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Application of a new atmospheric pressure ionization source for double focusing sector instruments

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Abstract

In three application examples we demonstrate how the capabilities of a modern atmospheric pressure ionization (API) source, as a universal LC–MS coupling tool, are supplemented by the high mass resolution available on double focusing sector instruments. The examples include charge state determination in complex electrospray ionization–collisional induced dissociation (ESI–CID) spectra of multiply charged ions, mixture analysis of multicomponent samples without prior chromatographic separation, and very sensitive and highly specific detection of pesticides using atmospheric pressure chemical ionization (APCI).

1. Introduction

The utilization of electrospray ionization (ESI) [1–3] and atmospheric pressure chemical ionization (APCI), also known as heated pneumatic nebulizer/corona discharge ionization [4,5], has induced a breakthrough for the online LC–MS coupling. On the one hand the generation of multiple charged ions from large biomolecules by ESI has opened entirely new fields of application for mass spectrometry, on the other hand the relatively simple operation of the new ionization techniques ESI and APCI in combination with their broad application kDa range has made them to the preferred LC–MS techniques over the previously developed techniques like thermospray (TSP), particle beam interface (PBI) and continuous-flow fast-atom bombardment (CF-FAB).

In both techniques, ESI and APCI, ions are generated from liquid sprays at atmospheric pressure and transferred through several differential pumping stages into the mass analyzer, a principle, which was introduced as atmospheric pressure ionization (API) by Horning et al. [6], but initially applied with a different type of ionization. During the last years the abbreviation API has been used for this principle, independent of the type of ionization applied [7].

Most ESI and APCI applications are performed on quadrupole instruments. The relatively low mass range of these instruments, typically up to m/z 2000, is sufficient for most analytic measurements, because the high charge state z of the generated ions causes the m/z values to fall into this mass range even for molecular masses in the M_r 100 kDa range [1]. The low resolving power of quadrupoles, however, limits their analytical capabilities and therefore ESI was also combined with high-res-

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olution mass analyzers, like double focusing sector instruments [8–17], Fourier transform ion cyclotron resonance (FTICR) instruments [18] and quadrupole ion traps, modified for high-resolution operation [19], but up to now only ESI on sector instruments has reached such a mature state that it is applied for daily routine analysis.

The new API source for sector instruments is based on a similar design for quadrupoles [20]. The main components are (Fig. 1): a heated metal capillary, patented by Chowdhury et al. [21], a tube lens, a skimmer and a radio frequency (rf)-only octapole. The octapole has a high transmission for ions and is nearly transparent for neutrals, such as liquid vapor- and residual gas-molecules, and therefore is the ideal focusing device for this type of source. It is responsible for the main contribution to the about ten-fold increase in sensitivity compared to the previous API source for sector instruments, based on the Analytica design [11].

The source manifold is entirely built from plastic material, allowing to keep the source elements at ± 5 kV ion acceleration potential and the vacuum pumps in the source region, a 16 m^3/h forepump and two 260 l/s turbomolecular pumps at ground potential.

The API source can be equipped either with an ESI- or with an APCI-sprayer, accepting a large range of flow-rates (1 μl/min up to 1 ml/min or in case of APCI up to 2 ml/min), thus allowing coupling with all modern liquid chromatographic techniques ranging from CZE to HPLC.

Three typical examples from the daily practice are chosen in order to demonstrate the capabilities of API combined with high-resolution sector instruments. These examples cover a relative low molecular mass range (below M_r 4000). An application of this API source for accurate mass determination at a resolution of $R = 20\,000$ on a protein (myoglobin) with a higher M_r resulting in a mass deviation of 1 ppm has already been published [24].

2. Experimental

The new API source in combination with the MAT 95 (example 1) and the MAT 900 (exam-

