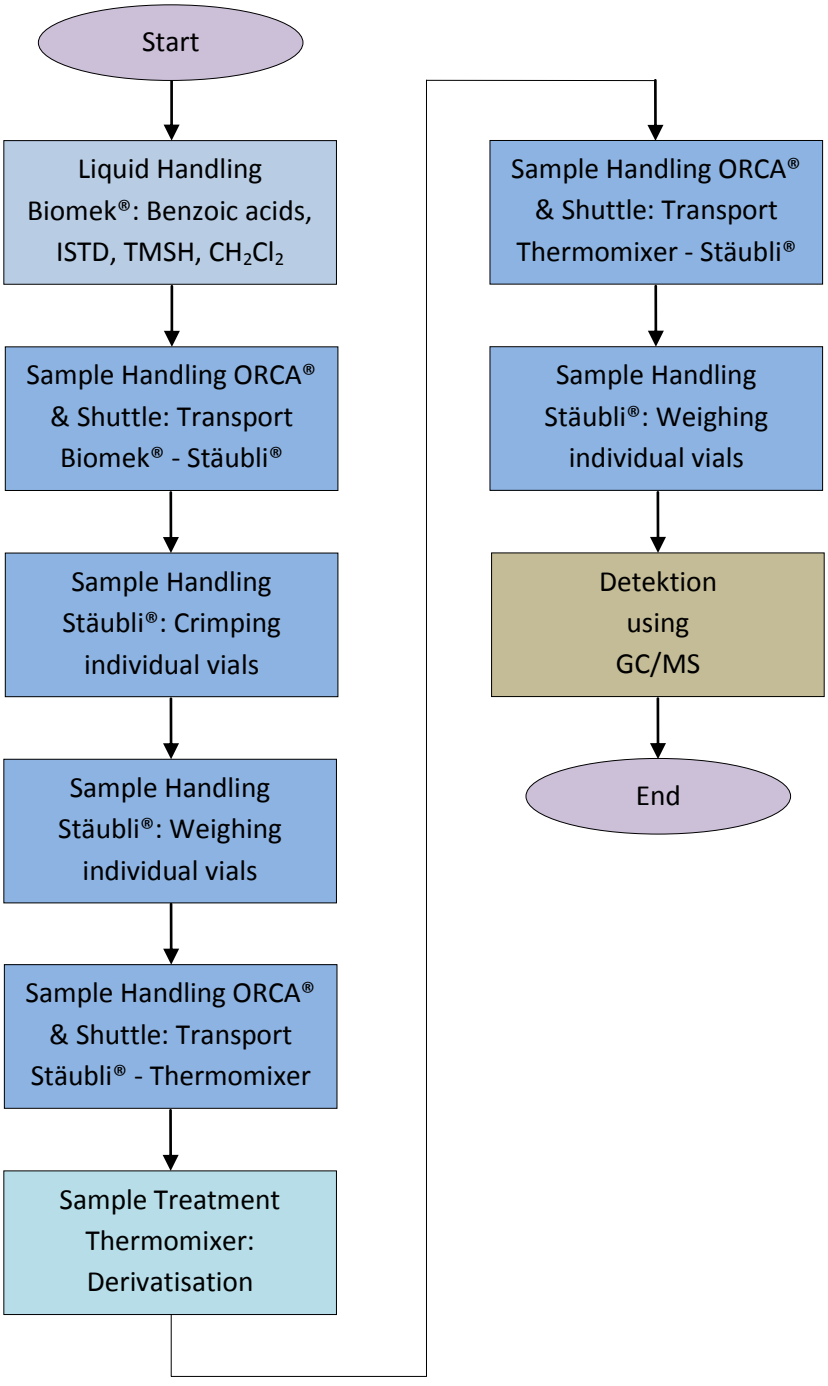


Fig 54: Flowchart – Automated sample preparation and measurement procedure for benzoic acids analysis

7.3 Results: Manual Validation Sequence – Structure-Specific Measurement

7.3.1 Bias Error of the Measuring Instrument

Bias error of the measuring instrument (GC/MS) was 0.42mg/l corresponding to a STD [%] of 1.3%.

7.3.2 Repeatability Standard Deviation

Repeatability STD [%] of the manual procedure was between 3.48% and 3.86%. The calculated STD [%] did not exceed the maximum value according to the Horwitz` definition (STD [%] = 6.39%).

7.3.3 Reproducibility Standard Deviation

Reproducibility STD [%] of the manual procedure was between 2.77% and 3.15%. The calculated STD [%] did not exceed the maximum value according to the Horwitz´ definition.

7.3.4 Discrimination Threshold

Discrimination threshold was 0.36mg/l.

7.4 Results: Automated Validation Sequence – Structure-Specific Measurement

7.4.1 Bias Error of the Measuring Instrument

Bias error [%] of the measuring instrument (GC/MS) was 0.8%.

7.4.2 Repeatability Standard Deviation

Repeatability STD [%] of the automated process was between 3.36% and 4.14%. The calculated STD [%] did not exceed the maximum value according to the Horwitz` definition (STD [%] = 6.39%).

7.4.3 Reproducibility Standard Deviation

Reproducibility STD [%] of the automated process was between 2.76% and 3.1%. The calculated STD [%] did not exceed the maximum value according to the Horwitz´ definition.

7.4.4 Discrimination Threshold

Discrimination threshold was 0.43mg/l.

7.5 Comparison and Conclusion of the Validation Sequences – Structure-Specific Measurement

The system's functionality has been confirmed in the validation sequence using a structure-specific measurement [165]. Verified by the David test, all measured values followed normal distribution. Performing repeatability and reproducibility testing, the automated sample preparation is as precise as the manual testing as shown in the following Fig. 55 and Fig. 56. Qualitative results are shown in Fig. 57. The developed system facilitates the analysis for up to 576 samples per day.

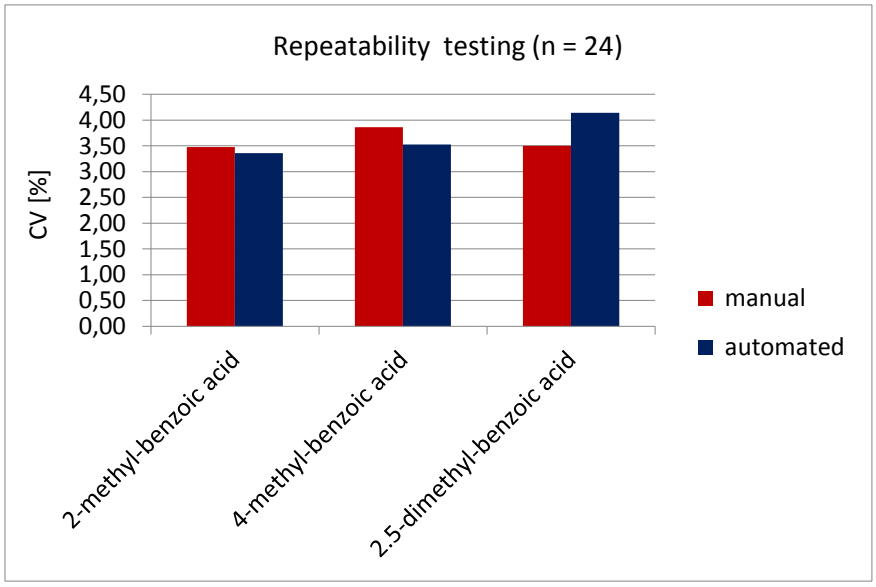


Fig 55: Comparison of the automated and the manual validation sequence for benzoic acid analysis using repeatability testing

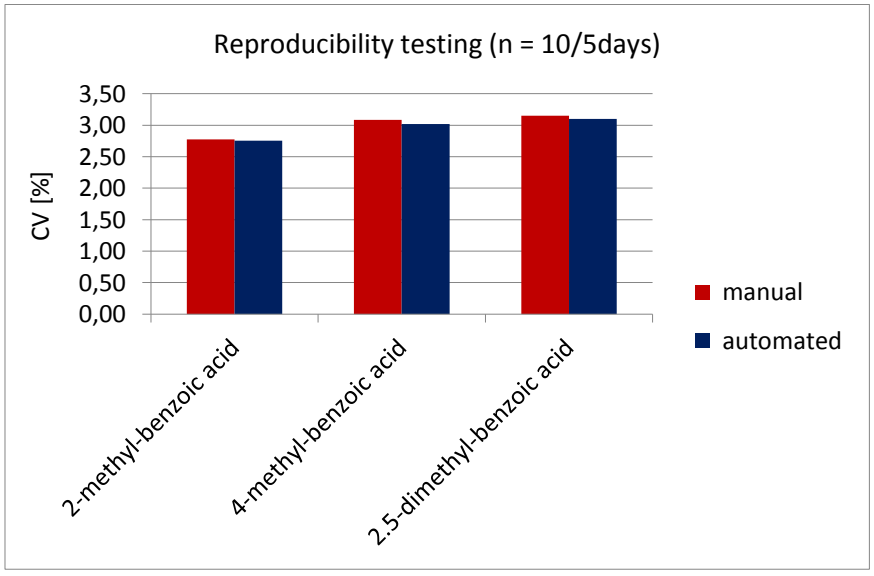


Fig 56: Comparison of the automated and the manual validation sequence for benzoic acid analysis using reproducibility testing

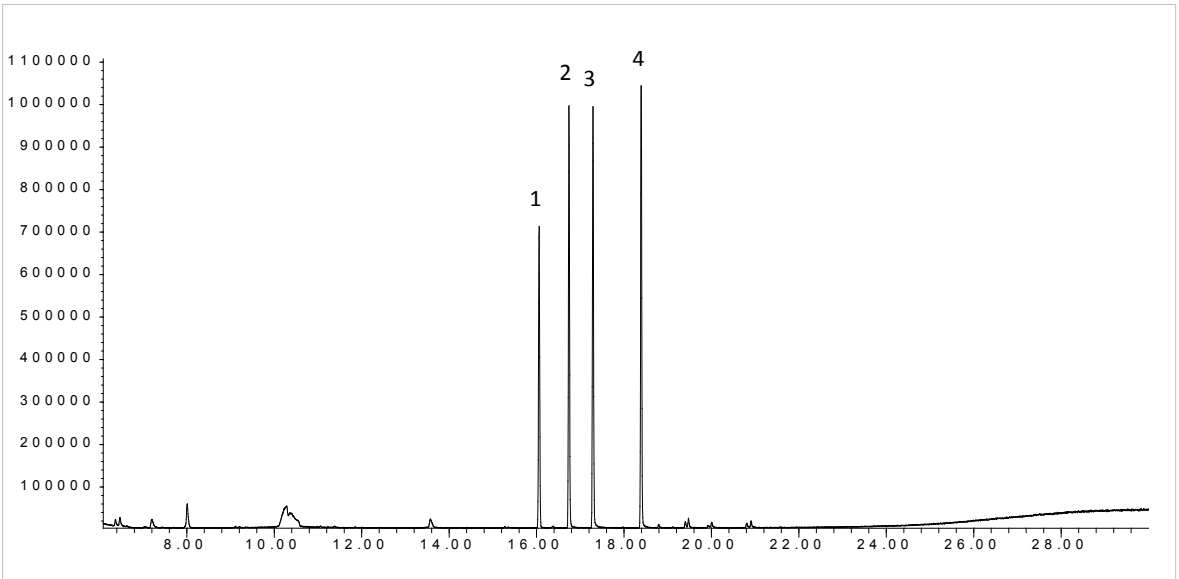


Fig 57: Qualitative analysis using structure specific measurement (GC/MS) for benzoic acid analysis. The chromatogram represents the total ion current (TIC). (1) ISTD; (2) 2-methyl benzoic acid, methyl ester; (3) 4-methyl benzoic acid, methyl ester; (4) 2.5-dimethyl benzoic acid, methyl ester (C = 30ppm), axis: Abundance vs. Acquisition Time [min.]

7.6 Further Structure-Specific Measurements

Cyclophosphamide has been widely used in patients with kidney diseases, such as vasculitis, steroid-resistant nephrotic syndrome, and progressive IgA-nephropathy. In addition incidences are increasing for specific kinds of tumors, such as bladder carcinoma, lymphomas, and tumors of the skin [166]. However, anticancer drugs are harmful substances that can have carcinogenic, mutagenic, teratogenic, genotoxic, and cytotoxic effects even at low concentrations [167]. Moreover, due to the increased consumption of chemotherapeutic agents, the occurrence of cytostatic drugs in the aquatic environment must be properly evaluated. Therefore, analytical methods based on online SPE and coupled to liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) have been developed and validated [168], [169], [170].

SPE enables the effective removal of sample matrix and allows for the pre-concentration of the target substances [134] while using target-specific columns. However, allowing for cyclophosphamide analysis, structure-specific analytical measurements have been performed that provide the validation sequence.

Performing the manual and the automated sample preparation process, phenyl-modified silica gel columns were used for the established analysis scheme. The columns were conditioned with methanol and water first. After sample loading, the washing step was performed with water again. Finally, the analyte was successively eluted with methanol.

Considering the MTP-formatted labware requirements for the automated sample preparation process, the Positive Pressure SPE Unit allows for the utilization of commercially available filter plates and for a designed labware adapter (as shown Fig. 58) facilitating the embedding and the usability of 24 individual columns.

Fig 58: Positive Pressure SPE Unit adapter

After performing the automated positive pressure SPE process, which attains a discrimination threshold of 6.3µg/l, the eluted substances have been measured using the HPLC/MS. Bias error of the measuring instrument was 1.4%. However, using the manual procedure, discrimination threshold was 6.9µg/l. Bias error of the measuring instrument was 2.6%. Finally, the manual and the automated processes were evaluated quantitatively and compared with each other [171]. Verified by the David test, all measured values followed normal distribution.

Repeatability testing was conducted using repeatability conditions. STD [%] were calculated and compared with each other. Providing excellent repeatability, STD [%] of the automated SPE process for both using the commercially available format with 96 columns and the designed adapter with 24 individual columns did not exceed the maximum value according to the Horwitz´ definition. In detail, performing 96 samples per run, repeatability STD [%] of the automated positive pressure SPE including the commercially available format was 6.1%. Performing 24 samples per run, repeatability STD [%] of the automated positive pressure SPE including the designed adapter was 4.4%.

Reproducibility testing was conducted using reproducibility conditions. STD [%] were calculated and compared with each other. Providing excellent reproducibility, STD [%] of the automated SPE process for both using the commercially available format with 96 columns and the designed adapter with 24 individual columns did not exceed the maximum value according to the Horwitz´ definition. In detail, performing 96 samples on five consecutive days, reproducibility STD [%] of the automated positive pressure SPE including the commercially available format was 6.1%. Performing 24 samples on five consecutive days, reproducibility STD [%] of the automated positive pressure SPE including the designed adapter was 5.5%. Results are shown in Table 12.

