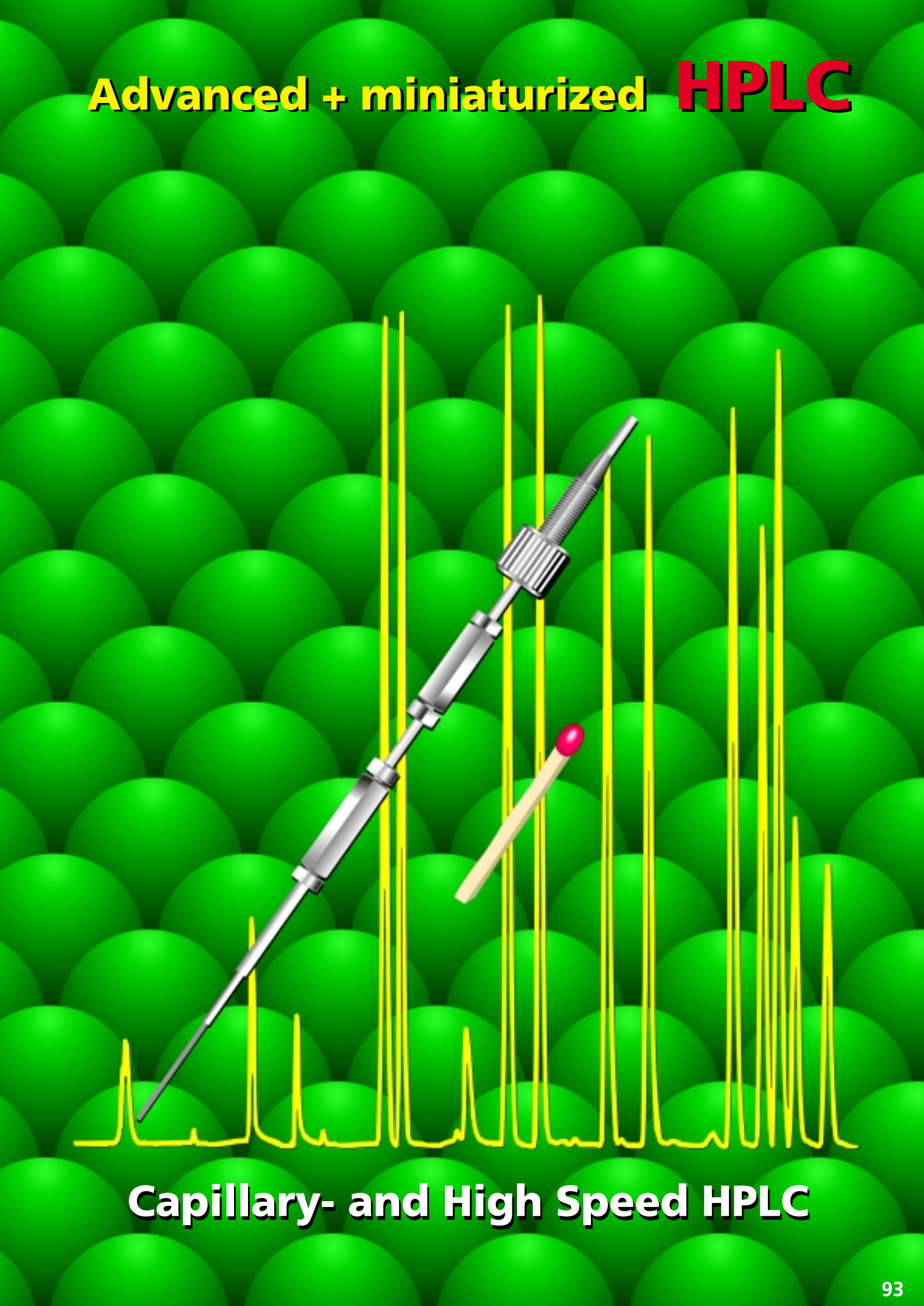
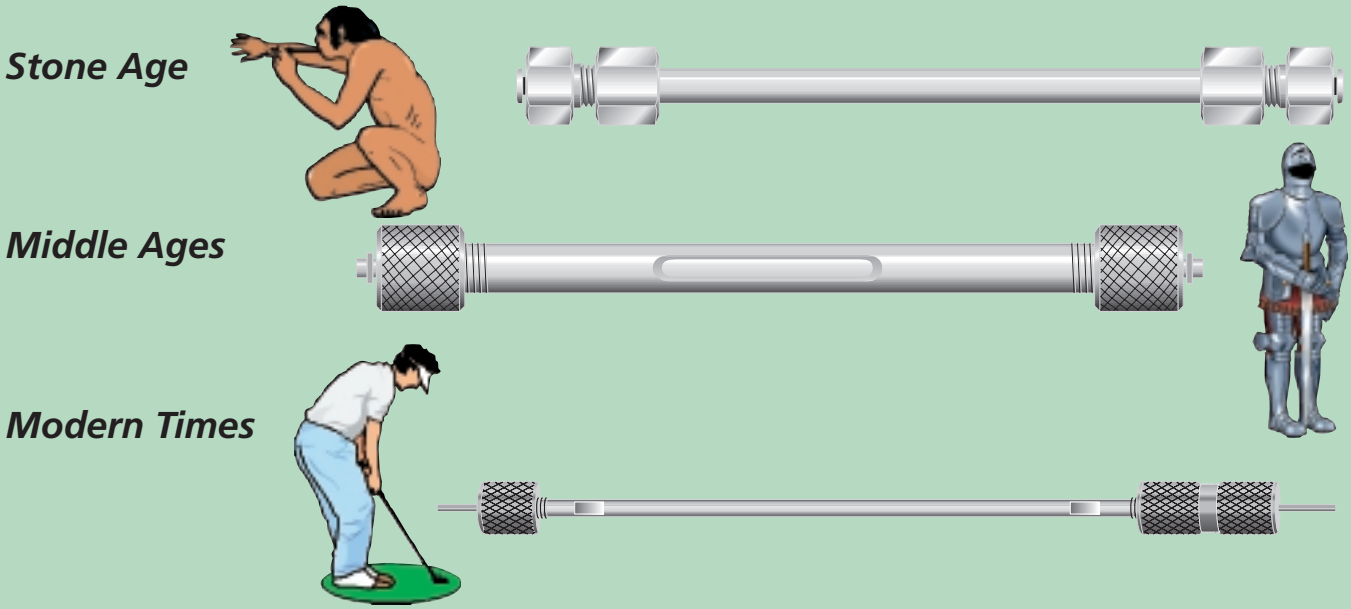
Advanced + miniaturized HPLC