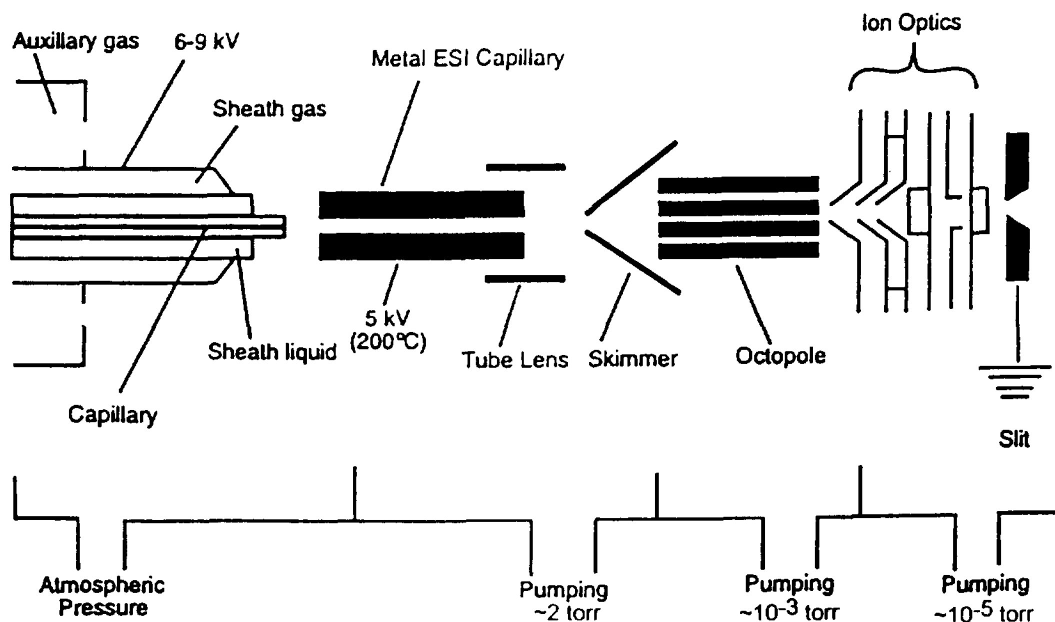

Fig. 1. Schematics of the new API source.

ples 2 and 3) double focusing mass spectrometers, has been used in these experiments. The API source was either equipped with the ESI-sprayer (examples 1 and 2) or with the APCI-sprayer (example 3). In the following examples a resolution $R = 3000$ to $10\,000$ (at 5% peak height) was applied, which was found to be a good compromise between peakwidth and sensitivity for the individual analytical problem; this resolution is much lower than the maximum resolution obtainable in the ESI mode.

3. Results and discussion

3.1. Example 1

Sequence elucidation of peptides by means of ESI–MS, combined with collisional induced dissociation (CID), has become an attractive technique since the introduction of ESI [27]. The following example demonstrates how the specificity of this type of experiment is increased, if a high-resolution sector instrument is applied. The Kaplan peptide with the following sequence (one letter code) was chosen for this demonstration:

S-A-I-S-L-D-G-E-K-V-D-F-N-B-F-R-G-R-A-V-K

N-terminus: free amino; C-terminus: free acid; B: acetylated Thr; the elemental composition (M_r 2352.6) is: $C_{103}H_{166}N_{30}O_{33}$.

With the mass spectrometer tuned to $R = 3000$, the ESI spectrum (Fig. 2) was measured under the following conditions: flow-rate 2 μl/min; solvent water–methanol (1:1); sample concentration 5 pmol/μl, continuous infusion of sample. Transforming the mass-to-charge data from the measured spectrum (Fig. 2) to a plot of relative abundance vs. mass, as per the deconvolution algorithm Biomass™ [25] (see also Ref. [26]), is presented in Fig. 3. It shows the resolved isotope pattern of the peptide in accordance with the theoretical isotope pattern.