Table 12: Comparison of the automated (24 vs. 96 columns) and the manual validation sequence for cyclophosphamid analysis using reproducibility and repeatability testing

	Manual method	Automated SPE using adapter with 24 individual columns	Automated SPE using MTP-format with 96 columns
Repeatability STD [%]	4.1	4.4	6.1
Reproducibility STD [%]	4.3	5.5	6.1

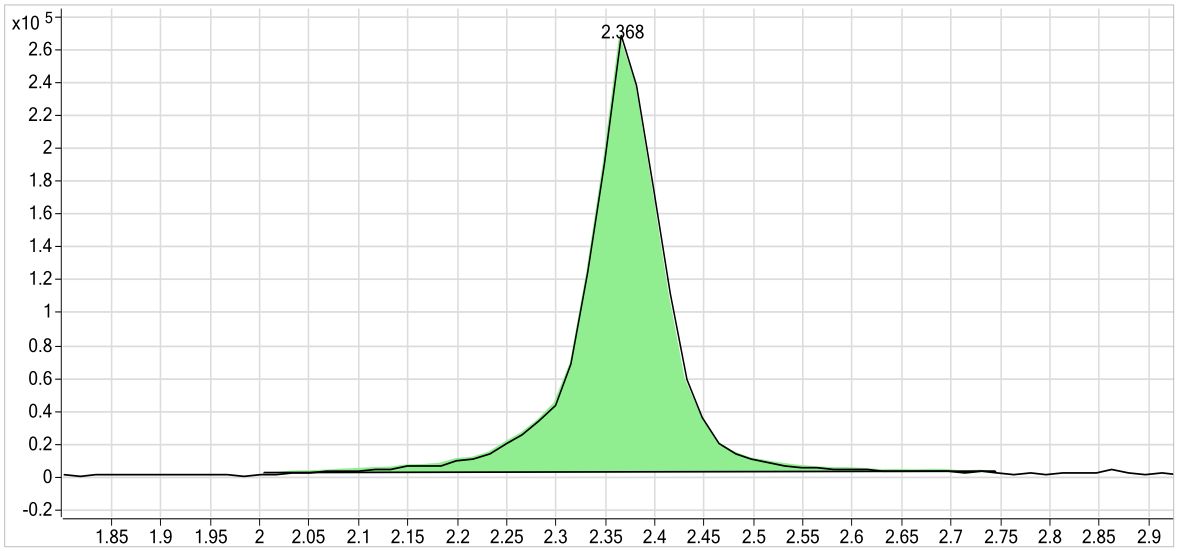


Fig 59: Qualitative analysis using structure specific measurement (HPLC/MS) for cyclophosphamide analysis after the effective removal of sample matrix. The chromatogram represents the extracted ion current (EIC) of caffeine (C = 200ppb), axis: Counts vs. Acquisition Time [min.]

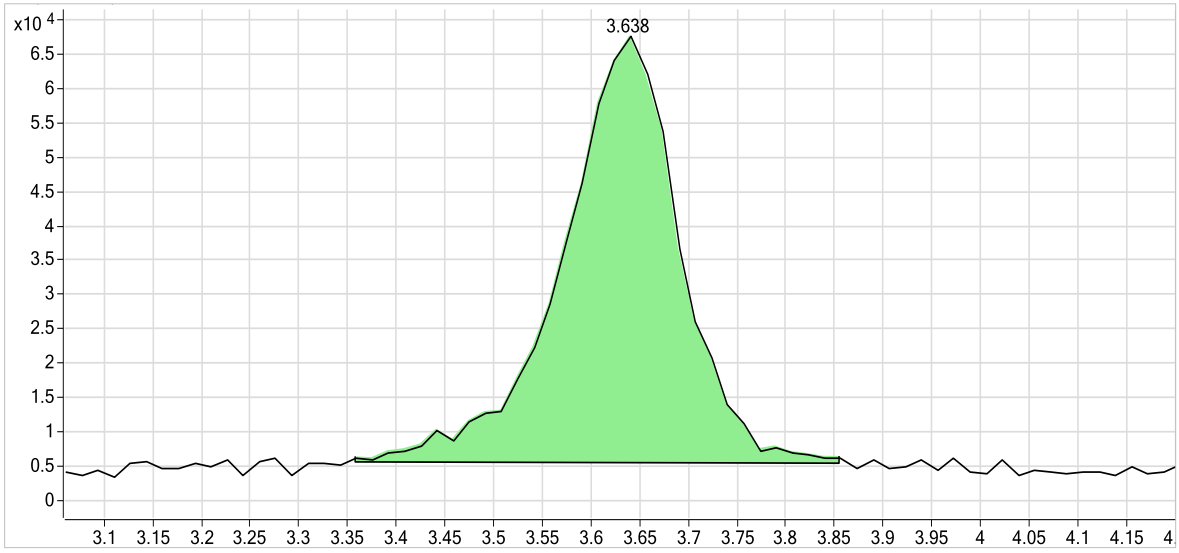


Fig 60: Qualitative analysis using structure specific measurement (HPLC/MS) for cyclophosphamide analysis after the effective removal of sample matrix. The chromatogram represents the EIC of cyclophosphamide (C = 200ppb), axis: Counts vs. Acquisition Time [min.]

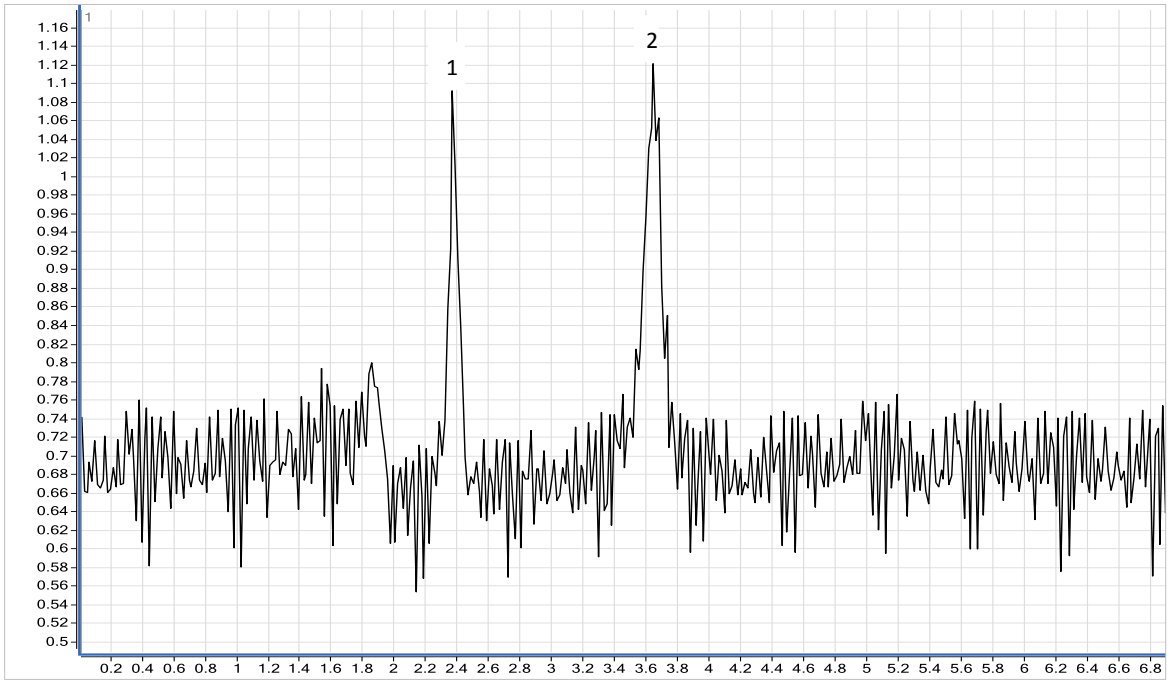
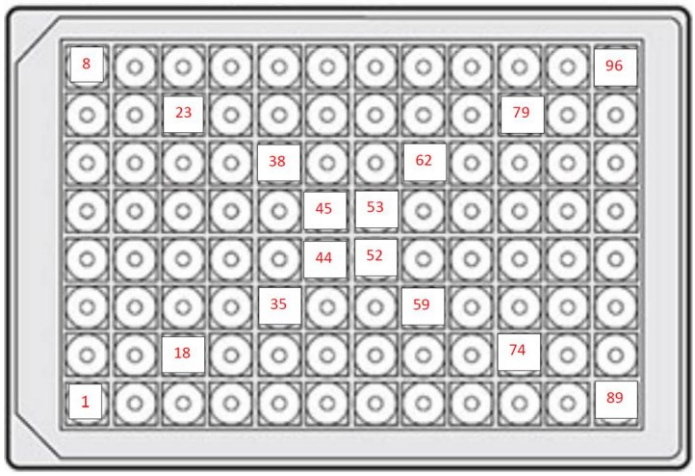


Fig 61: Qualitative analysis using structure specific measurement (HPLC/MS) for cyclophosphamide analysis after the effective removal of sample matrix. The chromatogram represents the TIC. (1) ISTD, (2) cyclophosphamide (C = 200ppb), axis: Counts x10^6 vs. Acquisition Time [min.]

However, results indicated leakages and loss of pressure, especially in the marginal columns while using the 96-column format. Therefore, using a test adapter with 96 ports, inconsistent pressurization was detected while using a contact pressure of 51psi (0.35MPa) and pressurization of 44psi (0.3MPa). The following figure represents the chosen positions – exemplifying the inner and the marginal positions. Moreover, depicting the initial and the final measurement of pressure at the columns` outlet, results identify leakages and inconsistent pressurization after a period of 10sec for both the inner and outer positions. Nevertheless, regarding the marginal column positions, loss of pressure is observed immediately. Therefore, the plate sealing material, thickness, and durability have to be improved performing further investigations.

7. Application II: Structure Specific Analytical Measurements



contact pressure 0.35MPa		Pressurization 0,3 Mpa																							
	Pos.Nr.	1		8		18		23		35		38		44		45		52		53		59		62		74		79		89		96	
	Pressurization start - stop	0,17	0,12	0,083	0,021	0,296	0,124	0,295	0,127	0,296	0,128	0,296	0,123	0,296	0,122	0,295	0,126	0,299	0,111	0,299	0,126	0,266	0,126	0,216	0,131	0,193	0,123	0,19	0,123	0,1	0,118	0,05	0,113
	Loss of pressure	0,13	0,18	0,217	0,279	0,004	0,176	0,005	0,173	0,004	0,172	0,004	0,177	0,004	0,178	0,005	0,174	0,001	0,189	0,001	0,174	0,034	0,174	0,084	0,169	0,107	0,177	0,11	0,177	0,2	0,182	0,25	0,187

Fig 62: Initial and final measurement of pressure at the columns` outlet

8. Summary and Outlook

Allowing for applied and basic research applications, all life science areas require specific sample pretreatment steps, such as stirring, diluting, evaporation, and sample extraction techniques. However, since sample preparation techniques are the rate-limiting step in many testing processes, *automated* sample pretreatment allows for improving the analysis´ efficiency, robustness, and reliability. In addition automated systems ensure the safety of the analysts by assigning risk-involving procedures.

However, supplying automated sample preparation techniques, commercially available workstations are usually configured for handling the standardized MTP-format. Accomplishing various assays types, biological applications use especially the MTP-format and represent, therefore, the prime candidates for laboratory automation. Nevertheless, ensuring the analysis of single elements, small molecules, and mixtures of molecules, analytical requirements differ significantly from biological applications.

Thus, accomplishing non-standard temperature and pressure procedures using higher liquid levels and, furthermore, highly active substances, the use of specific, inert vessels has remained indispensable. Moreover, automating analytical sample preparation calls for individual sample handling, tailored liquid handling and sample treatment steps, and in addition for extensive detection processes. Therefore, commercially available automated workstations are not suitable for analytical sample pretreatment. Furthermore, regarding the state of the art, existing systems either handle only a few steps (mostly at the end) of a complex analytical scheme or they offer a single solution for a fixed process.

Consequently, the purpose of the present dissertation was the design, the realization, and evaluation of an automated system that ensures an optimal balance between automating the most important steps (while regarding the variety of analytical sample preparation processes) and still providing high flexibility for easy upgrading and performance adaption (while dealing with the wide range of required vessels).

However, embedding these vessels into the standardized MTP-footprint represents a suitable solution that ensures the availability of existing workstations for multistep analytical sample pretreatment. Moreover, providing a flexible solution, this concept idea is applicable for a wide range of vessels and ensures the performance of different kinds of applications. In addition embedding allows for simultaneous handling of vessels and, thus, for less-cost and time-consuming automation

steps. Nevertheless, to ensure the entire task performance of the adapted workstations, the embedded vessels have to meet the specifications of the devices´ editor software.

However, acting as a system integrators and transport systems the central element of the automated system are two classical laboratory robots (ORCAs®). Allowing for the transfer of all sample types between both ORCA® robots, a re-grip station has been designed. Moreover, the samples are stored in a flexible sample-hotel until they are processed within the system. In addition a webcam has been implemented providing sample identification while using 2D-barcode reading.

Due to the utilization of the standardized labware design, the presented concept idea enables the ORCA® robots to place every kind of designed MTP-formatted labware type on any existing labware position throughout the integrated system. Thus, using the concept idea provides more flexibility during the transportation steps and enables the utilization of the system for various kinds of applications without any changing of labware positions. Therefore, conceiving the designed labware types represents the first stage of developing automated analytical sample pretreatment.

Pipetting steps are provided by the Biomek® 2000 due to the concept idea of system adaption while meeting the spezifications of the liquid handler software. The Biomek® 2000 enables precise and reliable pipetting steps up to 1,000µl. Supplying higher volume ranges and the required accuracy, a diluting station has been designed providing Cartesian configuration, MTP-formatted labware postions, and an Hamilton® Dispenser. Ensuring handling of evaporating solvents, the Biomek® 2000 has been covered with a housing and can be exhausted in addition.

Moreover, the presented concept comprises the idea of capping the whole MTP-footprint with just one lid. Thus, irrespective of the port diameter of the vessels, the Biomek® gripper tool is capable of gripping this lid in one single step providing simultaneous opening and covering of up to 24 vessels. However, individual capping enabling a very tight seal is still required in order to ensure concentration stability while handling volatile components.

Individual sealing, such as crimping, requires individual sample handling due to the fact that crimp caps and vessels have to be transferred to a crimp-tightening robot one by one. Thus, individual sample handling is enabled using the Stäubli® TS60 SCARA robot assembly that allows for high speed and high precise gripping and crimping. Also the weighing station is loaded by the high precise SCARA robot. Moreover, facilitating the weighing steps, the ionizer ANTISTAT 2000 avoids electrostatic charge of the samples. Ensuring the transport to the SCARA robot assembly, a shuttle transport has been integrated into the automation system.

Furthermore, sample treatment steps provide an appropriate form of the analyte for the detection or separation systems. Therefore, facilitating sample treatment steps, the heating and shaking device MHL 23 has been integrated allowing for derivatization reactions and homogenization of the samples. In addition enabling positive pressure SPE applications, the designed Positive Pressure Unit can be simply assembled on the Biomek®. Moreover, analytical sample preparation necessitates microwave digestion steps. Nevertheless, due to safety reasons, this steps have to be performed under a separated hood. Hence, a mobile robot system has been developed providing sample transport between the automated system, the microwave hood, and further external stations, such as the analytical devices.