Capillary- and High Speed HPLC

Take advantage of the benefits of miniaturization by employing state-of-the-art HPLC

All modern HPLC-pumps and, in conjunction with a solvent-splitting device, even any traditional HPLC-pump can easily maintain the low flow rates required for isocratic (often on-line coupled to a mass-spectrometer) or gradient elution in capillary HPLC.
Also, micro flow cells of UV detectors with 1.2 µl or even 3 nl volume and 3 mm, respectively 8 mm path length are currently available. They can readily be used in place of the standard analytical flow cells of HPLC detectors commonly used today. The risk of back-mixing caused by large volume detector cells is thus eliminated. Further, in addition to the benefits of drastically reduced solvent consumption, Micro HPLC may offer more than 200-fold enhanced sensitivity in accordance with Beer-Lambert´s law when using such tiny micro flow cells.



Enhanced sensitivity plus drastic solvent savings by using *smaller i.d. columns*

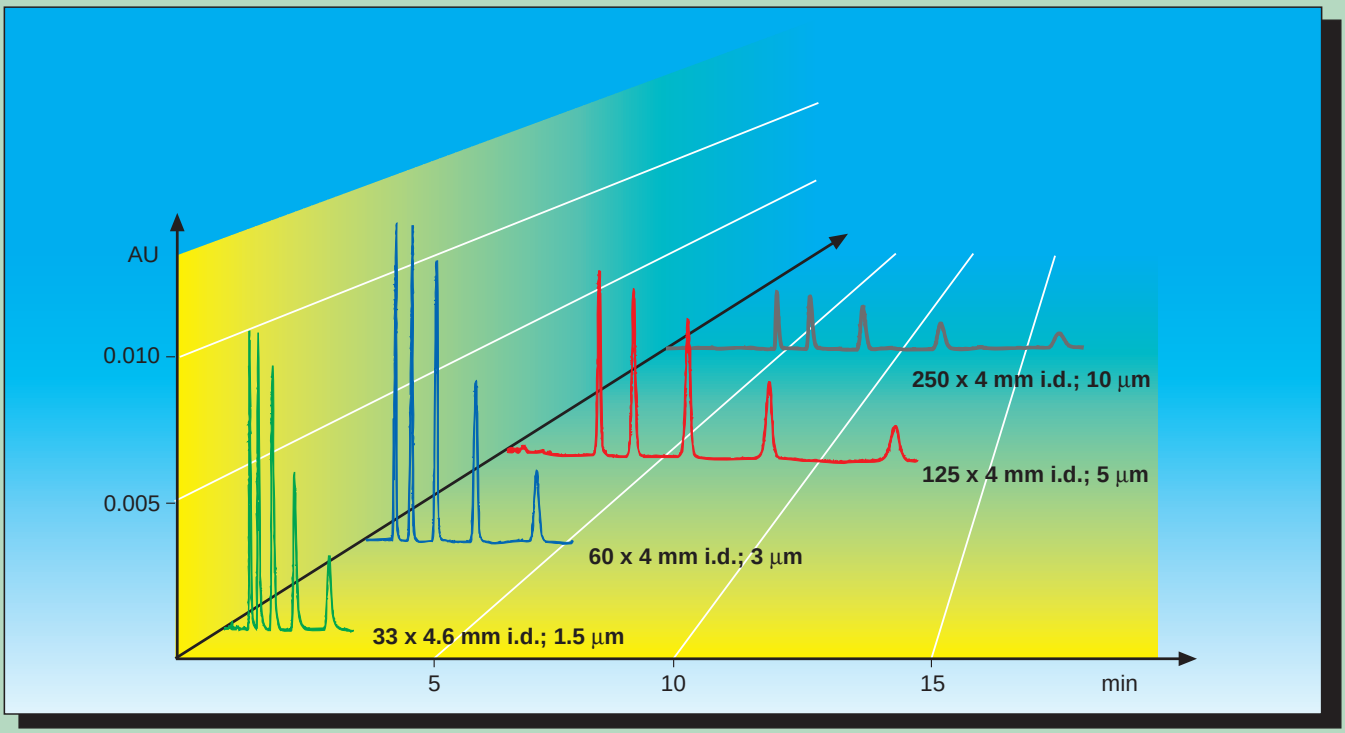
in. diameter [mm]	cross section [mm²]	flow [µl/min]	solvent consumption	flowcell volume [µl]	path length [mm]	gain in sensitivity **
4.6	16.6	1 200	100%	15	10	1
4.0	12.6	910	75%	15	10	1.3
3.0	7.1	510	42%	15	10	2.4
2.0	3.1	224	19%	15 *	10	5.3
				5	6	3.2
				1.2	3	1.6
1	0.8	56	5%	15 *	10	21
				5	6	12.6
				1.2	3	6.3
500 µm	0.2	15	1.2%	15 *	10	80
				5 *	6	48
				1.2	3	24
250 µm	0.05	3.5	0.3%	15 *	10	340
				5 *	6	200
				1.2	3	100
				100 nl	0.3	10
				3 nl	8	270