A simple and effective method to obtain CID

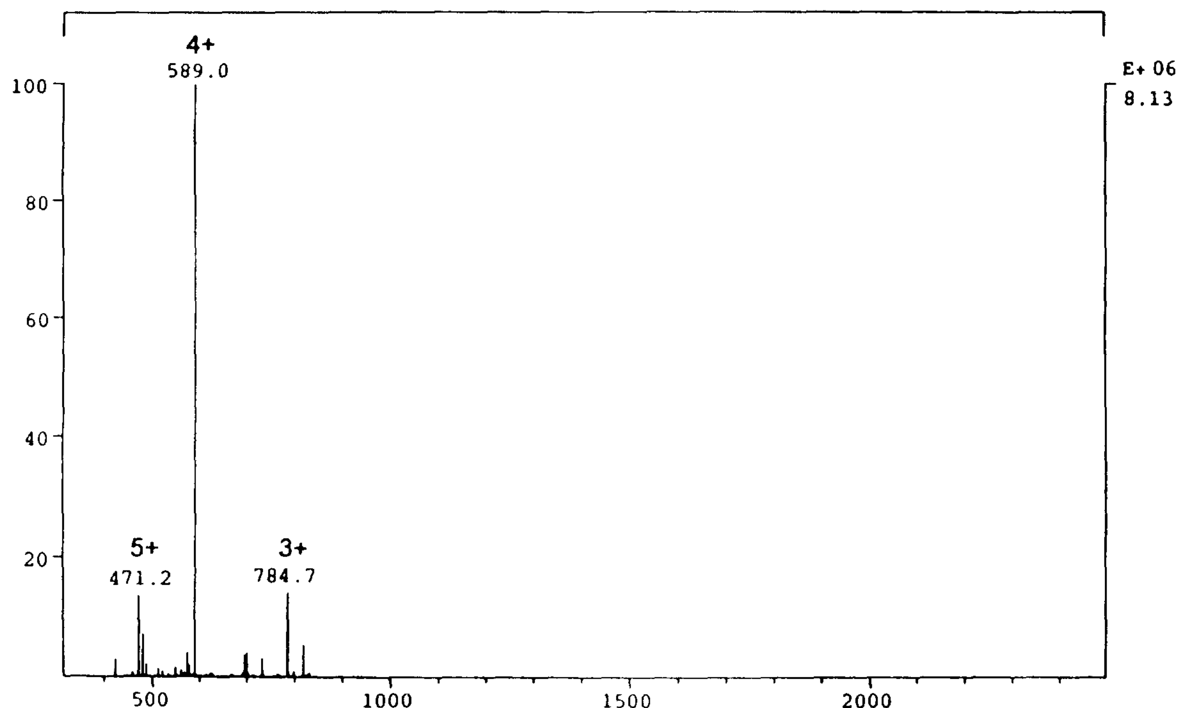

Fig. 2. ESI spectrum of Kaplan peptide, measured at resolution $R = 3000$.

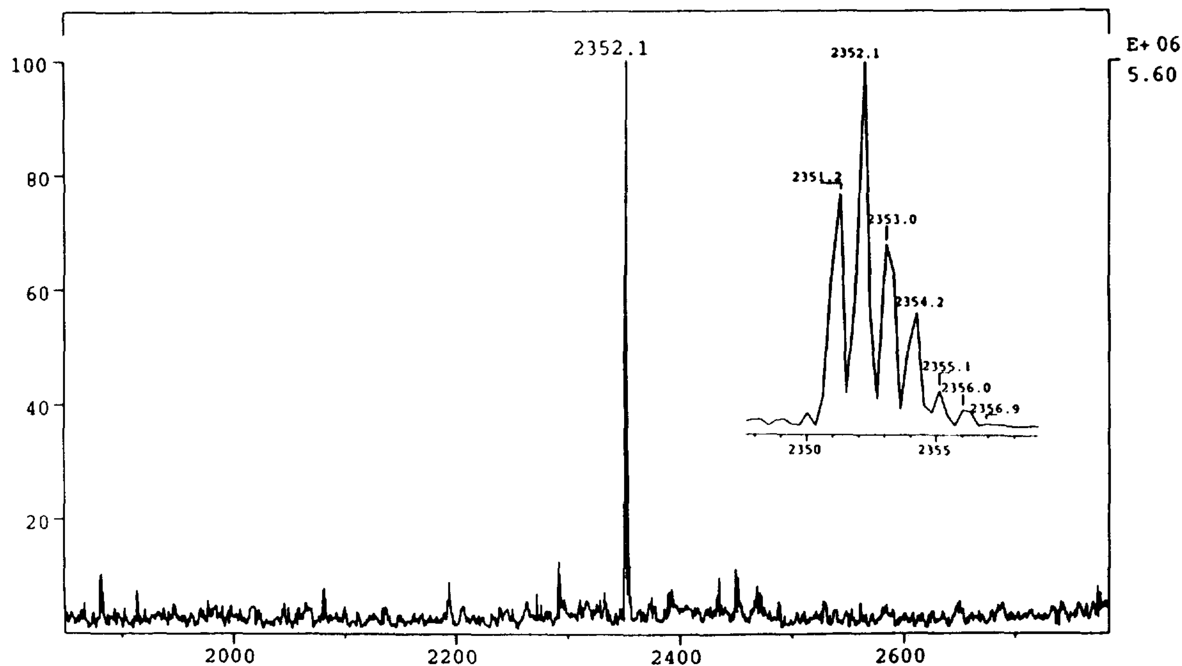

Fig. 3. The Biomass transformation of ESI spectrum from Fig. 2. Inset is expanded section of base peak. The corresponding calculated masses of the Kaplan peptide are: 2351.22 (76.6%), 2352.22 (100%), 2353.22 (69.7%), 2354.23 (34.2%), 2355.23 (13.2%), 2356.23 (4.2%)