Finally, the system's functionality has had to be confirmed in various validation sequences supplying element and structure-specific measurements. In detail, enabling element-specific measurements, the automated system fulfilled sample pretreatment for mercury, calcium, and phosphor analysis. Moreover, the system enabled automated sample preparation for benzoic acid analysis and SPE-based applications using cyclophosphamide analysis in order to ensure structure-specific measurements.

The results of the validation sequences have been evaluated and compared with the manual procedures. For validating the sample preparation processes, bias error of the measuring instrument (according to DIN 1319), repeatability standard deviation (STD) (according to DIN 1319), reproducibility STD (according to DIN 1319), and discrimination threshold (according to DIN 1319, also called limit of detection according to DIN 32645) have been calculated.

Furthermore, considering the automation requirements, the trend in laboratory automation is toward increasing miniaturization and simplification. Thereby, one quarter of the original sample mass and acid volume was merely applied for mercury, calcium, and phosphor analysis. In addition miniaturization improves throughput possibilities and reduces sample consumption.

Regarding the evaluation results, the fully automated system enables precise and reliable processes while performing automated sample preparation for mercury analysis: Repeatability STD [%] of the automated process was 25% lower than repeatability STD [%] of the miniaturized manual procedure and was, moreover, similar to the original process. Furthermore, reproducibility testing was similar to the miniaturized and the original manual processes. Using referenced wood material, the results of measurement were in excellent agreement with the true value as defined in the guide to the expression of uncertainty in measurement (ISO, 1993).

Performing a further element-specific application, the system's functionality has been confirmed using bone material and microwave digestion. Finally, dilution steps have been fulfilled in the ratio of 1:1,000. Ensuring precise sample preparation steps, repeatability and reproducibility STD [%] of the automated process were up to 50% lower than STD [%] of the original manual sample pretreatment.

Furthermore, using structure-specific measurements that provide benzoic acid analysis, the automated system enables precise analytical sample preparation processes: Performing repeatability and reproducibility testing, automated sample preparation was as precise as the manual procedure and provides sample pretreatment for up to 576 samples per day.

Performing a further structure-specific and automated SPE-based application, repeatability and reproducibility testing for both using the designed adapter for 24 individual columns and the commercially available 96-column format did not exceed the maximum value according to the Horwitz´ definition. Moreover, using the designed adapter with 24 individual columns, repeatability testing was as precise as the manual procedure.

Preparing 96 samples per run, the automated SPE process ensures high-throughput possibilities. However, results indicated leakages and loss of pressure especially in the marginal columns while using the 96-column format. Therefore, using a test adapter with 96 ports, inconsistent pressurization was detected. Therefore, the plate sealing material, thickness, and durability have to be improved requiring further investigations.

Moreover, in order to extend the provided application spectrum, further element and structure-specific measurements have to be performed using the automated system. All applications can be performed in a high-throughput manner supplying 24/7 investigations of real environmental and pharmaceuticals samples.

Bibliography

[1] H. WEINMANN, "Laboratory robot in combinatorial calalysis and method development," Nachrichten aus der Chemie, vol. 49, no. 2, pp. 150-154, 2001.

[2] Drug discovery technologies, San Jose, CA: Global Industry Analysts, October 2008.

[3] European markets for laboratory automation systems, London, GB: Frost and Sullivan Ltd., November 2008.

[4] Laboratory automation - a global outlook, San Jose, CA: Global Industry Analysts, January 2012.

[5] H. FLEISCHER and K. THUROW, "Fast mass spectrometry-based enantiomeric excess determination of proteinogenic amino acids," Amino acids, pp. 1-13, 2012.

[6] Chiral technology, San Jose, CA: Global Industry Analysts, April 2008.

[7] Forensic technologies - new and growing market, Wellesley, MA: BCC Research, February 2008.

[8] T. CHAPMAN, "Lab automation and robotics: Automation on the move," Nature, vol. 421, no. 6923, pp. 661, 663, 665-666, 2003.

[9] W. DE JONG, E. G. DE VRIES, and I. KEMA, "Current status and future developments of LC-MS/MS in clinical chemistry for quantification of biogenic amines," Clin. Biochem., vol. 44, no. 1, pp. 95-103, 2011.

[10] T. KNUDSEN, "Fully automated sample preparation and analysis in the mining industry," IFAC P. Vol. (IFAC-PapersOnline), pp. 153-158, 2007.

[11] D. TEITZ, S. KHAN, M. POWELL, and M. JEMAL, "An automated method of sample preparation of biofluids using pierceable caps to eliminate the uncapping of the sample tubes during sample transfer," J. Biochem. Biophys. Methods, vol. 45, no. 2, pp. 193-204, 2000.

[12] J. SIMS, A. SMITH, D. PATEL, R. BATCHELOR, and J. CARREIRA, "Automated sample preparation for icp analysis of active pharmaceutical ingredients and intermediates," J. Lab. Autom., vol. 16, no. 5, pp. 377-380, 2011.

[13] L. COUCHMAN, "Turbulent flow chromatography in bioanalysis: A review," Biomed. Chromatogr., vol. 26, no. 8, pp. 892-905, 2012.

[14] K. OZAWA, P. SCHNIPESLKY, H. PARDUE, J. PLACE, and A. TRUCHAUD, "The expanding role of robotics in the clinical laboratory," J. Autom. Chem., vol. 14, no. 1, pp. 9-15, 1992.

[15] S. SAITOH and T. YOSHIMORI, "Fully automated laboratory robotic system for automating sample preparation and analysis to reduce cost and time in drug development process," J. Assoc. Lab. Autom., vol. 13, no. 5, pp. 265-274, 2008.

[16] W. MULLETT, "Determination of drugs in biological fluids by direct injection of samples for liquid-chromatographic analysis," J. Biochem. Biophys. Methods, vol. 70, no. 2, pp. 263-273, 2007.

[17] N. SURUGAYA, T. HIYAMA, and M. WATAHIKI, "Automated gravimetric sample pretreatment using an industrial robot for the high-precision determination of plutonium by isotope dilution mass spectrometry," Anal. Sci., vol. 24, no. 6, pp. 739-744, 2008.

[18] P. HOFMEYR and B. MORRISON, "The benefits of automation for mining metallurgical plant analytical laboratories," Australas. Inst. Min. Metall. Publ. Ser., pp. 481-491, 2008.

[19] J. HONG, Y. MIKI, K. HONDA, and H. TOITA, "Development of the automated cleanup system for the analysis of PCDDs, PCDFs and DL-PCBs," Chemosphere, vol. 88, no. 11, pp. 1287-1291, 2012.

[20] F. RAYMOND, S. METAIRON, R. BORNER, M. HOFMANN, and M. KUSSMANN, "Automated target preparation for microarray-based gene expression analysis," Anal. Chem., vol. 78, no. 18, pp. 6299-6305, 2006.

[21] E. PLISE, D. TRAN, and L. SALPHATI, "Semi-automated protein binding methodology using equilibrium dialysis and a novel mixed-matrix cassette approach," J. Pharm. Sci., vol. 99, no. 12, pp. 5070-5078, 2010.

[22] V. TURNIA JR., T. GORE, S. SINGH, and R. ARUMUGHAM, "Automation of the anthrone assay for carbohydrate concentration determinations," Anal. Chem., vol. 82, no. 5, pp. 1786-1792, 2010.

[23] F. SCHMITT, B. COCHAND-PRIOLLET, M. TOETSCH, B. DAVIDSON, A. BONDI, and P. VIELH, "Immunocytochemistry in europe: Results of the european federation of cytology societies (EFCS) inquiry," Cytopathology, vol. 22, no. 4, pp. 238-242, 2011.

[24] M. THEVARAJAH, S. NADARAJAH, Y. CHEW, and P. CHAN, "Evaluation of a urinary metanephrines reagent kit: An automated approach," Singapore Med. J., vol. 49, no. 6, pp. 454-457, 2008.

[25] W. JANZEN and I. POPA-BURKE, "Review: Advances in improving the quality and flexibility of compound management," J. Biomol. Screen., vol. 14, no. 5, pp. 444-451, 2009.

[26] R. JIANG, E. CARASEK, S. RISTICEVIC, E. CUDJOE, J. WARREN, and J. PAWLISZYN, „Evaluation of a completely automated cold fiber device using compounds with varying volatility and polarity," Anal. Chim. Acta, vol. 742, pp. 22-29, 2012.

[27] L. GREENE, I. ISAAC, D. GRAY, and S. SCHWARTZ, "Streamlining plant sample preparation: The use of high-throughput robotics to process Echinacea samples for biomarker profiling by MALDI-TOF mass spectrometry," J. Biomol. Tech., vol. 18, no. 4, pp. 238-244, 2007.

[28] M. WENLOCK, R. AUSTIN, T. POTTER, and P. BARTON, "A highly automated assay for determining the aqueous equilibrium solubility of drug discovery compounds," J. Lab. Autom., vol. 16, no. 4, pp. 276-284, 2011.

[29] L. CANELLE, C. PIONNEAU, A. MARIE, J. BOUSQUET, J. BIGEARD, D. LUTOMSKI, T. KADRI, M. CARON, and R. JOUBERT-CARON, "Automating proteome analysis: Improvements in throughput, quality and accuracy of protein identification by peptide mass fingerprinting," Rapid Commun. Mass Spectrom., vol. 18, no. 23, pp. 2785-2794, 2004.

[30] O. HØRNING, S. THEODORSEN, O. VORM, and O. JENSEN, "Solid phase extraction-liquid chromatography (SPE-LC) interface for automated peptide separation and identification by tandem mass spectrometry," Int. J. Mass Spectrom., vol. 268, no. 2-3, pp. 147-157, 2007.

[31] M. OLAJOS, P. HAJÓS, G. BONN, and A. GUTTMAN, "Sample preparation for the analysis of complex carbohydrates by multicapillary gel electrophoresis with light-emitting diode induced fluorescence detection," Anal. Chem., vol. 80, no. 11, pp. 4241-4246, 2008.

[32] A. BARROS, V. LY, T. CHANDO, Q. RUAN, S. DONENFELD, D. HOLUB, and L. CHRISTOPHER, "Development and evaluation of a multiple-plate fraction collector for sample processing: Application to radioprofiling in drug metabolism studies," J. Pharm. Biomed. Anal., vol. 54, no. 5, pp. 979-986, 2011.

[33] A. LUIPPOLD, T. ARNHOLD, W. JÖRG, B. KRÜGER, and R. SÜSSMUTH, „Application of a rapid and integrated analysis system (RIAS) as a high-throughput processing tool for in vitro ADME samples by liquid chromatography/tandem mass spectrometry," J. Biomol. Screen., vol. 16, no. 3, pp. 370-377, 2011.

[34] D. GODE, M. MARTIN, F. STEINER, C. HUBER, and D. VOLMER, "Rapid narrow band elution for on-line SPE using a novel solvent plug injection technique," Anal. Bioanal. Chem., vol. 404, no. 2, pp. 433-445, 2012.

[35] N. BLOW, "Lab automation: Tales along the road to automation," Nat. Methods, vol. 5, no. 1, pp. 109-112, 2008.

[36] D. HOOK, "Case study of the automation options and decisions made in implementing a high-throughput cell based screen using the FLIPR(TM)," J. Autom. Methods Manage. Chem., vol. 22, no. 5, pp. 139-142, 2000.

[37] R. MARKIN and S. WHALEN, "Laboratory automation: Trajectory, technology, and tactics," Clin. Chem., vol. 46, no. 5, pp. 764-771, 2000.

[38] D. CHELIUS, G. XIAO, A. NICHOLS, A. VIZEL, B. HE, T. DILLON, D. REHDER, G. PIPES, E. KRAFT, A. OROSKA, M. TREUHEIT, and P. BONDARENKO, "Automated tryptic digestion procedure for HPLC/MS/MS peptide mapping of immunoglobulin gamma antibodies in pharmaceutics," J. Pharm. Biomed. Anal., vol. 47, no. 2, pp. 285-294, 2008.

[39] P. MIRSKY, A. CHATTERJEE, A. SAUER-BUDGE, and A. SHARON, "An automated, parallel processing approach to biomolecular sample preparation," J. Lab. Autom., vol. 17, no. 2, pp. 116-124, 2012.

[40] A. WATT, D. MORRISON, K. LOCKER, and D. EVANS, "Higher throughput bioanalysis by automation of a protein precipitation assay using a 96-well format with detection by LC-MS/MS," Anal. Chem., vol. 72, no. 5, pp. 979-984, 2000.

[41] X. TONG, J. WANG, S. ZHENG, and J. PIVNICHNY, "High-throughput pharmacokinetics screen of VLA-4 antagonists by LC/MS/MS coupled with automated solid-phase extraction sample preparation," J. Pharm. Biomed. Anal., vol. 35, no. 4, pp. 867-877, 2004.

[42] C. APOSTOLOU, Y. DOTSIKAS, C. KOUSOULOS, and Y. LOUKAS, "Quantitative determination of donepezil in human plasma by liquid chromatography/tandem mass spectrometry employing an automated liquid-liquid extraction based on 96-well format plates. Application to a bioequivalence study," J. Chromatogr. B, vol. 848, no. 2, pp. 239-244, 2007.

[43] M. ZARAGOZA-SUNDQVIST, H. ERIKSSON, M. ROHMAN, and P. GREASLEY, "High-quality cost-effective compound management support for HTS," J. Biomol. Screen., vol. 14, no. 5, pp. 509-514, 2009.

[44] M. CAPELLE and T. ARVINTE, "High-throughput formulation screening of therapeutic proteins," Drug Discov. Today, vol. 5, no. 2-3, pp. e71-e79, 2009.

[45] J. ALSENZ, E. MEISTER, and E. HAENEL, "Development of a partially automated solubility screening (PASS) assay for early drug development," J. Pharm. Sci., vol. 96, no. 7, pp. 1748-1762, 2007.

[46] J. KIM, J. FLICK, M. REIMER, R. RODILA, P. WANG, J. ZHANG, Q. JI, and T. EL-SHOURBAGY, "LC-MS/MS determination of 2-(4-((2-(2S,5R)-2-Cyano-5-ethynyl-1- pyrrolidinyl)-2-oxoethylamino)-4-methyl-1-piperidinyl)-4- pyridinecarboxylic acid (ABT-279) in dog plasma with high-throughput protein precipitaion sample preparation," Biomed. Chromatogr., vol. 21, no. 11, pp. 1118-1126, 2007.

[47] Y. ALELYUNAS, L. PELOSI-KILBY, and R. SPREEN, "Application of an automated multi-pH and multi-temperature platform for accelerated solution stability testing in supporting drug discovery," Anal. Methods, vol. 3, no. 10, pp. 2392-2399, 2011.

[48] Q. ZHANG, M. DING, B. WANG, H. PANG, and W. HE, "Application of DNA extraction automation workstation in the forensic area," Chinese J. Forensic Med., vol. 25, no. 1, pp. 40-42, 2010.