* when optimal resolution is needed these flow cells must not be used for these columns
** theoretical values

Why use short(er) columns? ...

... High Speed HPLC with short columns offers numerous advantages and benefits:

- short analysis time – only 1/7 of standard HPLC → high sample through put, with tremendously reduced costs per analysis
- low solvent consumption – only 1/5 of standard HPLC → cost savings and improved environmental compatibility
- decreased diffusion/dispersion → increased sensitivity – ≥ 2-fold –
- increased performance from minimum band broadening → higher number (N) of higth equivalents of theoretical plates (HETP) per meter (≥ 4.5-fold)



Stationary phase: *GROM*-SIL 100 ODS-2 FE; **Linear velocity:** 0.8 mm/s; **Eluent:** ACN:H_2O = * 65:35, resp. ** 60:40; **Flow cell:** 1.2 µl / 3 mm with quick-connector (100 x 0.1 mm capillary); **Injection:** 5 µl benzoate test mixture / 1:100 dil. (methyl-, ethyl-, propyl-, butyl-, pentyl benzoate; 10–20 mg/ml)

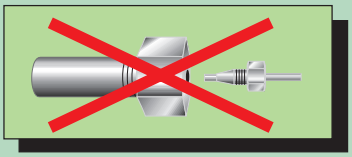
Varying column length and particle size can substantially reduce runtimes and solvent consumption without compromising sensitivity or resolution

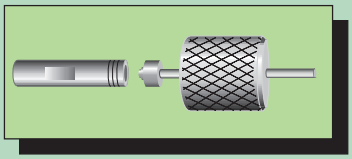
Column Length Particle Size	Analysis Time [min]	Solvent Consumption [ml]	Sensitivity (1st peak) [AU]	Resolution (1. + 2nd peak)	N/m (5th peak)
250 x 4 mm * 10 µm	18.1	10.9	0.004	4.8	29 000
125 x 4 mm * 5 µm	9.4	5.6	0.008	5.2	72 200
60 x 4 mm ** 3 µm	4.8	2.9	0.013	4.6	129 300
33 x 4.6 mm * 1.5 µm	2.5	2.0	0.010	2.5	130 900

To achieve the very best results in modern HPLC ...

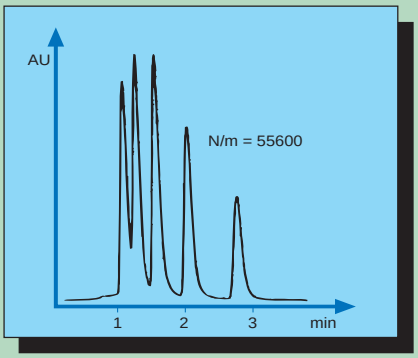
For making use of microbore, capillary and high speed liquid chromatography only minor hardware changes are needed, resp. „conditio sine qua non“. Critical components include:

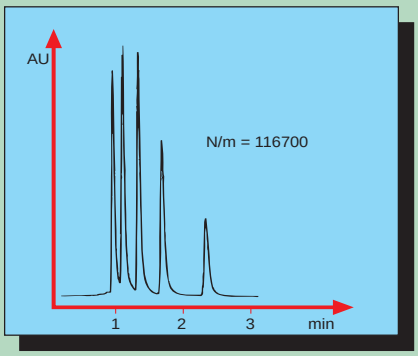
- **small flow cells** with appropriate internal volume and pathlength
- **connecting capillaries** (nearly dead volume-free) as short as possible,
- **low dispersion column hardware** guaranteeing maximum of chromatographical performance
- **flow splitter** or **high performance, low µl/min pump**

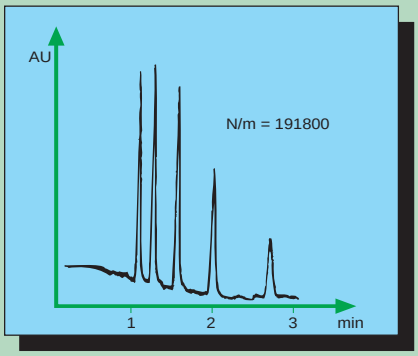




Note! It is seriously recommended to use exlusively capillaries with fitting adapters and quick-connectors rather than with ferrule-type fittings







Same chromatographical conditions, exept ...

analytical flow cell with (9 µl / 6mm) with inlet capillariy as supplied by manufacturer

analytical flow cell (9 µl / 6mm) with fitting adapter capillaries (100 x 0.12 mm)