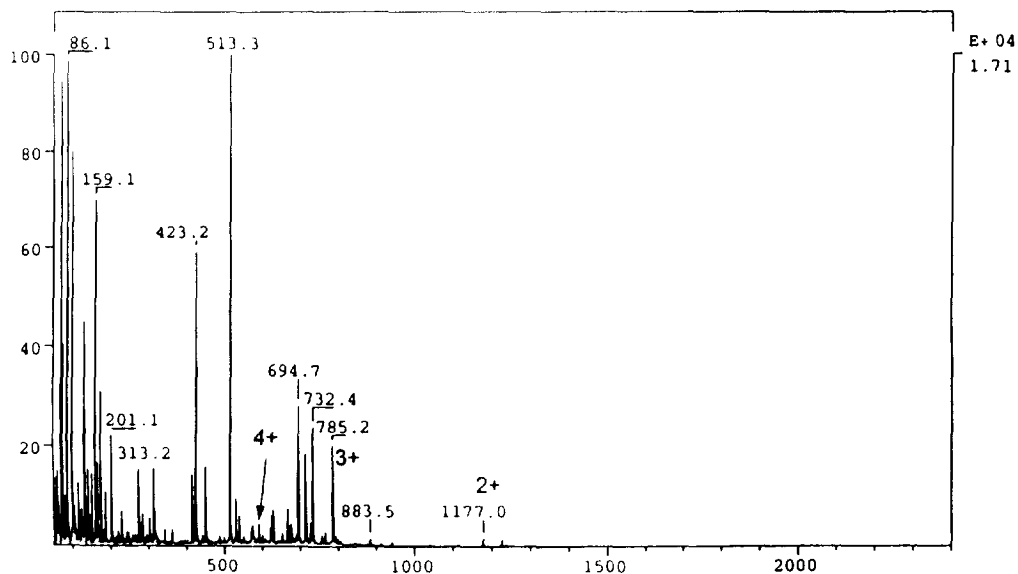

Fig. 4. Octapole CID spectrum of Kaplan peptide at $R = 3000$, mother ions marked by charge number.