[49] W. XIE, W. MULLETT, C. MILLER-STEIN, and J. PAWLISZYN, "Automation of in-tip solid-phase microextraction in 96-well format for the determination of a model drug compound in human plasma by liquid chromatography with tandem mass spectrometric detection," J. Chromatogr. B, vol. 877, no. 4, pp. 415-420, 2009.

[50] A. MARCONI, M. BALESTRIERI, G. COMASTRI, F. PULVIRENTI, W. GENNARI, S. TAGLIAZUCCHI, M. PECORARI, V. BORGHI, D. MARRI, and M. ZAZZI, "Evaluation of the Abbott Real-Time HIV-1 quantitative assay with dried blood spot specimens," Clin. Microbiol. Infec., vol. 15, no. 1, pp. 93-97, 2009.

[51] E. FARIAS-HESSON, J. ERIKSON, A. ATKINS, P. SHEN, R. DAVIS, C. SCHARFE, and N. POURMAND, "Semi-automated library preparation for high-throughput DNA sequencing platforms," J. Biomed. Biotechnol., vol. 2010, 2010.

[52] T. WU and Y. ZHOU, "Intelligent Automation Platform for Bioprocess Development," Comput. Aided Chem. Eng., vol. 30, pp. 1227-1231, 2012.

[53] L. BARKAWI, Y. TAM, J. TILLMAN, B. PEDERSON, J. CALIO, H. AL-AMIER, M. EMERICK, J. NORMANLY, and J. COHEN, "A high-throughput method for the quantitative analysis of indole-3-acetic acid and other auxins from plant tissue," Anal. Biochem., vol. 372, no. 2, pp. 177-188, 2008.

[54] S. BRIEM, S. MARTINSSON, T. BUETERS, and E. SKOGLUND, "Combined approach for high-throughput preparation and analysis of plasma samples from exposure studies," Rapid Commun. Mass Spectrom., vol. 21, no. 13, pp. 1965-1972, 2007.

[55] H. JIANG, Z. OUYANG, J. ZENG, L. YUAN, N. ZHENG, M. JEMAL, and M. ARNOLD, "A user-friendly robotic sample preparation program for fully automated biological sample pipetting and dilution to benefit the regulated bioanalysis," J. Lab. Autom., vol. 17, no. 3, pp. 211-221, 2012.

[56] J. PALANDRA, D. WELLER, G. HUDSON, J. LI, S. OSGOOD, E. HUDSON, M. ZHONG, L. BUCHHOLZ, and L. COHEN, "Flexible automated approach for quantitative liquid handling of complex biological samples," Anal. Chem., vol. 79, no. 21, pp. 8010-8015, 2007.

[57] C. JI, J. WALTON, Y. SU, and M. TELLA, "Simultaneous determination of plasma epinephrine and norepinephrine using an integrated strategy of a fully automated protein precipitation technique, reductive ethylation labeling and UPLC-MS/MS," Anal. Chim. Acta, vol. 670, no. 1-2, pp. 84-91, 2010.

[58] S. KNOX, J. HARRIS, L. CALTON, and A. WALLACE, "A simple automated solid-phase extraction procedure for measurement of 25-hydroxyvitamin D 3 and D 2 by liquid chromatography-tandem mass spectrometry," Ann. Clin. Biochem., vol. 46, no. 3, pp. 226-230, 2009.

[59] Z. KUKLENYIK, X. YE, L. NEEDHAM, and A. CALAFAT, "Automated solid-phase extraction approaches for large scale biomonitoring studies," J. Chromatogr. Sci., vol. 47, no. 1, pp. 12-18, 2009.

[60] M. BLADERGROEN, R. DERKS, S. NICOLARDI, B. DE VISSER, S. VAN BERLOO, Y. VAN DER BURGT, and A. DEELDER, "Standardized and automated solid-phase extraction procedures for high-throughput proteomics of body fluids," J. Proteomics, vol. 77, pp. 144-153, 2012.

[61] Y. DOTSIKAS, C. APOSTOLOU, C. KOUSOULOS, G. TSATSOU, and Y. LOUKAS, "An improved high-throughput liquid chromatographic/tandem mass spectrometric method for terbinafine quantification in human plasma, using automated liquid-liquid extraction based on 96-well format plates," Biomed. Chromatogr., vol. 21, No. 2, pp. 201-208, 2007.

[62] Y. DOTSIKAS, C. APOSTOLOU, S. SOUMELAS, F. KOLOCOURI, A. ZIAKA, C. KOUSOULOS, and Y. LOUKAS, "Validation of a novel, fully automated high throughput high-performance liquid chromatographic/tandem mass spectrometric method for quantification of pantoprazole in human plasma," J. AOAC Int., vol. 93, no. 4, pp. 1129-1136, 2010.

[63] J. TWEED, Z. GU, H. XU, G. ZHANG, P. NOURI, M. LI, and R. STEENWYK, "Automated sample preparation for regulated bioanalysis: An integrated multiple assay extraction platform using robotic liquid handling," Bioanalysis, vol. 2, no. 6, pp. 1023-1040, 2010.

[64] H. NORDBERG, G. JERNDAL, and R. THOMPSON, "Direct injection of lipophilic compounds in the organic phase from liquid-liquid extracted plasma samples onto a reversed-phase column," Bioanalysis, vol. 3, no. 17, pp. 1963-1973, 2011.

[65] S. IDDER, L. LEY, P. MAZELLIER, and H. BUDZINSKI, "Quantitative on-line preconcentration-liquid chromatography coupled with tandem mass spectrometry method for the determination of pharmaceutical compounds in water," Anal. Chim. Acta, vol. 805, pp. 107-115, 2013.

[66] H. VLČKOVÁ, J. JANÁK, T. GOTTVALD, F. TREJTNAR, P. SOLICH, and L. NOVÁKOVÁ, "How to address the sample preparation of hydrophilic compounds: Determination of entecavir in plasma and plasma ultrafiltrate with novel extraction sorbents," J. Pharm. Biomed. Anal., vol. 88, pp. 377-344, 2014.

[67] H. FLEISCHER and K. THUROW, "Determination of total mercury content in wood materials, part 2: Icp-ms-a multielement method," Am. Lab., vol. 45, no. 8, pp. 6-11, 2013.

[68] S. VERMEIR, B. NICOLAÏ, K. JANS, G. MAES, and J. LAMMERTYN, "High-throughput microplate enzymatic assays for fast sugar and acid quantification in apple and tomato," J. Agr. Food Chem., vol. 55, no. 9, pp. 3240-3248, 2007.

[69] Y. BANG-CE, L. SONGYANG, Z. PENG, and L. XIAO-HONG, "Simultaneous detection of sulfamethazine, streptomycin, and tylosin in milk by microplate-array based SMM-FIA," Food Chem., vol. 106, no. 2, pp. 797-803, 2008.

[70] C. ZU and J. WANG, "Tumor-colonizing bacteria: A potential tumor targeting therapy," Crit. Rev. Microbiol., vol. 40, no. 3, pp. 225-235, 2014.

[71] J. NAHALKA, "Protein-RNA recognition: Cracking the code," J. Theor. Biol., vol. 343, pp. 9-15, 2014.

[72] E. SABBIONI, S. FORTANER, M. FARINA, R. DEL TORCHIO, I. OLIVATO, C. PETRARCA, G. BERNARDINI, R. MARIANI-COSTANTINI, S. PERCONTI, L. DI GIAMPAOLO, R. GORNATI, and M. DI GIOACCHINO, "Cytotoxicity and morphological transforming potential of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts: An in vitro model," Nanotoxicology, vol. 8, no. 4, pp. 455-464, 2014.

[73] J. WAHRHEIT and E. HEINZLE, "Quenching methods for the analysis of intracellular metabolites," Methods Mol. Biol., vol. 1104, pp. 211-221, 2014.

[74] A. HALDAR, R. PAUL, S. PAN, A. MITRA, C. BISWAS, D. MAJUMDAR, S. GHOSH, N. SINGH, S. NGACHAN, K. BAJURBHORUEA, and B. PRAKASH, "Validation of a simple, sensitive enzyme immunoassay (EIA) for the determination of caprine plasma LH," Small Ruminant Res., vol. 84, no. 1-3, pp. 22-27, 2009.

[75] S. H. LÓPEZ, M. J. GÓMEZ, M. D. HERNANDO, and A. R. FERNÁNDEZ-ALBA, "Automated dynamic headspace followed by a comprehensive two-dimensional gas chromatography full scan time-of-flight mass spectrometry method for screening of volatile organic compounds (VOCs) in water," Anal. Methods, vol. 5, no. 5, pp. 1165-1177, 2013.

[76] A. ABERL and M. COELHAN, "Determination of sulfur dioxide in wine using headspace gas chromatography and electron capture detection," Food Addit. Contam., vol. 30, no. 2, pp. 226-233, 2013.

[77] A. TOLOKÁN, L. GÖDÖRHÁZY, V. HORVÁTH, A. HRABÉCZY-PÁLL, Z. NIEGREISZ, E. KOCSI, G. HORVAI, I. KLEBOVICH, and K. BALOGH-NEMES, "Economic approach to robotic sample pretreatment in high-performance liquid chromatography," J. Chromatogr. A, vol. 771, no. 1-2, pp. 35-43, 1997.

[78] M. SADOWSKY, W. KOSKINEN, J. SEEBINGER, B. BARBER, and E. KANDELER, "Automated robotic assay of phosphomonoesterase activity in solis," Soil Sci. Soc. Am. J., vol. 70, no. 2, pp. 378-381, 2006.

[79] C. BRINKMANN and A. EISENTRAEGER, "Completely automated short-term genotoxicity testing for the assessment of chemicals and characterisation of contaminated soils and waste waters," Environ. Sci. Pol. Res. Int., vol. 50, no. 3, pp. 211-217, 2008.

[80] I. TSINA, F. CHU, K. HAMA, M. KALOOSTIAN, Y. TAM, T. TARNOWSKI, and B. WONG, "Manual and automated (robotic) high-performance liquid chromatography methods for the determination of mycophenolic acid and its glucuronide conjugate in human plasma," J. Chromatogr. B, vol. 675, no. 1, pp. 119-129, 1996.

[81] C. JOHNSON, C. CHRISTIANSON, C. SHEAFF, D. LAINE, J. ZIMMER, and S. NEEDHAM, "Use of conventional bioanalytical devices to automate DBS extractions in liquid-handling dispensing tips," Bioanalysis, vol. 3, no. 20, pp. 2303-2310, 2011.

[82] C. APOSTOLOU, C. KOUSOULOS, Y. DOTSIKAS, G. SOUMELAS, F. KOLOCOURI, A. ZIAKA, and Y. LOUKAS, "An improved and fully validated LC-MS/MS method for the simultaneous quantification of simvastatin and simvastatin acid in human plasma," J. Pharm. Biomed. Anal., vol. 46, no. 4, pp. 771-779, 2008.

[83] C. HELLMUTH, M. WEBER, B. KOLETZKO, and W. PEISSNER, "Nonesterified fatty acid determination for functional lipidomics: Comprehensive ultrahigh performance liquid chromatography-tandem mass spectrometry quantitation, qualification, and parameter prediction," Anal. Chem., vol. 84, no. 3, pp. 1483-1490, 2012.

[84] C. APOSTOLOU, Y. DOTSIKAS, C. KOUSOULOS, G. TSATSOU, F. COLOCOURI, G. SOUMELAS, and Y. LOUKAS, "Application of a semi-automated 96-well format solid-phase extraction, column-switching, fluorescence detection protocol for the determination of alendronate in human urine samples obtained from a bioequivalence study," J. Pharm. Biomed. Anal., vol. 43, no. 3, pp. 1151-1155, 2007.

[85] A. EL-BEQQALI, A. KUSSAK, and M. ABDEL-REHIM, "Determination of dopamine and serotonin in human urine samples utilizing microextraction online with liquid chromatography/electrospray tandem mass spectrometry," J. Sep. Sci., vol. 30, no. 3, pp. 421-424, 2007.

[86] D. WHIGAN and A. SCHUSTER, "Manual and automated determination of D-arabinofuranosyl-E-5-(2-bromovinyl)uracil and its metabolite (E)-5-(2-bromovinyl)uracil in urine," J. Chromatogr. B, vol. 664, no. 2, pp. 357-363, 1995.

[87] R. SMITH, "Before the injection - Modern methods of sample preparation for separation techniques," J. Chromatogr. A, vol. 1000, no. 1-2, pp. 3-27, 2003.

[88] N. VAN DE MERBEL, "Membrane-based sample preparation coupled on-line to chromatography or electrophoresis," J. Chromatogr. A, vol. 856, no. 1-2, pp. 55-82, 1999.

[89] M. SEIPP, M. HERRMANN, and C. WITTWER, "Automated DNA extraction, quantification, dilution, and PCR preparation for genotyping by high-resolution melting," J. Biomol. Tech., vol. 21, no. 4, pp. 163-166, 2010.

[90] M. BREVNOV, J. MUNDT, J. BENFIELD, L. TREAT-CLEMONS, G. KALUSCHE, J. MEREDITH, G. PORTER, M. FURTADO, and J. SHEWALE, "Automated extraction of DNA from forensic sample types using the PrepFiler automated forensic DNA extraction kit," J. Assoc. Lab. Autom., vol. 14, no. 5, pp. 294-302, 2009.

[91] J. VAN DE BILT, J. DERKS, and J. JANSE, "Automated, high-throughput immunofluorescence staining: A new approach," Eur. J. Plant Pathol., vol. 120, no. 1, pp. 91-96, 2008.

[92] J. MA, J. SHI, H. LE, R. CHO, J. HUANG, S. MIAO, and B. WONG, "A fully automated plasma protein precipitation sample preparation method for LC-MS/MS bioanalysis," J. Chromatogr. B, vol. 862, no. 1-2, pp. 219-226, 2008.

[93] G. THEODORIDIS, C. ZACHARIS, and A. VOULGAROPOULOS, "Automated sample treatment by flow techniques prior to liquid-phase separations," J. Biochem. Biophys. Methods, vol. 70, no. 2, pp. 243-252, 2007.

[94] M. CHIU, W. LAWI, S. SNYDER, P. WONG, J. LIAO, and V. GAU, "Matrix effects - A challenge toward automation of molecular analysis," J. Assoc. Lab. Autom., vol. 15, no. 3, pp. 233-242, 2010.

[95] M. JEMAL, Y. XIA, and D. WHIGAN, "The use of high-flow high performance liquid chromatography coupled with positive and negative ion electrospray tandem mass spectrometry for quantitative bioanalysis via direct injection of the plasma/serum samples," Rapid Commun. Mass Spectrom., vol. 12, no. 19, pp. 1389-1399, 1998.

[96] M. JEMAL, Z. OUYANG, Y. XIA, and M. POWELL, "A versatile system of high-flow high performance liquid chromatography with tandem mass spectrometry for rapid direct-injection analysis of plasma samples for quantitation of a β-lactam drug candidate and its open-ring biotransformation product," Rapid Commun. Mass Spectrom., vol. 13, no. 14, pp. 1462-1471, 1999.