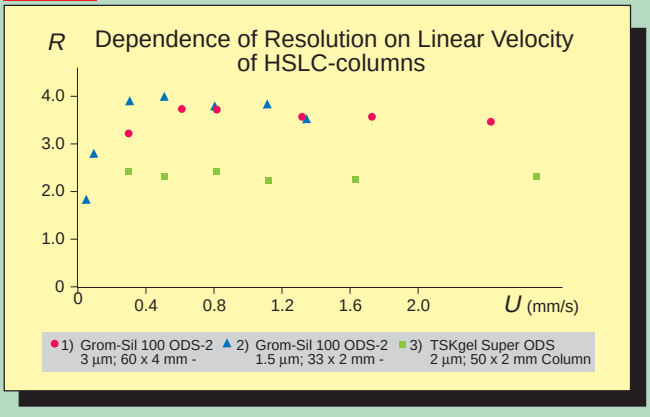
capillary flow cell (0.1 µl / 0.3 mm) with fused silica inlet

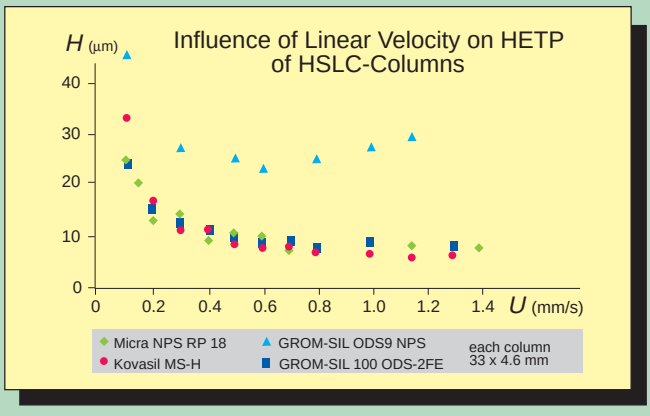
Different Flow Cells – Influence of Volume and Pathlength

Cell Volume [µl]	Pathlength [mm]	Sensitivity [AU]	Resolution (1st + 2nd peak)	N/m (5th peak)	Assymetry factor (5th Peak)
15	10	0.030	1.3	91 800	2.9
9	6	0.023	1.4	116 700	2.5
5	6	0.025	1.55	131 600	2.6
1.2	3	0.013	1.7	139 600	2.1
1.2*	4	0.016	2.6	118 800	1.1
0.009*	8	0.008	3.3	164 500	1.2

Stationary phase: *GROM*-SIL 100 ODS-2 FE; **Column:** 33 x 2.0 mm; **Linear velocity** 0.8 mm/s; **Eluent:** ACN:H_2O = 65:35; each flow cell connected via quick-connector (100 x 0.1 mm stainless steel capillary), * except capillary flowcells (150 mm x 100 µm „fused-silica“-capillaries); **Injection:** 1 µl benzoate test sample (dil. 1:100)