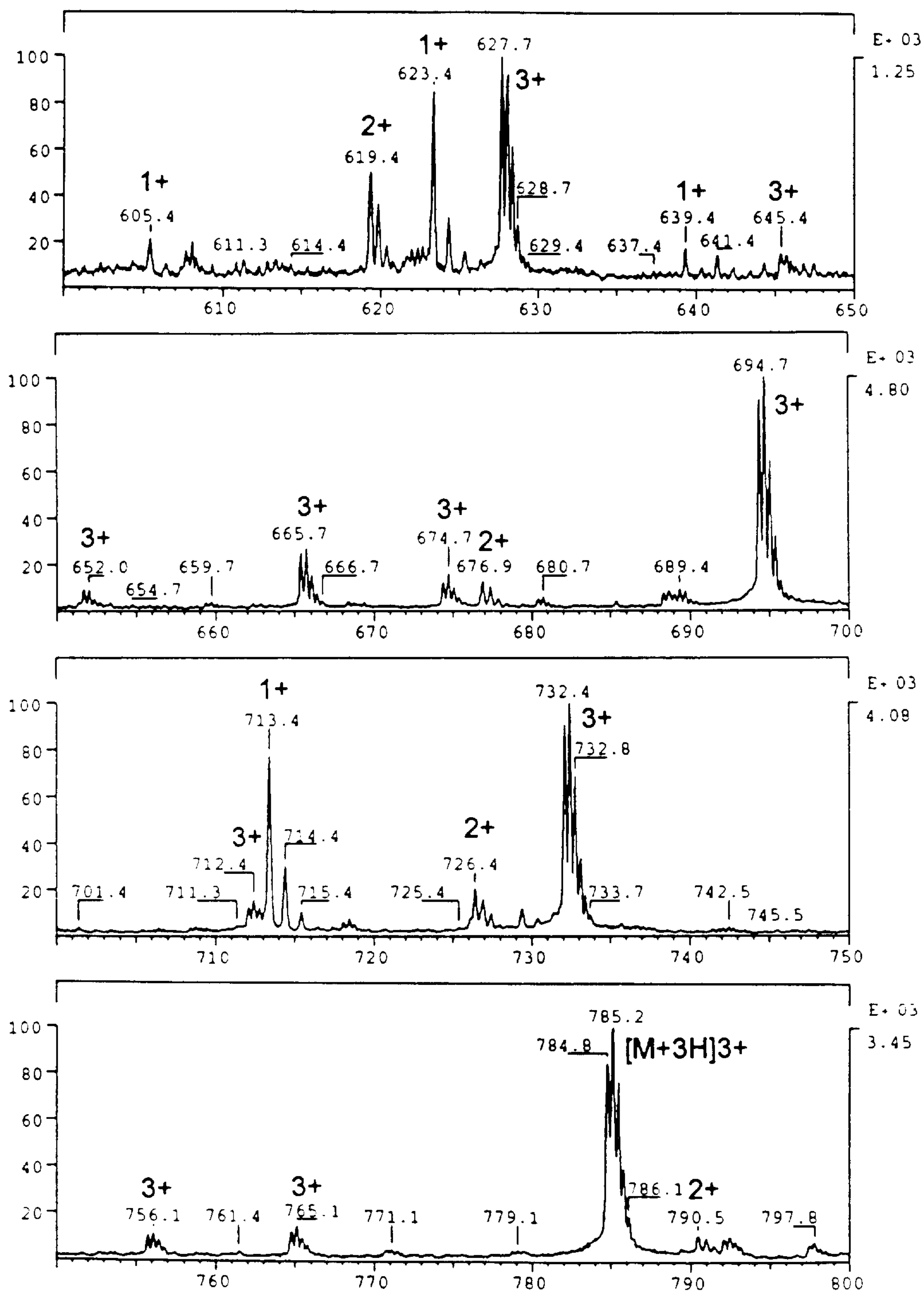

Fig. 5. Enlarged section (higher mass range) of CID spectrum from Fig. 4 showing triply charged mother ion (marked $[M+3H]^{3+}$) and numerous fragment ions with charges between 1+ and 3+

spectra with an API source is octapole CID. For this purpose the potentials of the heated ESI capillary and the tube lens (see Fig. 1) are raised by approximately 50 V for positive ions (lowered by 50 V for negative ions), so that the ions enter the rf-only octapole with an increased translational energy and undergo collisions with the residual gas molecules, a process similar to the one applied in triple-stage quadrupoles.

The daughter ions generated by this CID method appear with the parent ions in the same spectrum, because both types of ions are accelerated in the ion optics of the source to the same final energy (5 kV in case of MAT 95 and MAT 900).

The octapole CID spectrum of the Kaplan peptide (Fig. 4) shows parent ions with charge states 2+ to 4+ as well as numerous daughter ions. The charge state of the parents in this spectrum is one charge unit lower than the charge state of the ions in the normal ESI spectrum (Fig. 2), because of a charge-stripping process within the rf-only octapole.

The daughter ions at the low mass end of the spectrum are all singly charged, so the spectrum is relatively simple to interpret, but the situation changes if one looks at higher masses (Fig. 5). Charge states between 1+ and 3+ appear in the daughter spectrum and spectra interpretation is made much more straightforward on the basis of a high-resolution measurement, where the charge state is made visible by the spacing of the isotope peaks, than on a low-resolution spectrum, where no indication of the charge state is available in the spectrum. In addition the mass values from the high-resolution spectrum are much more exact due to the resolved isotope pattern than the mass values from quadrupole instruments, which very much improves the capability for correct assignment of the fragment ions to fragments of the amino acid chain.

We leave the method of structure elucidation of peptides at this point and refer to the literature (e.g. [27]) for more details.

3.2. Example 2

The sample "*Haemophilus influenzae* A2 LOS" (Table 1) is a complex mixture of 11 lipo-oligosaccharides (LOS); these components behave like detergents in HPLC and CZE and therefore the chromatographic separation is very difficult [28]. High-resolution ESI–MS, without prior chromatographic separation, can help to verify the proposed composition much more easily.