[97] M. FERNÁNDEZ, S. WILLE, N. SAMYN, M. WOOD, M. LÓPEZ-RIVADULLA, and G. DE BOECK, "High-throughput analysis of amphetamines in blood and urine with online solid-phase extraction-liquid chromatography-tandem mass spectrometry," J. Anal. Toxicol., vol. 33, no. 9, pp. 578-587, 2009.

[98] X. CHEN, J. FRANKE, K. ENSING, J. WIJSBEEK, and R. DE ZEEUW, "Pitfalls and solutions in the development of a fully automated solid- phase extraction method for drug screening purposes in plasma and whole blood," J. Anal. Toxicol., vol. 17, no. 7, pp. 421-426, 1993.

[99] J. ALLANSON, R. BIDDLECOMBE, A. JONES, and S. PLEASANCE, "The use of automated solid phase extraction in the '96 well' format for high throughput bioanalysis using liquid chromatography coupled to tandem mass spectrometry," Rapid Commun. Mass Spectrom., vol. 10, no. 7, pp. 811-816, 1996.

[100] T. PARKER III, D. WRIGHT, and D. ROSSI, "Design and evaluation of an automated solid-phase extraction method development system for use with biological fluids," Anal. Chem., vol. 68, no. 14, pp. 2437-2441, 1996.

[101] J. JANISZEWSKI, R. SCHNEIDER, K. HOFFMASTER, M. SWYDEN, D. WELLS, and H. FOUDA, „Automated sample preparation using membrane microtiter extraction for bioanalytical mass spectrometry," Rapid Commun. Mass Spectrom., vol. 11, no. 9, pp. 1033-1037, 1997.

[102] R. PLUMB, R. GRAY, and C. JONES, "Use of reduced sorbent bed and disk membrane solid-phase extraction for the analysis of pharmaceutical compounds in biological fluids, with applications in the 96-well format," J. Chromatogr. B, vol. 694, no. 1, pp. 123-133, 1997.

[103] H. SIMPSON, A. BERTHEMY, D. BUHRMAN, R. BURTON, J. NEWTON, M. KEALY, D. WELLS, and D. WU, "High throughput liquid chromatography/mass spectrometry bioanalysis using 96-well disk solid phase extraction plate for the sample preparation," Rapid Commun. Mass Spectrom., vol. 12, no. 2, pp. 75-82, 1998.

[104] S. STEINBORNER and J. HENION, "Liquid-liquid extraction in the 96-well plate format with SRM LC/MS quantitative determination of methotrexate and its major metabolite in human plasma," Anal. Chem., vol. 71, no. 13, pp. 2340-2345, 1999.

[105] J. COMLEY, "Secondary screening workstations - The emergence of compact sreening workstations that works!," Drug Discov. World, vol. 3, no. 4, pp. 31-40, 2002.

[106] D. DREXLER, K. EDINGER, and J. MONGILLO, "Improvements to the sample manipulation design of a LEAP CTC HTS PAL autosampler used for high-throughput qualitative and quantitative liquid chromatography-mass spectrometry assays," J. Assoc. Lab. Autom, vol. 12, no. 3, pp. 152-156, 2007.

[107] P. PROFOS and T. PFEIFER, Handbuch der industriellen Messtechnik, München Wien: R. Oldenbourg Verlag, 1994.

[108] M. OTTO, Analytische Chemie, Weinheim: VCH Verlagsgesellschaft, 1995.

[109] J. HOFFMANN, Taschenbuch der Messtechnik, Leipzig: Fachbuchverlag, 1998.

[110] M. KRACHLER, "Microwave digestion methods for the determination of trace elements in brain and liver samples by inductively coupled plasma mass spectrometry," Fresenius' J. Anal. Chem., vol. 355, no. 2, pp. 120-128, 1996.

[111] J. DJEDJIBEGOVIC, T. LARSSEN, A. SKRBO, A. MARJANOVIĆ, and M. SOBER, "Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS)," Food Chem., vol. 131, no. 2, pp. 469-476, 2012.

[112] F. ANTES, F. DUARTE, M. MESKO, M. NUNES, V. PEREIRA, E. MÜLLER, V. DRESSLER, and E. FLORES, "Determination of toxic elements in coal by ICP-MS after digestion using microwave-induced combustion," Talanta, vol. 83, no. 2, pp. 364-369, 2010.

[113] K. LINGE and K. JARVIS, "Quadrupole ICP-MS: Introduction to instrumentation, measurement techniques and analytical capabilities," Geostand. Geoanal. Res., vol. 33, no. 4, pp. 445-467, 2009.

[114] R. HOUK, "Inductively coupled argon plasma as an ion source for mass spectrometric determination of trace elements," Anal. Chem., vol. 52, no. 14, pp. 2283-2289, 1980.

[115] W. RICHLY, Mess- und Analyseverfahren, Würzburg: Vogel Buchverlag, 1992.

[116] F. W. FIFIELD and D. KEALEY, Principles and Practice of Analytical Chemistry, London: Blackie Acadamic and Professional, 1995.

[117] C. GIACOVAZZO, Fundamentals of Crystallography, Oxford University Press, 3rd ed. 2011.

[118] J. BÖCKER, Chromatographie, Würzburg: Vogel Buchverlag, 1997.

[119] H. EL-SHARIF, Q. PHAN, and S. REDDY, "Enhanced selectivity of hydrogel-based molecularly imprinted polymers (HydroMIPs) following buffer conditioning," Anal. Chim. Acta, vol. 809, pp. 155-161, 2014.

[120] M. MARINOVA, C. ARTUSI, L. BRUGNOLO, G. ANTONELLI, M. ZANINOTTO, and M. PLEBANI, „Immunosuppressant therapeutic drug monitoring by LC-MS/MS: Workflow optimization through automated processing of whole blood samples," Clin. Biochem., vol. 46, no. 16-17, pp. 1723-1727, 2013.

[121] J. COMLEY, „Serial vs direct dilution - Time to apply new thinking to IC50 determination and dose-response analysis?," Drug Discov. World, vol. 8, no. 2, pp. 36-37+39+41+43-44+46-50, 2007.

[122] L. MAYR and D. BOJANIC, "Novel trends in high-throughput screening," Curr. Opin. Pharmacol., vol. 9, no. 5, pp. 580-588, 2009.

[123] T. FITZGERALD and R. MCQUALTER, "The quantitative real-time polymerase chain reaction for the analysis of plant gene expression," Methods Mol. Biol., vol. 1099, pp. 97-115, 2014.

[124] X. AMOURETTI, "Impact of HTS on multidetection microplate readers and benefits for life science research laboratories," Am. Biotechnol. Lab., vol. 24, no. 8, p. 16, 2006.

[125] X. AMOURETTI, "Multidetection microplate readers: Filter-based, monochromator-based...or both?," Am. Biotechnol. Lab. , vol. 25, no. 7, pp. 22-23, 2007.

[126] M. HENSHALL and P. HELD, "Assay: Hybrid bridges the microplate reader gap.," Genet. Eng. Biotechnol. News, vol. 28, no. 2, pp. 1-2, 2008.

[127] T. EISSA, E. GONZÁLEZ-BURGOS, M. CARRETERO, and M. GÓMEZ-SERRANILLOS, "Biological activity of HPLC-characterized ethanol extract from the aerial parts of Haplophyllum tuberculatum," Pharm. Biol., vol. 52, no. 2, pp. 151-156, 2014.

[128] O. SHOUAKAR-STASH, S. FRAPE, and R. DRIMMIE, "Determination of bromine stable isotopes using continuous-flow isotope ratio mass spectrometry," Anal. Chem., vol. 77, no. 13, pp. 4027-4033, 2005.

[129] M. ESPINO and J. LEON, "Determination of tri-heptabrominated diphenyl ethers in indoor dust by chromatoprobe injection and gas chromatography-mass spectrometry," Microchem. J., vol. 114, pp. 1-7, 2014.

[130] S. FERREIRA, W. DOS SANTOS, I. DOS SANTOS, M. JUNIOR, L. SILVA, U. BARBOSA, F. DE SANTANA, and A. QUEIROZ, "Strategies of sample preparation for speciation analysis of inorganic antimony using hydride generation atomic spectrometry," Microchem. J., vol. 114, pp. 22-31, 2014.

[131] K. JENKINS, R. ANGELES, M. QUINTOS, R. XU, D. KASSEL, and R. ROURICK, "Automated high throughput ADME assays for metabolic stability and cytochrome P450 inhibition profiling of combinatorial libraries," J. Pharm. Biomed. Anal., vol. 34, no. 5, pp. 989-1004, 2004.

[132] M. PLEBANI, "Laboratory medicine: Value for patients is the goal [7]," Clin. Chem., vol. 53, no. 10, pp. 1873-1874, 2007.

[133] K. JANSEN, D. HIGGINS, D. WOLF, and W. BATES, "Laboratory automation. A strategic step in laboratory integration," Lab. Inform. Manage., vol. 17, no. 2, pp. 171-180, 1992.

[134] M. VACLAVIKOVA, Z. DZUMAN, O. LACINA, M. FENCLOVA, Z. VEPRIKOVA, M. ZACHARIASOVA, and J. HAJSLOVA, „Monitoring survey of patulin in a variety of fruit-based products using a sensitive UHPLC-MS/MS analytical procedure," Food Control, vol. 47, pp. 577-584, 2015.

[135] J. P. ALLANSON, R. A. BIDDLECOMBE, A. E. JONES, and S. PLEASANCE, "The Use of Automated Solid Phase Extraction in the '96 well' Format for High Throughput Bioanalysis using Liquid Chromatography Coupled to Tandem Mass Spectrometry," Rapid Commun. Mass Spectrom, pp. 811–816, 1996.

[136] D. T. ROSSI and N. ZHANG, "Automating solid-phase extraction: Current aspecs and future prospekts," J. Chromatogr. A, vol. 885, pp. 97–113, 2000.

[137] „Waters," [Online]. Available: http://www.waters.com/waters/en_US/Waters-Positive-Pressure-96-Processor/nav.htm?cid=10161512. [9 January 2015].

[138] M. STANGEGAARD, C. TOMAS, A. HANSEN, R. FRANK-HANSEN, C. BØRSTING, and N. MORLING, "Biomek-3000 and GenPlex SNP genotyping in forensic genetics," J. Assoc. Lab. Autom., vol. 13, no. 5, pp. 297-303, 2008.

[139] J. DAWSON, "High-throughput screening at biosafety level 2 with high flexibility: MicroStar BSL2," J. Assoc. Lab. Autom. , vol. 14, no. 3, pp. 133-140, 2009.

[140] B. DE JONG, M. ANTONIE, A. HARDON, D. VAN DEN BERGEN, and A. KNEPPERS, "Successful validation of a fully automated sample lysis workstation.," Forensic Sci. Int., vol. 4, no. 1, pp. e93-e94, 2013.

[141] H. FLEISCHER and K. THUROW, „Rapid enantiomeric excess determination of D- and L-proline using electrospray ionization-mass spectrometry," Am. Lab., vol. 43, no. 9, pp. 32-36, 2011.

[142] W. HORWITZ, "Evaluation of analytical methods used for regulation of foods and drugs," Anal. Chem., vol. 54, no. 1, pp. 67A-76A, 1982.

[143] M. HOJDOVÁ, T. NAVRÁTIL, J. ROHOVEC, K. ŽÁK, A. VANĚK, V. CHRASTNÝ, R. BAČE, and M. SVOBODA, "Changes in mercury deposition in a mining and smelting region as recorded in tree rings," Water, Air, Soil Pollut., vol. 216, no. 1-4, pp. 73-82, 2011.

[144] H. CHENG and Y. HU, "Understanding the paradox of mercury pollution in China: High concentrations in environmental matrix yet low levels in fish on the market," Environ. Sci. Technol., vol. 46, no. 9, pp. 4695-4696, 2012.

[145] D. KEIL, J. BERGER-RITCHIE, and G. MCMILLIN, "Testing for toxic elements: A focus on arsenic, cadmium, lead, and mercury," Lab. Med., vol. 42, no. 12, pp. 735-742, 2011.

[146] S. CHATTERJEE, A. PILLAI, and V. GUPTA, "Spectrophotometric determination of mercury in environmental sample and fungicides based on its complex with o-carboxy phenyl diazoamino p-azobenzene," Talanta, vol. 57, no. 3, pp. 461-465, 2002.

[147] G. JARZYŃSKA and J. FALANDYSZ, "The determination of mercury in mushrooms by CV-AAS and ICP-AES techniques," J. Environ. Sci. Health., Part A, vol. 46, no. 6, pp. 569-573, 2011.

[148] J. WUILLOUD, R. WUILLOUD, J. SALONIA, R. OLSINA, and L. MARTINEZ, "Modified gas-liquid separator for the determination of mercury with cold vapor flow injection-inductively coupled plasma optical emission spectrometry," J. Anal. Chem., vol. 57, no. 9, pp. 799-801, 2002.

[149] D. LAMBROPOULOU and T. ALBANIS, "Liquid-phase micro-extraction techniques in pesticide residue analysis," J. Biochem. Biophys. Methods, vol. 70, no. 2, pp. 195-228, 2007.

[150] J. MOREDA-PIÑEIRO, E. ALONSO-RODRÍGUEZ, P. LÓPEZ-MAHÍA, S. MUNIATEGUI-LORENZO, D. PRADA-RODRÍGUEZ, A. MOREDA-PIÑEIRO, and P. BERMEJO-BARRERA, "Determination of major and trace elements in human scalp hair by pressurized-liquid extraction with acetic acid and inductively coupled plasma-optical-emission spectrometry," Anal. Bioanal. Chem., vol. 388, no. 2, pp. 441-449, 2007.

[151] J. QUINTANA, M. MIRO, J. ESTELA, and V. CERDÀ, "Automated on-line renewable solid-phase extraction-liquid chromatography exploiting multisyringe flow injection-bead injection lab-on-valve analysis," Anal. Chem., vol. 78, no. 8, pp. 2832-2840, 2006.

[152] H. TAN, D. SEMIN, M. WACKER, and J. CHEETHAM, "An automated screening assay for determination of aqueous equilibrium solubility enabling SPR study during drug lead optimization," J. Assoc. Lab. Autom, vol. 10, no. 6, pp. 364-373, 2005.

[153] H. FLEISCHER, E. VORBERG, and K. THUROW, "Determination of total mercury content in wood materials-Part 3: Miniaturization using ICP-MS," Am. Lab., vol. 46, no. 6, 2014.

[154] E. VORBERG, H. FLEISCHER, S. JUNGINGER, N. STOLL, and K. THUROW, "Automated sample preparation for mercury analysis in wood materials," Proceeding of the 5th IMEKO TC19 Symposium on Environmental Instrumentation and Measurements, pp. 66-70, Chemnitz (D), 2014.