Note! experiments always have to be done at optimal linear velocity



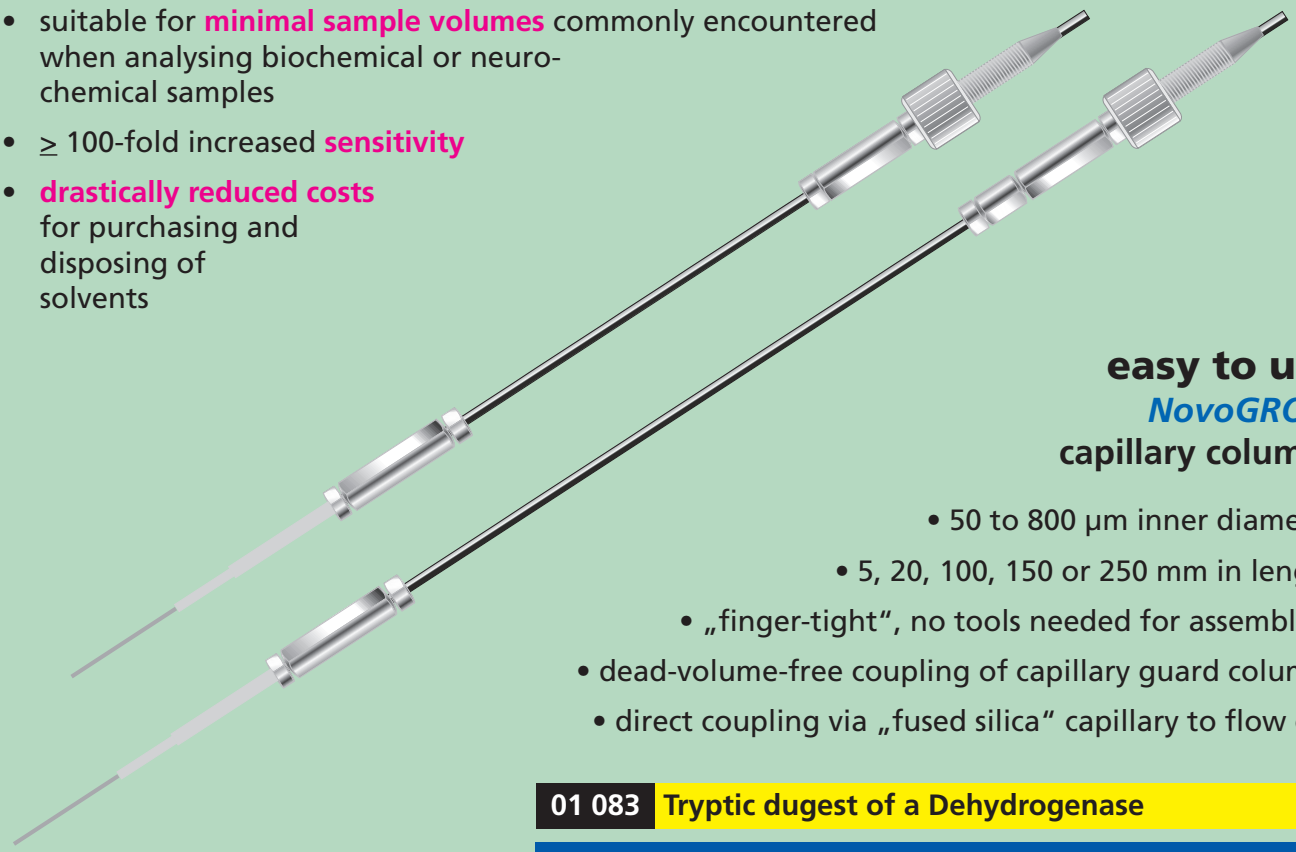


* High speed liquid chromatography (HSLC)

Don't be afraid of Capillary-HPLC

There is no sound reason for not taking advantage of the unique benefits of this exciting technique:

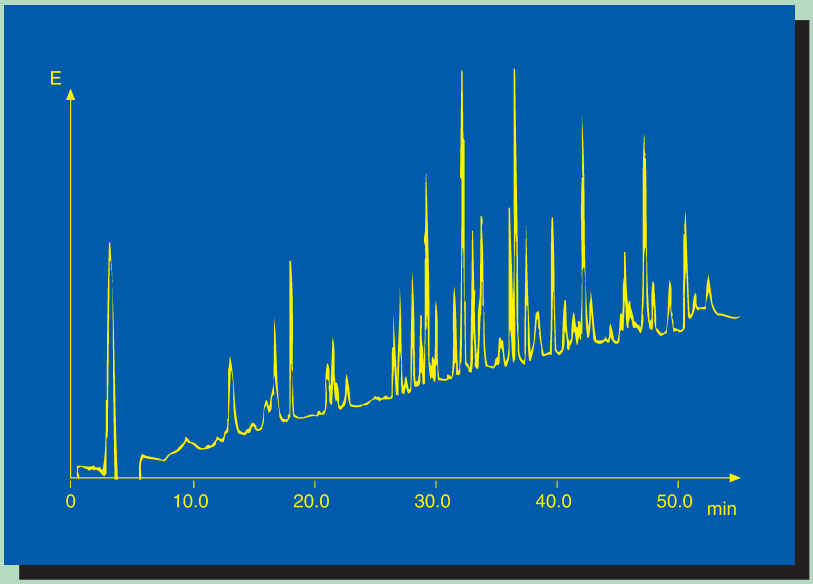
- convinient **„on-line"** coupling to mass spectrometer
- suitable for **minimal sample volumes** commonly encountered when analysing biochemical or neuro-chemical samples
- ≥ 100-fold increased **sensitivity**
- **drastically reduced costs** for purchasing and disposing of solvents



easy to use *NovoGROM* capillary columns

- 50 to 800 µm inner diameter
- 5, 20, 100, 150 or 250 mm in length
- „finger-tight", no tools needed for assembling
- dead-volume-free coupling of capillary guard columns
- direct coupling via „fused silica" capillary to flow cell

01 083 **Tryptic dugest of a Dehydrogenase**

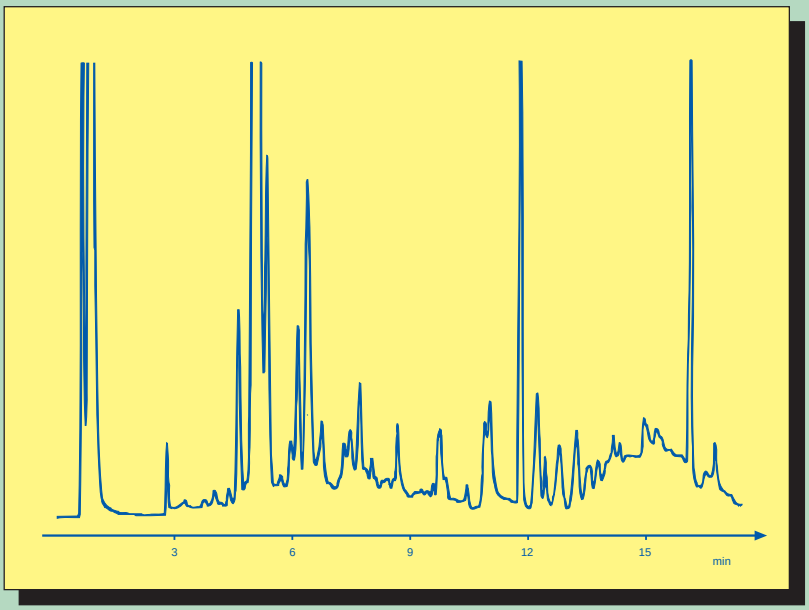


Column phase:	GROM-SIL 100 ODS-2 FE, 5 µm
Column size:	250 mm x 300 µm
Eluent:	A: 0.1% TFA in H_2O
	B: 0.1% TFA in ACN
Gradient:	10–60% B (0–90 min)
Flow rate:	5 µl/min
Pressure:	16–7 MPa
Temperature:	RT
Detection (UV):	214 nm
Sample:	1 µl (2 pMol)

Note! Any modern HPLC system may easily be converted to a capillary HPLC by means of a splitter, microinjector (only needed when working in isocratic mode) and capillary detector cell.
NovoGROM capillary columns (for further information see pages 12/13 and 144) can be packed with any of the stationary phases listed or with the customer's stationary phase.