In a first analysis step, using a low-resolution instrument in negative ESI mode, an overlapping of 2 pairs of components in the multiple charged ion peaks was proposed due to the postulated

Table 1
Proposed composition of *Haemophilus influenzae* A2 LOS

LOS	M_r	Proposed composition
A	2277.8	2 Hex, 3 Hep, PEA, P, KDO, lipid A*
B	2438.4	3 Hex, 3 Hep, PEA, P, KDO, lipid A*
C	2561.1	3 Hex, 3 Hep, 2 PEA, P, KDO, lipid A*
D	2600.8	4 Hex, 3 Hep, PEA, P, KDO, lipid A*
E	2723.1	4 Hex, 3 Hep, 2 PEA, P, KDO, lipid A*
F	2762.4	5 Hex, 3 Hep, PEA, P, KDO, lipid A*
G	2925.9	6 Hex, 3 Hep, PEA, P, KDO, lipid A*
H	3086.4	7 Hex, 3 Hep, PEA, P, KDO, lipid A*
I	3249.0	8 Hex, 3 Hep, PEA, P, KDO, lipid A*
J	3256.2	NANA, HexNAc, 5 Hex, 3 Hep, PEA, P, KDO, lipid A*
K	3416.4	NANA, HexNAc, 6 Hex, 3 Hep, PEA, P, KDO, lipid A*

Abbrevations used [28]: Hex = hexose (glucose or galactose); Hep = heptose; PEA = phosphoethanolamine; P = phosphate; KDO = 2-keto-3-deoxy-D-*mano*-octulosonic acid; NANA = 5-N-acetyl-neuraminic acid (sialic acid); HexNAc = N-acetyl-hexosamine; lipid A* = diphosphorylated O-deacylated lipid A

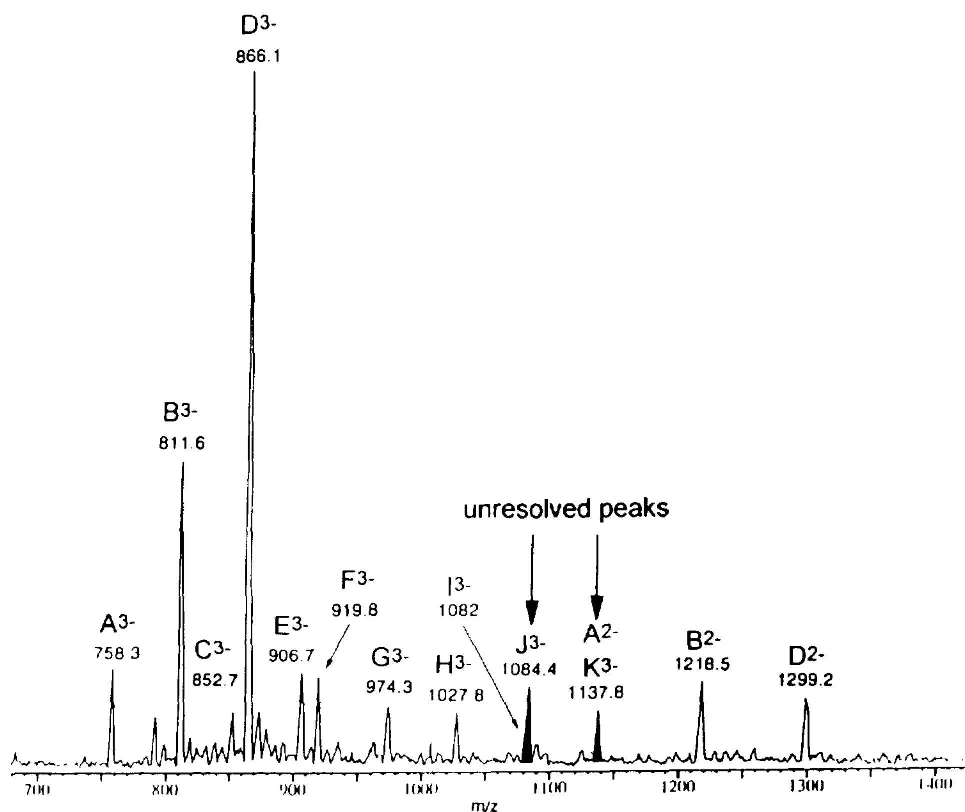

Fig. 6. Low-resolution negative ion ESI spectrum of *H. influenzae* A2 LOS.