[155] M. TOFANELLI, L. PARDINI, M. BORRINI, F. BARTOLI, A. BACCI, A. D'ULIVO, E. PITZALIS, M.C. MASCHERPA, S. LEGNAIOLI, G. LORENZETTI, S. PAGNOTTA, G. DE HOLANDA CAVALCANTI, M. LEZZERINI, and V. PALLESCHI, "Spectroscopic analysis of bones for forensic studies," Spectrochim. Acta, Part B, vol. 99, pp. 70-75, 2014.

[156] A. HANĆ, A. OLSZEWSKA, and D. BARAŁKIEWICZ, "Quantitative analysis of elements migration in human teeth with and without filling using LA-ICP-MS," Microchem. J., vol. 110, pp. 61-69, 2013.

[157] S. SIVAKUMAR, C. KHATIWADA, and J. SIVASUBRAMANIAN, "Studies the alterations of biochemical and mineral contents in bone tissue of mus musculus due to aluminum toxicity and the protective action of desferrioxamine and deferiprone by FTIR, ICP-OES, SEM and XRD techniques," Spectrochim. Acta, Part A, vol. 126, pp. 59-67, 2014.

[158] H. FLEISCHER, E. VORBERG, M. WARKENTIN, and K. THUROW, "Qualitative and quantitative determination of methacrylates in dental filling materials," Am. Lab., vol. 46, no. 2, 2014.

[159] H. FLEISCHER, E. VORBERG, M. WARKENTIN, D. BEHREND, and K. THUROW, "Determination of calcium and phosphor in bones using microwave digestion and ICP-MS," Proceeding of the 5th IMEKO TC19 Symposium on Environmental Instrumentation and Measurements, pp. 94-99, Chemnitz (D), 2014.

[160] X. NING, M. LIN, L. SHEN, J. ZHANG, J. WANG, Y. WANG, Z. YANG, and J. LIU, "Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants," Environ. Res., vol. 132, pp. 112-118, 2014.

[161] S. ANDLEEB, N. ATIQ, A. PARMAR, G. ROBSON, and S. AHMED, "An HPLC method development for the assessment of degradation products of anthraquinone dye," Environ. Monit. Assess., vol. 176, no. 1-4, pp. 597-604, 2011.

[162] A. TANG, L. WANG, and R. ZHOU, "Gibbs energies of formation of chlorinated benzoic acids and benzoates and application to their reductive dechlorination," THEOCHEM, vol. 960, no. 1-3, pp. 31-39, 2010.

[163] V. BESSONNEAU, B. BOJKO, and J. PAWLISZYN, "In situ chemical exploration of underwater ecosystems with microsampling/enrichment device," J. Chromatogr. A, vol. 1328, pp. 113-117, 2014.

[164] A. GÖREN, G. BILSEL, A. ŞIMŞEK, M. BILSEL, F. AKC¸ADA1, K. TOPAL, and H. OZGEN, "HPLC and LC-MS/MS methods for determination of sodium benzoate and potassium sorbate in food and beverages: Performances of local accredited laboratories via proficiency tests in Turkey," Food Chem., vol. 175, pp. 273-279, 2015.

[165] E. VORBERG, H. FLEISCHER, S. NEUBERT, N. STOLL, and K. THUROW, "A highly flexible, automated system providing reliable sample preparation in element- and structure-specific measurements, " J. Lab. Autom., in press.

[166] M. HAUBITZ, "Acute and long-term toxicity of cyclophosphamide," Transplantationsmedizin: Organ der Deutschen Transplantationsgesellschaft, vol. 19, no. 2, pp. 26-31, 2007.

[167] C. LUTTERBECK, T. MACHADO, and K. KÜMMERER, "Photodegradation of the antineoplastic cyclophosphamide: A comparative study of the efficiencies of UV/H2O2, UV/Fe2+/H2O2 and UV/TiO2 processes," Chemosphere, vol. 120, pp. 538-546, 2015.

[168] F. RABII, P. SEGURA, P. FAYAD, and S. SAUVÉ, "Determination of six chemotherapeutic agents in municipal wastewater using online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry," Sci. Total Environ., vol. 487, no. 1, pp. 792-800., 2014.

[169] N. NEGREIRA, M. DE ALDA, and D. BARCELO´, "Cytostatic drugs and metabolites in municipal and hospital wastewaters in Spain: Filtration, occurrence, and environmental risk," Sci. Total Environ., vol. 497, pp. 68-77, 2014.

[170] C. SOTTANI, L. FUGNOLI, J. BUSINARO, P. POMA, A. RONCHI, G. MICOLI, and C. MINOIA, "Antineoplastic profile assessment for the evaluation of occupational exposure in hospital personnel involved in handling cytotoxic agents," Giornale italiano di medicina del lavoro ed ergonomia , vol. 34, no. 3, pp. 294-298, 2012.

[171] C. GALLERT, E. VORBERG, T. RODDELKOPF, S. JUNGINGER, H. FLEISCHER, and K. THUROW, "Evaluation of an automated solid-phase extraction method using positive pressure," Am. Lab., vol. 47, no. 2, pp. 28-33, 2015.

Experimental Description

Experimental Description for Element-Specific Measurements Providing Mercury Analysis

1. Sample Preparation

Sample preparation was performed using the Mars 5 Microwave Digestion System (CEM, Kamp-Lintfort, Germany) including the self-venting Xpress vessels (CEM, vol. 25ml, PFA). After adding 62.5mg of the referenced wood material ERM-CD100 (BAM, Berlin, Germany) and 2ml of aqua regia (hydrochloric acid : nitric acid, 3:1, both from Merck, Darmstadt, Germany) including the rhenium ICP Standard CertiPUR® (1.70344.0100) from Merck with a concentration of 1.25ppm, the samples were predigested for a period of 20min. One blank sample was included at every digestion run. Moreover, PTFE magnetic stirrers from VWR (Darmstadt, Germany) were used providing mixing and homogenization during the microwave digestion steps. The microwave digestion procedure included a temperature–time ramp of 20min. The temperature-time ramp started at room temperature and provided a final temperature of 180°C (356 °F) with a 25min holding-time at 600W. Finally, the samples were cooled down to room temperature. The vessels were uncapped and 1ml of the clear sample solution was transferred to a polypropylene vessel and filled with 11.5ml of ultra-pure water (Millipore, Merck).

Calibration solutions were prepared using diluted aqua regia (8% v/v), the mercury (1.70333.0100) and the rhenium ICP Standard CertiPUR® and the ICP multi-element standard IV CertiPUR® (1.11355.0100) from Merck**.** The mercury standard was diluted 1:100 using 80µl of the mercury standard (1,000ppm) and 7,920µl of the diluted aqua regia (8% v/v). Providing the standard solution, the diluted mercury standard (10ppm), the rhenium standard (1,000ppm), and the multi-element standard (1,000ppm) were mixed and diluted 1:1,000 using aqua regia (8% v/v). Calibration solutions were prepared as shown in the following table.

Table 1: Pipetting scheme of the calibration solutions for the mercury analysis

No.	c_{Hg} [µg/L]	c_{Re} / c_{Multi} [µg/L]	$V_{standard\ solution}$ [ml]	$V_{aqua\ regia}$ [ml]	V_{total} [ml]
4	1.00	100.00	1.000	9.000	10.000
3	0.50	50.00	0.500	9.500	10.000
2	0.10	10.00	0.100	9.900	10.000
1	0.05	5.00	0.050	9.950	10.000
Blank	-	-	-	50.000	50.000

Allowing for the correction of the sample introduction effects while using the peristaltic pump of the ICP-MS, a further ISTD was prepared using 25µl of the lutetium ICP Standard CertiPUR® (1.70330.0100) from Merck (1,000ppm) and 49,975µl of diluted aqua regia (5% v/v). Thus, the lutetium standard supplied a final concentration of 500µg/l. All solutions were prepared and stored in vessels made of polyethylene or polypropylene.

2. Automated Sample Preparation

The automated method was performed using the SAMI® Ex Workstation method "Pre-Digestion". In order to perform this method for one so-called family, the tray with two reservoirs for high concentrated acid (as depicted in chapter 6) supplied 16ml of aqua regia including the rhenium standard (c = 1,25ppm). During the method "Pre-Digestion" the high concentrated acid was added to the samples. The samples (for each 62.5mg of referenced wood material, ERM-CD100) were provided by the tray with six vessels for microwave digestion treatment (as depicted in chapter 6).

After microwave digestion, the SAMI® Ex Workstation method "Dilution" was performed on the automated system. The tray with six vessels for microwave digestion treatment was required in order to supply the already digested sample solutions. In addition the tray with two reservoirs for water supplied 20ml of ultra-pure water (Millipore, Merck). The tray with six analysis vessels (empty in the beginning) provided the analysis solutions finally. The home position of every tray is defined by the SAMI® method, but can be adjusted if necessary. The reservoir of the 2nd in-house designed liquid handler was filled with 200ml of ultra-pure water.

3. Analytical Measurement

Sample solution analysis was performed using the ICP-MS 7700x (Agilent Technologies, Waldbronn, Germany) including the following parameters: radiofrequency power – 1,550W; sample depth – 10mm; carrier gas – 0.65l/min; nebulizer pump – 0.10rps; spray chamber temperature – 13°C (55.4°F) and dilution gas – 0.40l/min. Argon Bip® ultra-pure gas (Air Products GmbH, Hattingen, Germany) was used as plasma, carrier, and dilution gas. Helium Bip® ultra-pure gas flow (Air Products GmbH) in the collision cell was used as follows: 0ml/min in NoGas-tuning mode; 4.3ml/min in He-tuning mode and 10ml/min in HEHe-tuning mode. Measurements were done using three replicates. Moreover, rinsing time was set to 60sec at 0.3rps using the nebulizer pump, followed by 30sec at 0.4rps. Sample introduction was performed using the ASX-500 autosampler (Cetac, Omaha, NE).

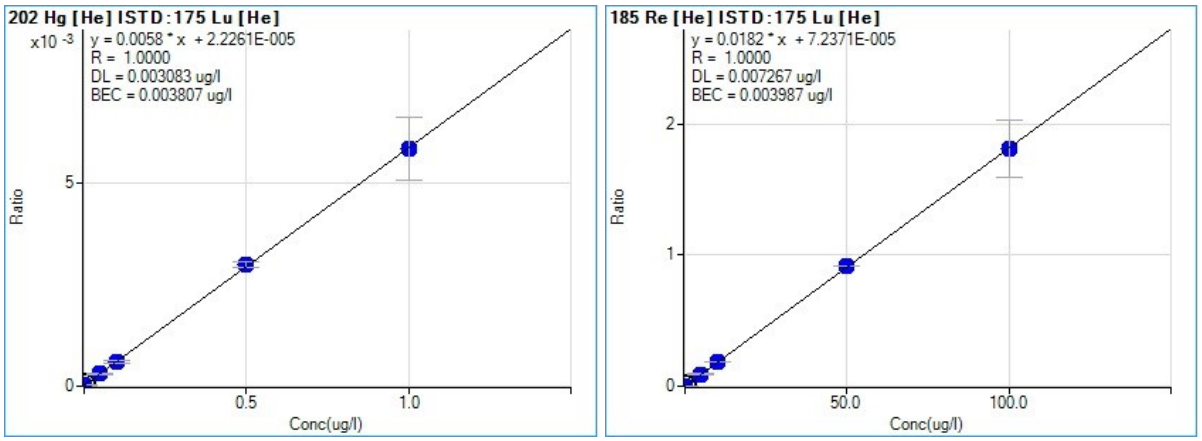


Fig 1: Calibration curves of mercury (Hg) and rhenium (Re)

Experimental Description for Element-Specific Measurements Providing Calcium and Phosphor Analysis

1. Sample Preparation

The solid bone samples were prepared using the Mars 5 Microwave Digestion System (CEM, Kamp-Lintfort, Germany) including the self-venting Xpress vessels (CEM, vol. 25ml, PFA). After adding 62.5mg of the referenced bone material (NIST, Gaithersburg, MD) and 2ml of the high concentrated nitric acid from Merck (Darmstadt, Germany) including the rhenium ICP Standard CertiPUR® (1.70344.0100) from Merck with a concentration of 50ppm, the samples were predigested for a period of 20min including one blank sample at every digestion run. PTFE magnetic stirrers from VWR (Darmstadt, Germany) were used providing mixing and homogenization during the microwave digestion steps.

The microwave digestion procedure included a temperature–time ramp of 20min. The temperature-time ramp started at room temperature and provided a final temperature of 180°C (356 °F) with a 25min holding-time at 600W. Finally, the samples were cooled down to room temperature. The vessels were uncapped and 1ml of the clear sample solution was transferred to a polypropylene vessel filled with 11.5ml of ultra-pure water (Millipore, Merck).

In order to reduce the acid content, the generation of polyatomic ions during the analysis process, and the resulting interferences, the sample solutions were diluted in the ratio of 1:8 using ultra-pure water.

Finally, the sample solutions were adjusted using nitric acid (1% v/v) and a further dilution step (1:10 v/v). Thus, the final sample solution supplied a dilution ratio of 1:1,000 including a final acid concentration of 1% (v/v).

Calibration solutions were prepared using diluted nitric acid (1% v/v), the calcium (1.70308.0100), the phosphor (1.70340.0100), the strontium (1.70354.0100), the magnesium (1.70331.0100), and the rhenium (1.70344.0100) ICP Standard CertiPUR® from Merck. Providing the standard solution, 1ml of the calcium standard (1,000ppm), 1ml of the phosphor standard (1,000ppm), 10µl of the strontium standard (1,000ppm), 10µl of the magnesium standard (1,000ppm), and 10µl of the rhenium standard (1,000ppm) were mixed and diluted up to 10ml using diluted nitric acid (1% v/v). Calibration solutions were prepared as shown in the following table.

Table 2: Pipetting scheme of the calibration solutions for the calcium and phosphor analysis

No.	$c_{Ca/P}$ [mg/L]	$c_{Re}/c_{Sr}/c_{Mg}$ [µg/L]	$V_{standard\ solution}$ [ml]	$V_{aqua\ regia}$ [ml]	V_{total} [ml]
5	10.00	100.00	0.500	4.500	5.000
4	5.00	50.00	0.250	4.750	5.000
3	1.00	10.00	0.050	4.950	5.000
2	0.50	5.00	0.025	4.975	5.000
1	0.10	1.00	0.010	9.990	10.000
Blank	-	-	-	50.000	50.000

Allowing for the correction of the sample introduction effects while using the peristaltic pump of the ICP-MS, a further ISTD was prepared using 25µl of the lutetium ICP Standard CertiPUR® (1.70330.0100) from Merck (1,000ppm) and 49,975µl of diluted nitric acid (5% v/v). Thus, the lutetium standard supplied a final concentration of 500µg/l. All solutions were prepared and stored in vessels made of polyethylene or polypropylene.

2. Automated Method

The automated method was performed using the SAMI® Ex Workstation software method “Pre-Digestion”. In order to perform this method for one so-called family, the tray with two reservoirs for high concentrated acid (as depicted in chapter 6) supplied 16ml of high concentrated nitric acid including the rhenium standard (c = 50ppm). During the method “Pre-Digestion” the high concentrated nitric acid was added to the samples.