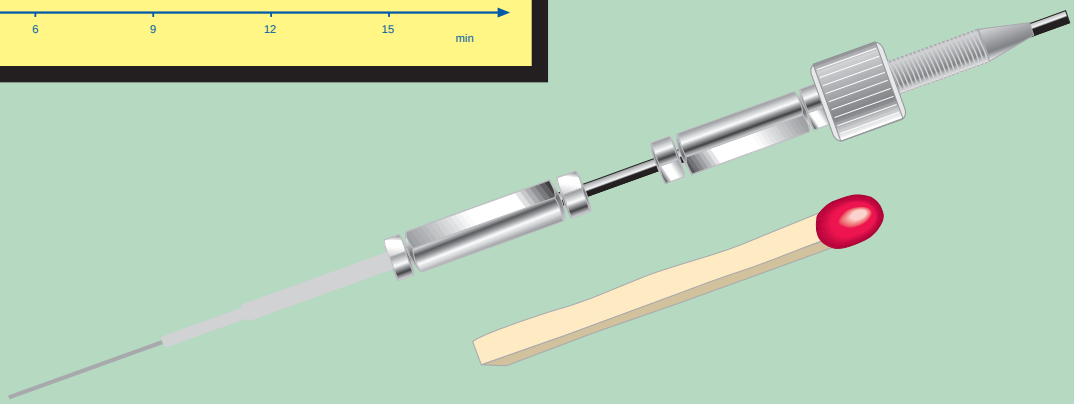
Capillary High Speed Liquid Chromatography

33 mm x 300 µm capillary column, 1.5 µm packing material



10 184 Control of peptide synthesis

Column phase:	GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size:	33mm x 300 µm
Eluent:	A: H_2O, 0.1% TFA B: Acetonitrile, 0.1% TFA
Gradient:	5–60% B (0–15 min)
Flow rate:	6 µl/min
Temperature:	RT
Detection (UV):	214 nm
Sample:	60 nl

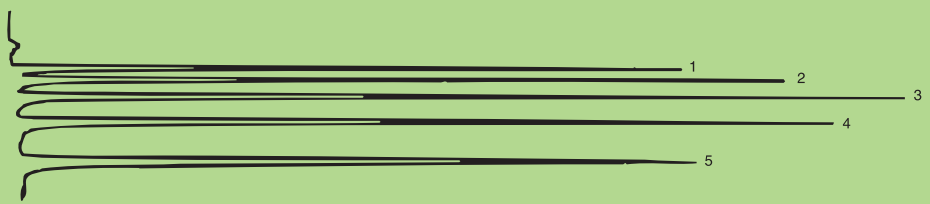


Typical computer printout of column test protocol

PACKING MATERIAL
GROM-SIL 100 ODS-2 FE
PARTICLE SIZE 1.5 um
COLUMN DIMENSIONS
33 mm x 300 µm ID
PART NO. GSQD20210C0430
SERIAL NO. 290995 1

TEST CONDITIONS
MOBILE PHASE
CH3CN:H2O 70:30

FLOW RATE 5 µL/min
PRESSURE 5 MPa
DETECTION: UV 254 nm



PEAK NO.	RETENTION TIME (MIN)	SOLUTES	ASYMMETRY FACTOR	THEORETICAL PLATES/COL	THEORETICAL PLATES/M
1	1.38	METHYLBENZOATE	1.2	3307	100221
2	1.65	ETHYLBENZOATE	1.2	3803	115236
3	2.07	PROPYLBENZOATE	1.4	4985	151072
4	2.71	BUTYLBENZOATE	1.3	5787	175371
5	3.71	PENTYLBENZOATE	1.3	7181	217592

