## Separation of organic acids by High Pressure Liquid Chromatography

Chromatographic conditions

- Column: Rezex ROH-Organic Acid H+, Phenomenex Ltd.
- Flow: 0.5 m²/min, $H_2SO_4$ c=0.005 mol/l
- Oven temperature: 30 °C
- RI-Detector Shodex RI-71
- HPLC Pump 420
- HPLC Autosampler 460
- HPLC Oven Controler 480
- Kontron Instruments Ltd.
- Cooling of the samples with Haake K20 Thermo at 2 °C
- Interpretation by an extern standard with 4 different concentrations and linear regression
- Running time: 45 min/sample

For the general characterisation of silages an acid decomposition was used.

| **DM** | **g FM + ml $H_2SO_4$** |
|---|---|
| < 25 % - 40 % | 50 g + 200 ml 0.1 n $H_2SO_4$ |
| > 40 % | 25 g + 200 ml 0.1 n $H_2SO_4$ |

The fresh silage was extracted with 0.1 n $H_2SO_4$ in a Stomacher blender for 4 min, then filtered and filled in HPLC vials.

For the characterisation of the silage media the aqueous solution was used as described in the text. SIEGFRIED *et al.*, 1984, also determined organic acids in aqueous extracts.

Calculation:
To convert the concentration [mg/ml] to [% FM] the following equation must be calculated:

$$\%FM = \frac{M}{E*10} * \left( V + E * \frac{100 - DM}{100} \right)$$

FM = Fresh matter
M = measured value [mg/ml]
V = extraction volume [ml]
E = weighted sample [g]
DM = Dry matter

# Reference List

SIEGFRIED, R., RÜCKEMANN, H. and STUMPF, G. (1984): Eine HPLC-Methode zur Bestimmung organischer Säuren in Silagen. Landwirtschaftliche Forschung, 37, 298-304