The samples (for each 62.5mg of referenced bone material, NIST) were provided by the tray with six vessels for the microwave digestion treatment (as depicted in chapter 6).

After microwave digestion, the process sequence “Dilution 1:1000” as provided by the SAMI® Ex Workstation software was performed on the automated system. Supplying the already digested sample solution, the tray with six vessels (each with 2ml solution) for microwave digestion treatment was required. The tray with two reservoirs for water supplied 50ml of ultra-pure water (Millipore, Merck). Three trays – each with six analysis vessels – were required in order to ensure the 1:1000-dilution sequence, which comprises three dilution steps. The tray with two reservoirs for acids supplied the diluted nitric acid (1%) for the final dilution step. The home position of every tray is defined by the SAMI® method, but can be adjusted if necessary. The reservoir of the 2nd in-house designed liquid handler was filled with 200ml of ultra-pure water.

3. Analytical Measurement

The analysis was performed using an ICP-MS 7700x (Agilent Technologies, Waldbronn, Germany) supplying the following parameters: radio frequency power – 1,550 W; sample depth – 10mm; carrier gas – 0.65l/min; nebulizer pump – 0.10rps; spray chamber temperature – 13 °C (55.4 °F) and dilution gas – 0.40l/min. Argon Bip® ultra-pure gas (Air Products GmbH, Hattingen, Germany) was used as plasma, carrier, and dilution gas. Helium Bip® ultra-pure gas flow (Air Products GmbH) of 4.3ml/min was used in the collision cell preventing polyatomic interferences. Measurements were performed with three replicates and a peak pattern of six points. Rinsing time was set to 55sec at 0.3rps using the nebulizer pump, followed by 45sec at 0.4rps. The automated sample introduction was performed using the ASX-500 autosampler (Cetac**,** Omaha, NE).

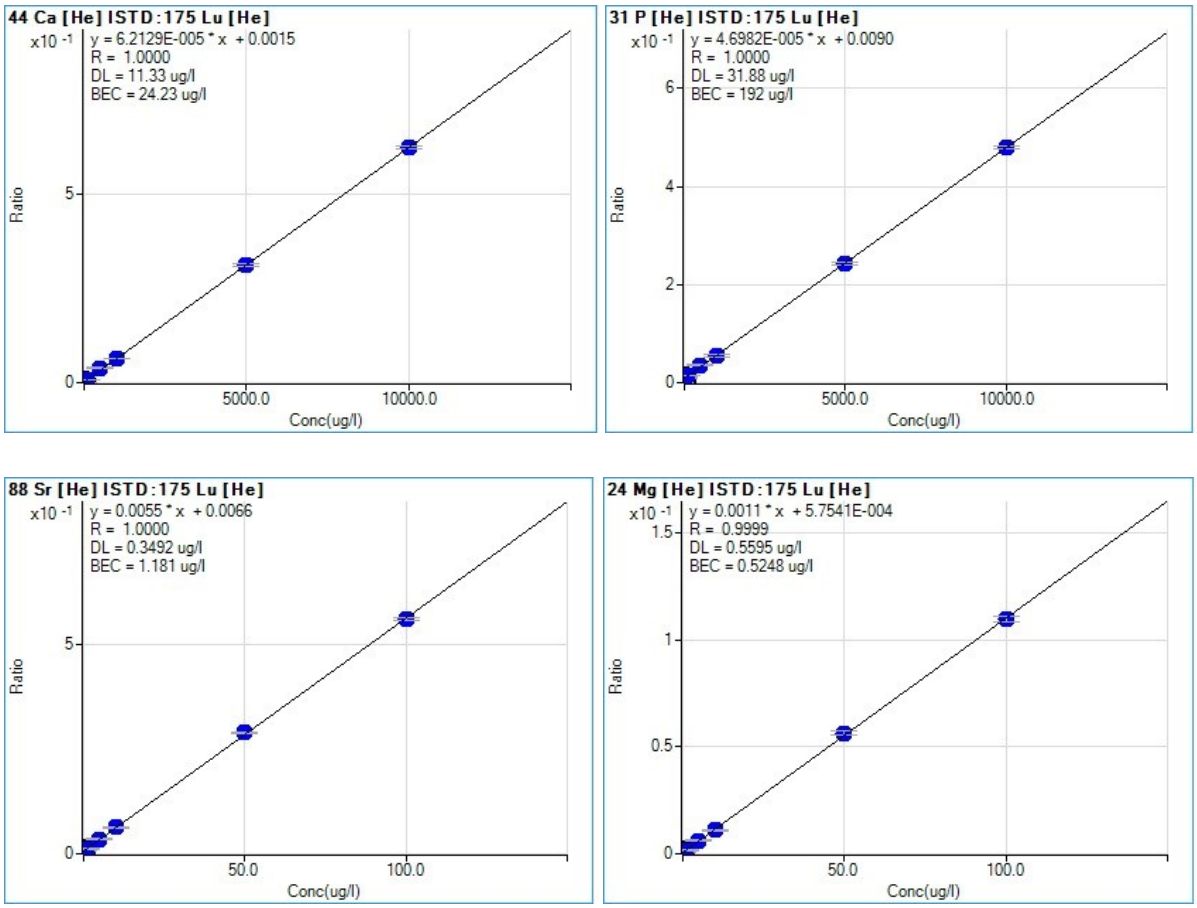


Fig 2: Calibration curves of calcium (Ca), phosphor (P), strontium (Sr), and magnesium (Mg)

Experimental Description for Structure-Specific Measurements Providing Benzoic Acids Analysis

1. Sample Preparation

100µl of 2-methyl, 4-methyl, and 2.5-dimethyl benzoic acid (c = 300ppm), (Merck, Darmstadt, Germany), respectively, were dissolved in 675µl of dichloromethane (Roth, Karlsruhe, Germany). Each benzoic acid solutions were prepared by dissolving 3mg of the substance in 1,000µl of dichloromethane. After adding 25µl of the ISTD cis-decahydronaphthalene (Sigma-Aldrich, St. Louis, MO) and 132µl of trimethylsulfonium hydroxide solution (TMSH, Sigma-Aldrich), the sample vessels were individually crimped and heated for a period of 30min (at 90°C).

2. Automated Method

The automated method was performed using the SAMI® Ex Workstation software method “Derivatization”. In order to perform this method for one so-called family, one tray with two reservoirs for high concentrated acids or organic solvents (as depicted in chapter 6) supplied 20ml of dichloromethane. 675µl of the organic solvent was transferred into each GC-vial (as provided by the

rack for six 2ml-GC-vials). A second rack for six 2ml-GC-vials supplied the three benzoic acids solution: Rack position A1 supplied 1.5ml of 2-methyl benzoic acid. Rack position A2 supplied 1.5ml of 4-methyl benzoic acid. Rack position A3 supplied 1.5ml of 2.5-dimethyl benzoic acid and rack position B1 supplied 1.5ml of the ISTD. 2ml of TMSH were provided by the tray for the calibration (or lower volume) solutions using rack position A1.

3. Analytical Measurement

Benzoic acid detection was accomplished using the HP Agilent (Agilent Technologies, Waldbronn, Germany) 6890 GC, the Agilent 5973Network Mass Selective Detector (MSD) and the Agilent 7673C-6890 autosampler.

Table 3: Parameter of the GC/MS in order to ensure benzoic acid analysis

Parameter	Settings
Analytical column	HP-1 19091Z-236; 60m x 250µm x 1µm
Injection volume	1µl
Inlet Heater	280°C
Inlet Split Ratio	Splitless
Column Flow	1ml/min
Oven	50°C/1min; Ramp: 10°C/min/300°C; 4min hold
Thermal Aux 2	300°C
MS Source	230°C
MS Quad	150°C
Solvent Delay	6min

Table 4: Quantifier and qualifiers of the quantification method

Parameter	2-methyl benzoic acid, methyl ester	4-methyl benzoic acid, methyl ester	2,5-dimethyl benzoic acid, methyl ester
Quantifier	119.00	119.00	133.00
Qualifier 1	91.00	150.00	164.00
Qualifier 1	150.00	91.00	105.00
Qualifier 1	65.00	65.00	77.00

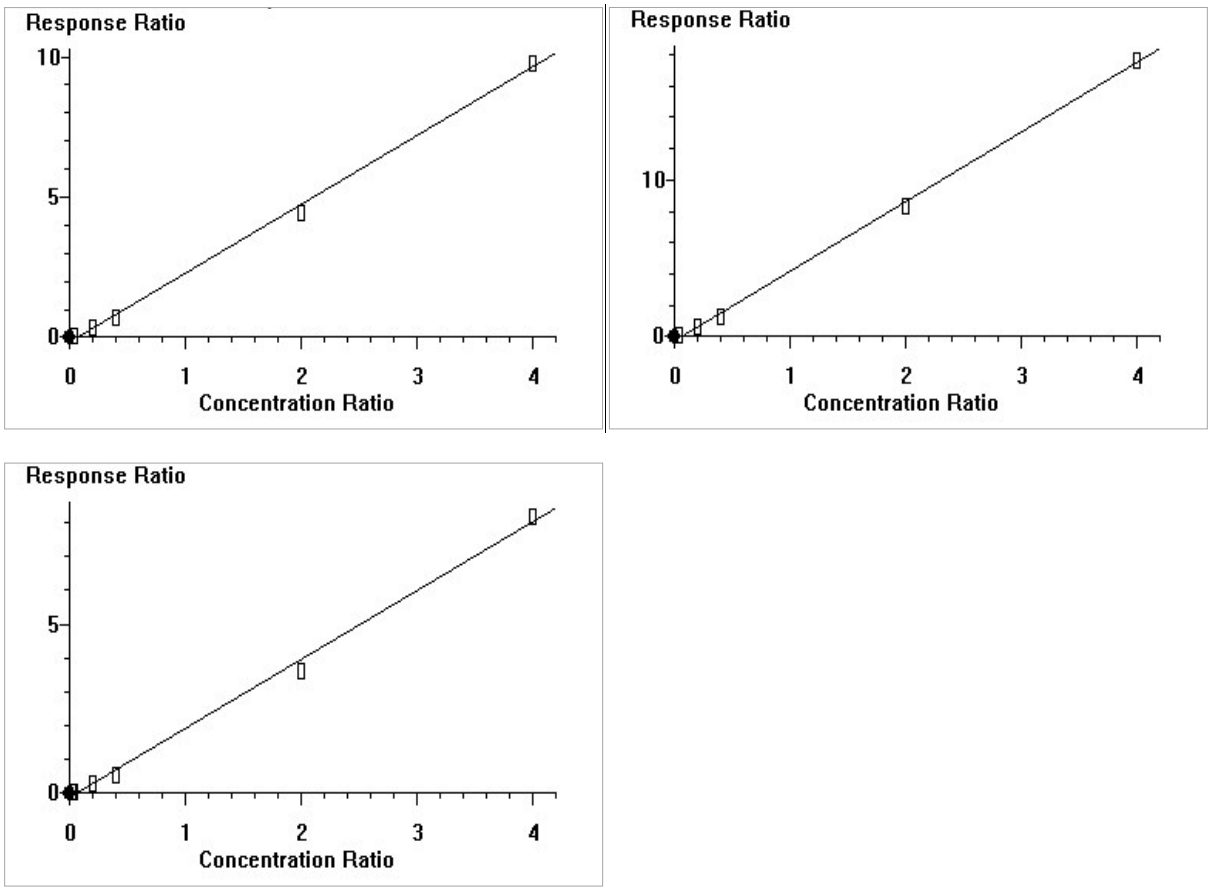


Fig 3: Calibration curves of 2-methyl benzoic acid, methyl ester (left above); 4-methyl benzoic acid, methyl ester (right above) and 2.5-dimethyl benzoic acid, methyl ester (below)

Experimental Description for Structure-Specific Measurements Providing Cyclophosphamide Analysis

1. Sample Preparation

Cyclophosphamide was dissolved in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St. Louis, MO), (20mg/l) including fetal calf serum (FCS), (10%); Penicillin/Streptomycin (50µg/ml); and Glutamin (2mM). The extraction of cyclophosphamide was ensured using a vacuum chamber (Chromabond®, Macherey-Nagel, Düren, Germany), (14.5psi) for 12 SPE columns or cartridges. The Strata® C18-E cartridges (Phenomenex, Aschaffenburg, Germany) were conditioned with 2ml of Methanol ROTISOLV® HPLC Gradient Grade (Roth, Karlsruhe, Germany) first. The conditioning step was followed by the pre-equilibration step using 2ml of ultra-pure water (Millipore, Merck, Darmstadt, Germany) and by the sample loading step using 1ml of the dissolved cyclophosphamide. Subsequently, the washing step (using 2ml of ultra-pure water) and the drying step (duration: 10min) were performed.

Finally, the purified cyclophosphamide was eluted using 2 times of 500µl Methanol ROTISOLV® HPLC Gradient Grade. Samples were diluted in the ration of 1:100 providing a final concentration of 200µg/l.

2. Automated Method

The automated method was performed using the software method "SPE_Adapter_24columns" or the software method "SPE_Adapter_96columns", respectively. In order to perform the method "SPE_Adapter_24columns" one tip box Span_8_1000µl_LLS (liquid level sensing, Beckman Coulter, Brea, CA) was required. Moreover, one Nunc® flat reservoir (Thermo Scientific, Waltham, MA) with 160,000µl of Methanol ROTISOLV® HPLC Gradient Grade, one Nunc® flat reservoir with 160,000µl of ultra-pure water, one Nunc® flat reservoir with 50,000µl of medium (including the dissolved cyclophosphamide), one Pressure_Processor adapter (celisca, Rostock, Germany), one Pressure_Processor cartridge block (celisca) with 24 Strata® C18-E columns, and one Greiner 96-round deep plate (Greiner Bio-One, Kremsmünster, Austria) were required.

In order to perform the method "SPE_Adapter_96columns" one tip box Span_8_1000µl_LLS (liquid level sensing, Beckman Coulter) was required. Moreover, two Nunc® flat reservoirs from Thermo Scientific with 250,000µl of Methanol ROTISOLV® HPLC Gradient Grade, two Nunc® flat reservoirs with 250,000µl of ultra-pure water, one Nunc® flat reservoir with 150,000µl of medium (including the dissolved cyclophosphamide), one Pressure_Processor adapter (celisca), one Strata® C18-E 96-well plate, and one Greiner 96-round deep plate (Greiner Bio-One) were required.

In order to perform the dilution sequence "SPE_Dilution_1:1000" one tip box Span_8_1000µl_LLS (liquid level sensing, Beckman Coulter) and one tip box AP96_20µl_LLS (liquid level sensing, Beckman Coulter), one Nunc® flat reservoir from Thermo Scientific with 200,000µl of Methanol ROTISOLV® HPLC Gradient Grade, one empty Greiner 96-round deep plate (Greiner Bio-One) and the processed Greiner 96-round deep plate from the previous sequence were required. The home position of every tip box, plate, or reservoir is defined by the Biomek® software, but can be adjusted if necessary.