On-line Coupling of Capillary HPLC and with Coordination Ion Spray

Separation and Detection of Unsat

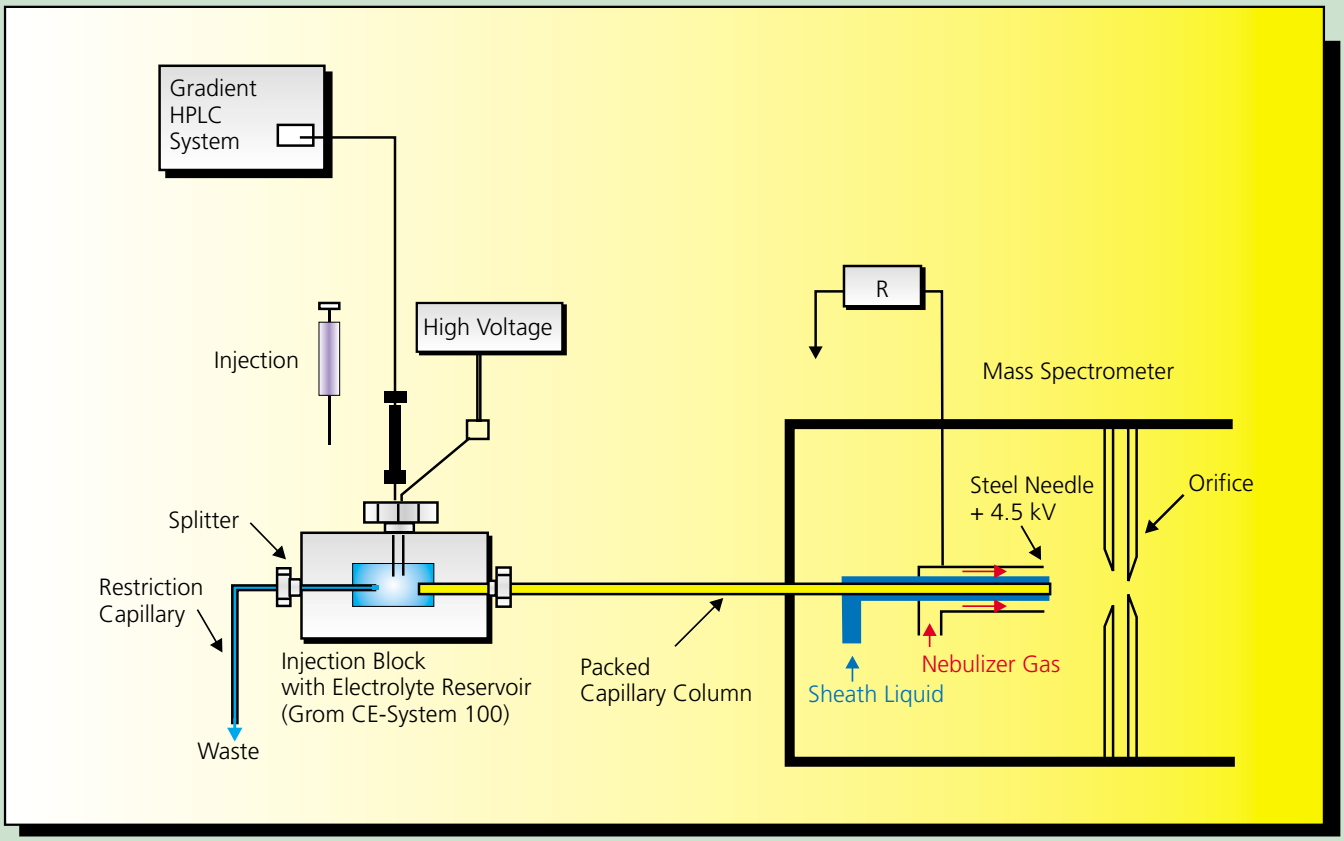
Electrospray mass spectrometry (ES-MS) especially in combination with capillary HPLC (CHPLC) and capillary electrochromatography (CEC) is one of the most powerful techniques for the structural elucidation of polar bio-polymeres (see also page 85).

Unfortunately electrospray ionisation so far is not applicable to non-polar substances and of limited significance for weakly polar substances. Recently a new ionization method adding (by sheath flow technique) a solution of a central ion capable of forming charged coordination compounds has been developed [1, 2].

For the hyphenation of CHPLC and CEC to coordination ion spray mass spectrometry (CIS-MS) the coaxial sheath flow interface described by Smith et al. [3] for capillary zone electro-phoresis mass spectrometry (CZE-MS) coupling was used [3]. Figure 1 shows a schematic drawing of the setup for CEC- and CHPLC-CIS-MS coupling. In this arrangement a concentric series of capillaries is used in which the central CEC/CHPLC column (100 µm i.d., 164 µm o.d.; length 25 cm) is surrounded by a stainless steel needle and the latter by the nebulizer gas.

The coordination ion solution is introduced in the interspace between steel needle and separation column by a syringe pump at a flow rate of 3 µ l/min. Thereby the coordination ion solution is acting as complexing reagent providing ionization as well as sheath flow ensuring stable spray conditions.

It is well known from coordination chemistry that silver ions have a high affinity for olefinic compounds. This affinity was utilized for the detection of unsaturated



fatty acid methyl esters (UFAMEs) by using an aqueous sheath flow containing $AgNO_3$. Figure 2 shows the comparative separations of four UFAMEs with CEC, pressurized with CEC, pressurized CEC (pCEC) and CHPLC with CIS-MS for detection. The reduction of analysis time is relatively small by changing from CHPLC to CEC (10 %). The combination of pressure and

Capillary Electrochromatography
Mass Spectrometry (CIS-MS)
urated Fatty Acid Methyl Esters (UFAMEs)

electroosmotic flow (EOF) in pressure CEC (pCEC) however decreases the analysis time by about 60 % and narrows the peaks drastically.