3. Analytical Measurement

Cyclophosphamide detection was accomplished using the Agilent 1200 Series (Agilent Technologies, Waldbronn, Germany) including the High-Performance Autosampler SL G1367C, the Binary Pump SL G1312B, the Thermostatted Column Compartment SL G1316B, and the LC/MSD TOF G1969A with the electrospray ionization-interface (ESI). The specific parameters are shown in the following table.

Table 5: Parameter of the HPLC/MS in order to ensure cyclophosphamide detection

Parameter	Settings
Analytical column	Zorbax Eclipse XDB-C18 (RP) 4,6x150mm particle size: 5µm; pore size: 80Å
Injection volume	10µl
Flow rate	0.75ml/min
Elution	Isocratic; MeOH : water (0,1% HCOOH) = 60% : 40% (v/v)
Column temperature	41°C
Ion source	Dual ESI
Ion polarity	Positive
Drying gas temperature	330°C
Drying gas flow rate	10l/min
Nebulizer pressure	20psig
Capillary	4000V
Fragmentor	200V
Collision energy	65V
Range	100 - 3000m/z

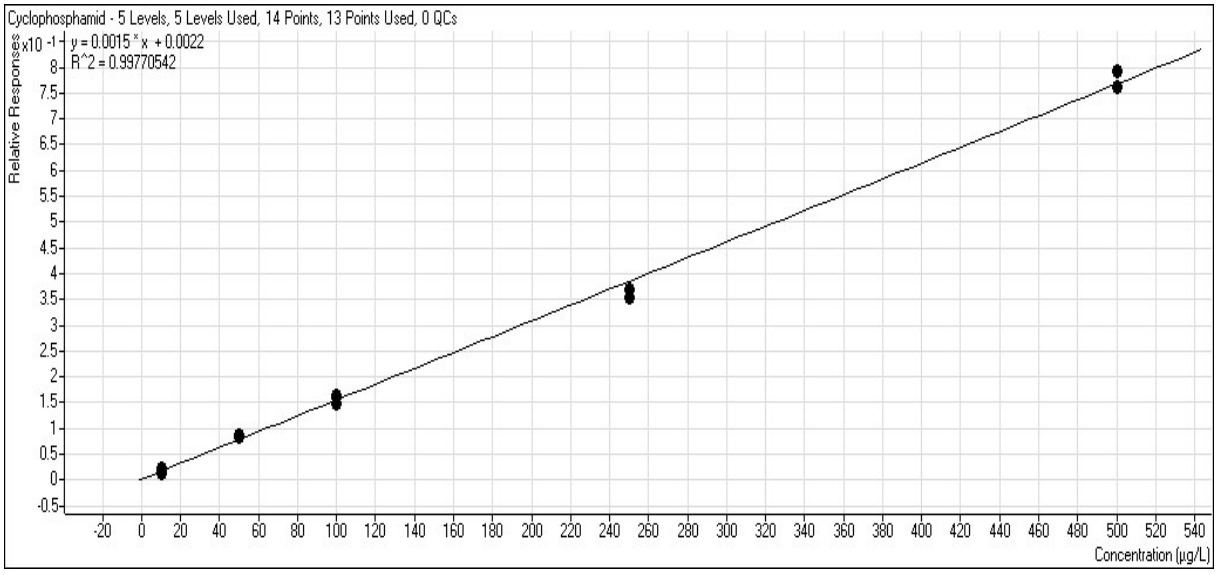


Fig 4: Calibration curve of cyclophosphamide

Thesis

1. Life Science areas require specific sample pretreatment allowing for research applications.

2. By automating these sample preparation steps laboratories will gain on improved quality and better economical results since sample pretreatment techniques are the rate-limiting step in many testing processes.

3. Enabling automated sample preparation, commercially available workstations are usually configured for handling the standardized MTP-format facilitating various assay types and the analysis of (therapeutic) proteins.

4. Allowing for the analysis of elements, small molecules, or mixtures of molecules, analytical processes differ significantly from biological applications.

5. Due to the non-homogeneity of solid samples, the higher volume ranges (allowing for sample dissolving), the non-standard temperature and pressure reactions, the highly active solvents and substances, and the resulting wide range of required inert vessels, current existing workstations are not suitable for flexible analytical sample pretreatment processes.

6. The most important challenge is the design, the realization, and the evaluation of an automated system that supplies multistep analytical sample pretreatment and, besides, high flexibility for easy upgrading and performance adaption.

7. Embedding the applicable vessels into the standardized MTP-footprint represents a suitable solution providing the availability of existing workstations for analytical sample preparation.

8. In order to ensure the entire task performance of the adapted workstations, the embedded vessels have to meet the dimension specifications of the devices´ editor software.

9. Due to the deviation of the top height parameter from the standard MTP-contours, embedding the applicable vessels into the standardized MTP-footprint is enabled and ensures any kind of pipetting and gripping while using the designed labware types.

10. Due to the standardized MTP-footprint, embedding enables the system integrator to place every kind of designed labware type on any existing labware position throughout the integrated system supplying more flexibility during transportation and sample storage steps.

11. Embedding provides less cost- and time-consuming steps while providing simultaneous handling, opening, and covering of up to 24 vessels (depends on the designed labware type).

12. Individual capping is still required in order to ensure concentration stability while handling volatile components and is provided by the Stäubli® TS60 robot assembly that supplies crimping steps.

13. Providing accurate weighing processes of individual vials, the weighing station BP 211D is loaded by high-precision SCARA robot motions and the individual finger design.

14. Facilitating derivarization reactions, the MTP-formatted thermo-block adapter design of the heating and shaking device MHL 23 matches with the aluminum-based labware types and enables precise stacking steps and connective heat transfer.

15. Matching with the specifications of the liquid handler, the designed SPE Unit can be simply assembled on the Biomek® providing fully automated positive pressure SPE processes avoiding vacuum manifold specific difficulties.

16. A specific, executable SILAS integration module has been developed for every custom integrated device of the automated system, which is controlled by the SAMI® software.

17. Ensuring the integration of external stations, such as hoods and detection systems, mobile robot transport has been provided by the Hierarchical Workflow Management System.

18. The system´s functionality has been confirmed in various validation sequences using established analysis schemes that have been validated using manual sample preparation first.

19. Automation requirements have been compared with the manual procedures resulting in miniaturization and simplification of the original handling, which facilitates automated sample pretreatment.

20. Considering the validation parameters, automated sample preparation was up to 50% more precise compared to the manual procedure using element-specific analytical measurement, and as precise as the manual procedure using structure-specific analytical measurement.

Abstract

Laboratories providing life science applications will gain on improved analysis´ efficiency, robustness, and reliability by automating sample pretreatment processes. However, commercially available automated systems are especially suitable for the standardized MTP-format allowing for biological assays, whereas automating analytical sample pretreatment is still an unsolved challenge. Therefore, the purpose of this presentation is the design, the realization, and evaluation of an automated system that supplies multistep analytical sample pretreatment and high flexibility for easy upgrading and performance adaption. The presented concept comprises the idea of embedding the wide range of required analytical vessels into the standardized MTP-footprint ensuring the availability of existing workstations for multistep analytical sample preparation while handling several vessels simultaneously and, thereby, providing less cost and time-consuming automation steps. Moreover, due to the standardized MTP-footprint, embedding enables the central element of the automated system (ORCA® robot) to place every kind of designed labware type on any existing labware position throughout the integrated system. Therefore, using the MTP-formatted labware types provides more flexibility during transportation steps and enables the utilization of the system for various kinds of applications without any changing of labware positions. Meeting the spezifications of the liquid handler software, the Biomek® 2000 enables precise and reliable pipetting steps up to 1,000µl while using the concept idea of embedding. Supplying higher volume ranges, a diluting station has been designed providing Cartesian configuration, MTP-formatted labware postions, and an Hamilton® Dispenser. Moreover, embedding allows for simultaneous opening and covering of up to 24 vessels. However, individual tight sealing is still required to ensure concentration stability while handling volatile components and is, therefore, enabled using the Stäubli® TS60 SCARA robot assembly, which ensures precise and high speed gripping, crimping, and placing. Facilitating the weighing steps, the ionizer ANTISTAT 2000 avoids electrostatic charge of the samples. Moreover, a shuttle system ensuring the transport to the SCARA robot assembly, a re-grip station allowing for labware transferring, a webcam providing 2D-barcode reading, and a flexible sample hotel supplying sample and labware storage have been integrated. Enabling specific sample treatment, the thermo-shaker MHL 23 and the Positive Pressure SPE Unit have been integrated allowing for derivatization and extraction reactions, respectively. Due to safety reasons, microwave digestion steps have to be performed under a separated hood, which is connected with the automated system using a mobile robot system. However, considering the validation parameters, automated sample preparation was up to 50% more precise than the manual procedure using element-specific analyses, and as precise as the manual procedure using structure-specific analyses.

Abstract

Durch den Einsatz der automatisierten Probenvorbereitung gewinnen Life Science Laboratorien an Analyseneffizienz, Robustheit und Zuverlässigkeit. Nichtdestotrotz sind automatisierte, kommerziell verfügbare Systeme nicht für den weitläufigen Bereich analytischer Applikationen geeignet, da diese Systeme das standardisierte MTP-Format verwenden und somit hauptsächlich biologische Assays unterstützen. Der Schwerpunkt der vorliegenden Dissertation lag daher in der Entwicklung, Realisierung und Evaluierung eines automatisierten Systems, das die vielfältige analytische Probenvorbereitung gewährleistet und gleichzeitig genügend Flexibilität besitzt, um das System zu erweitern und an sich ändernde Erfordernisse anzupassen. Das zugrundeliegende Konzept der Arbeit ermöglicht das Einbetten der spezialisierten, analytischen Probengefäße in das standardisierte MTP-Format, wodurch nicht nur die Verfügbarkeit von bereits existierenden Plattformen mittels Systemadaption, sondern auch das parallele Probenhandling gewährleistet wird. Durch das parallele Abarbeiten werden zudem die erforderlichen Prozessschritte minimierte und der Zugang zum Hochdurchsatz-Verfahren geschaffen. Darüber hinaus ermöglicht die konzeptionelle Idee hohe Flexibilität während der notwendigen Probentransportschritte, da es dem zentralen Roboter (ORCA®) des entwickelten Systems auf Basis der gleichbleibenden Standfläche ermöglicht wird, jegliche entwickelte Labware frei, innerhalb des Systems, auf allen vorhanden Labware-Positionen zu platzieren. Konzeptbasierend ermöglichen die eingesetzten Liquid Handler präzises Pipettieren im Bereich von 1µl bis zu 1ml (Biomek® 2000) oder aber, unter Nutzung einer entwickleten Plattform mit kartesischem Aufbau und integriertem Hamilton® Dispenser, das Dispensieren höherer Volumina (bis zu 10ml/Schritt). Weiterhin gewährleistet das Konzept bis zu 24 eingebettete Probengefäße gleichzeitig zu verschließen bzw. wieder zu öffnen. Um auch das Verschließen einzelner Probengefäße mittels Krimpen zu realisieren, wurde der Stäubli® TS60 SCARA implementiert, der auf Basis eines Endeffektors mit flexiblem Fingerdesign auch das individuelle Bestücken der Wiegestation verrichtet. Darüber hinaus wurde in das entwickelte System ein Ionisationsgerät zur Vermeidung elektrostatischer Aufladung, ein Shuttle zum weiteren Probentransport, eine Regrip-Station zur Übergabe der Probengefäße, eine Kamera zum Auslesen des Barcodes, eine Probenhotel zur Lagerung der benötigter Labware, ein Heizschüttler und eine mit Überdruck arbeitende SPE Einheit integriert, welche die Derivatisierung der Proben bzw. deren Extraktion unterstützen. Eine zum Aufschluss von Probenmaterial benötigte Mikrowelle wurde aus sicherheitstechnischen Gründen unter einem separaten Abzug eingesetzt und mittels mobilen Robotertransports in das entwickelte System eingebunden. Die Evaluierung des automatisierten Systems ergab eine bis zu 50% höhere Präzision der automatisierten Probenvorbereitung im Vergleich zum manuellen Prozedere unter Anwendung Element spezifischer und eine den manuellen Prozessen entsprechende Präzision für die Struktur spezifischen Applikationen.

Publikationen

E. VORBERG, H. FLEISCHER, S. JUNGINGER, L. HUI, N. STOLL, and K. THUROW, "A highly flexible, automated system providing reliable sample preparation in element- and structure-specific measurements, " J. Lab. Autom. , 2015, *in press.*

E. **VORBERG**, H. FLEISCHER, S. JUNGINGER, N. STOLL, and K. THUROW, "Automated sample preparation for mercury analysis in wood materials," IET Sci. Meas. Technol., 2015, *accepted.*

C. GALLERT, **E. VORBERG**, T. RODDELKOPF, S. JUNGINGER, H. FLEISCHER, and K. THUROW, "Evaluation of an automated solid-phase extraction method using positive pressure," Am. Lab., vol. 47, no. 2, pp. 28-33, 2015.

E. VORBERG, H. FLEISCHER, S. JUNGINGER, N. STOLL, K. THUROW, "Automated sample preparation for mercury analysis in wood materials," Proceeding of the 5th IMEKO TC19 Symposium on Environmental Instrumentation and Measurements, pp. 66-70, Chemnitz (D), 2014.

H. FLEISCHER, **E. VORBERG**, M. WARKENTIN, D. BEHREND, and K. THUROW, "Determination of calcium and phosphor in bones using microwave digestion and ICP-MS," Proceeding of the 5th IMEKO TC19 Symposium on Environmental Instrumentation and Measurements, pp. 94-99, Chemnitz (D), 2014.

H. FLEISCHER, **E. VORBERG**, and K. THUROW, "Determination of total mercury content in wood materials-Part 3: Miniaturization using ICP-MS," Am. Lab., vol. 46, no. 6, pp. 16-20, 2014.

H. FLEISCHER, **E. VORBERG**, M. WARKENTIN, and K. THUROW, "Qualitative and quantitative determination of methacrylates in dental filling materials," Am. Lab., vol. 46, no. 2, 2014.

M. SWANGNETR, D. KABER, **E. VORBERG**, H. FLEISCHER, and K. THUROW, "Workload Assessment for Manual and Automated Processes in Life Sciences," 5th International Conference on Applied Human Factors and Ergonomics AHFE 2014, pp. 121-128, Krakow (P), 2014.

M. SWANGNETR, D. KABER, **E. VORBERG**, H. FLEISCHER, and K. THUROW, "Identifying Automation Opportunities in a Life Science Process through Operator Task Modeling and Workload Assessment," 5th International Conference on Applied Human Factors and Ergonomics AHFE 2014, pp. 6248-6255, Krakow (P), 2014.

Selbständigkeitserklärung

Hiermit versichere ich, dass ich die eingereichte Dissertation zum Thema

"Process Automation for Analytical Measurements Providing High Precise Sample Preparation in Life Science Applications"

selbständig und ohne Hilfe verfasst, andere als die angegeben Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche gekennzeichnet habe.

Rostock, 25. Februar 2015

Ellen Vorberg