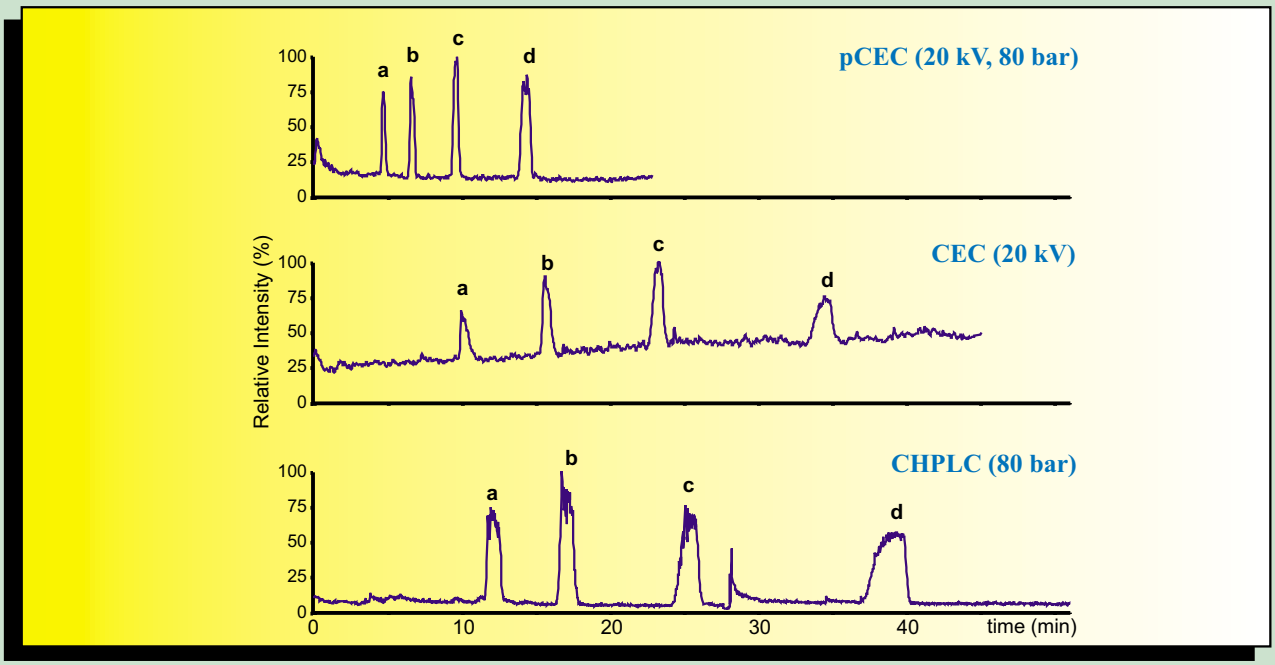


Figure 2: Separation of the methyl esters of (a) palmitoleic acid, (b) oleic acid, (c) eicosenoic acid and (d) erucic acid. Column: 100 µm i.d., 164 µm o.d., length 25 cm, packed with GROM-SIL ODS 0-AB, 100 Å, 3 µm. Eluent: 40 mM NH_4AC, pH 9 / ACN = 5 / 95. Sheath liquid: 100 µg/ml $AgNO_3$.

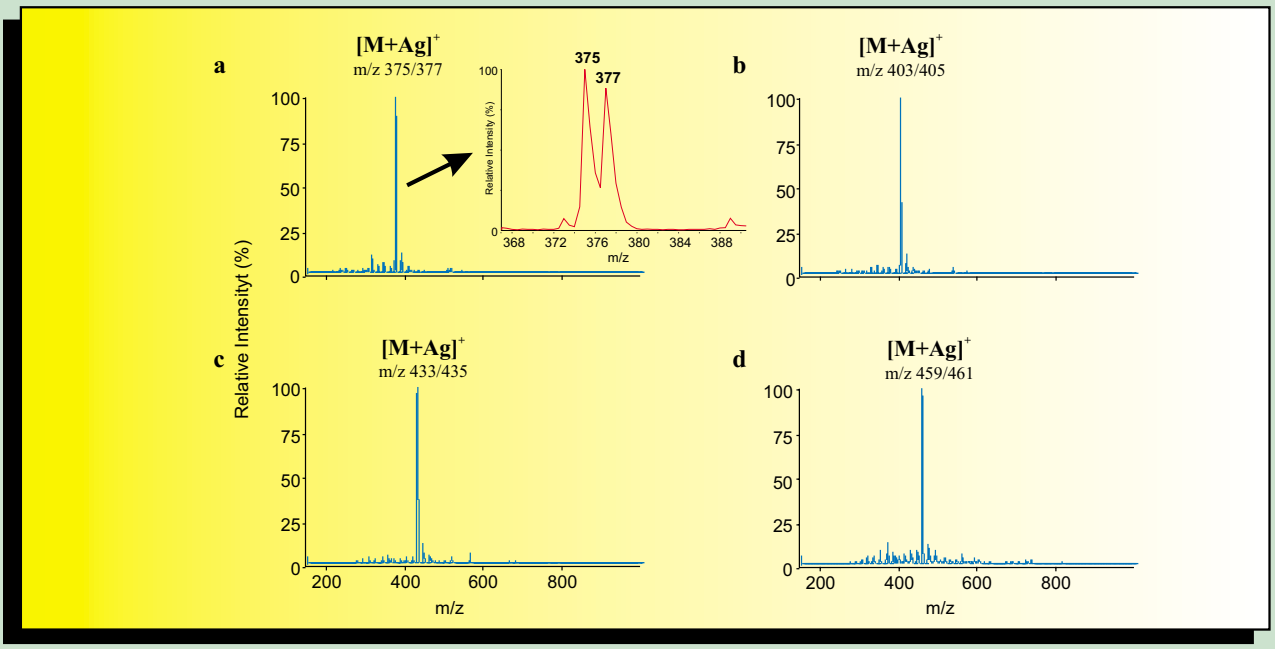


Figure 3: Shows the mass spectra taken from the peaks in figure 2. The Ag^+ complexes are readily recognized on account of the characteristic abundance ratio of silver isotopes (107Ag / 109Ag = 51.8 / 48.2). Therefore the magnification shows a double peak for the [M+Ag]+ ions.

[1] E. Bayer, P. Gfrörer, C. Rentel, Angew. Chem. Int. Ed. 38, 992-995 (1999).
[2] C. P. Gfrörer, E. Bayer, C. Rentel, Electrophoresis 20, 2329-2336 (1999).
[3] R. D. Smith, C. J. Barinaga, H. R. Udseth, Anal. Chem. 60, 1948-1952 (1988).