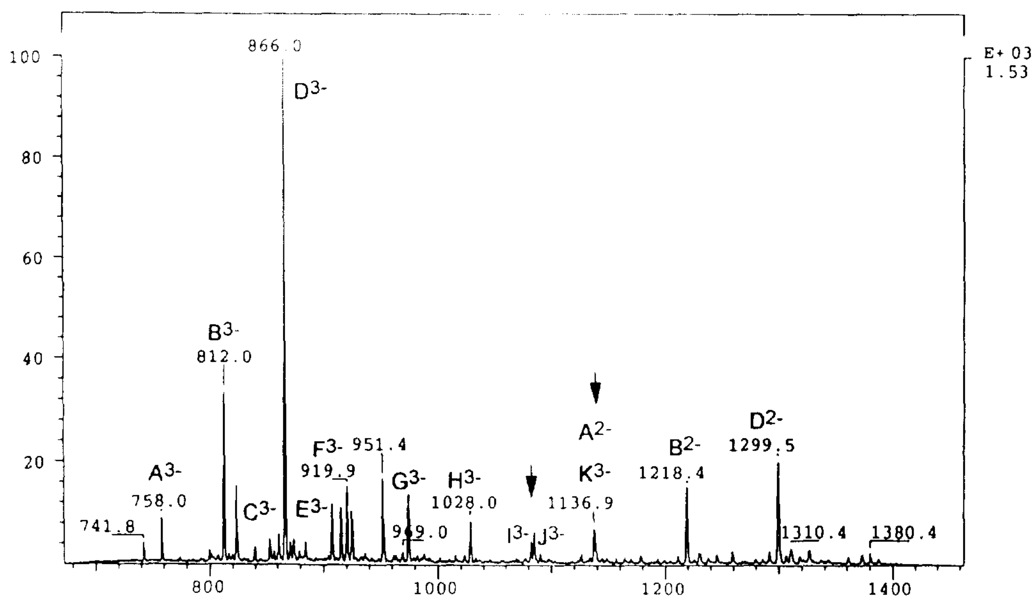

Fig. 7. Negative ion ESI spectrum of *H. influenzae* A2 LOS, obtained with sector instrument at $R = 2500$.

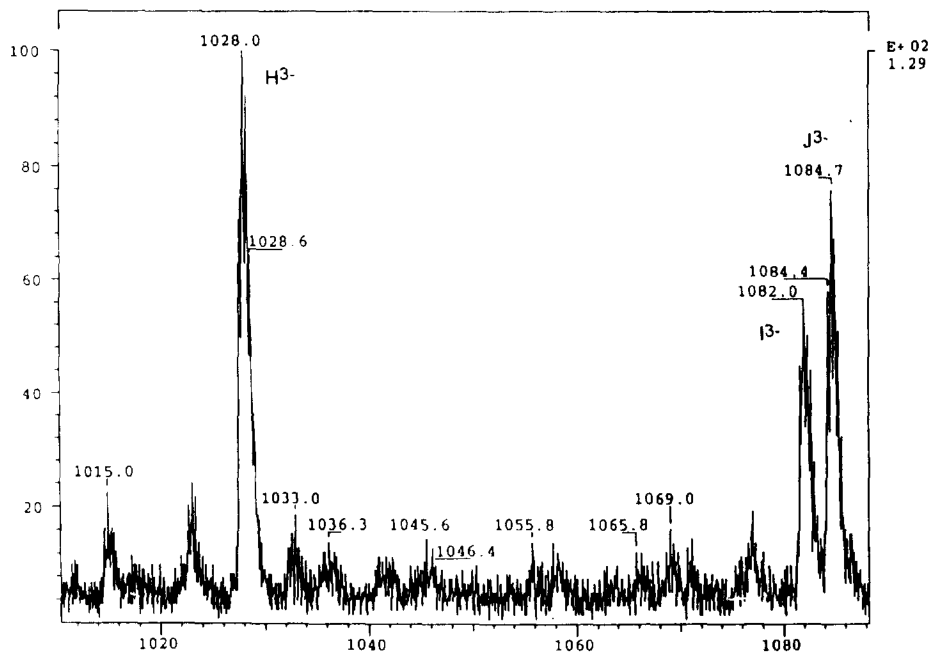

Fig. 8. Enlarged section of ESI spectrum from Fig. 7, showing mass resolved peaks I^{3-} and J^{3-}.

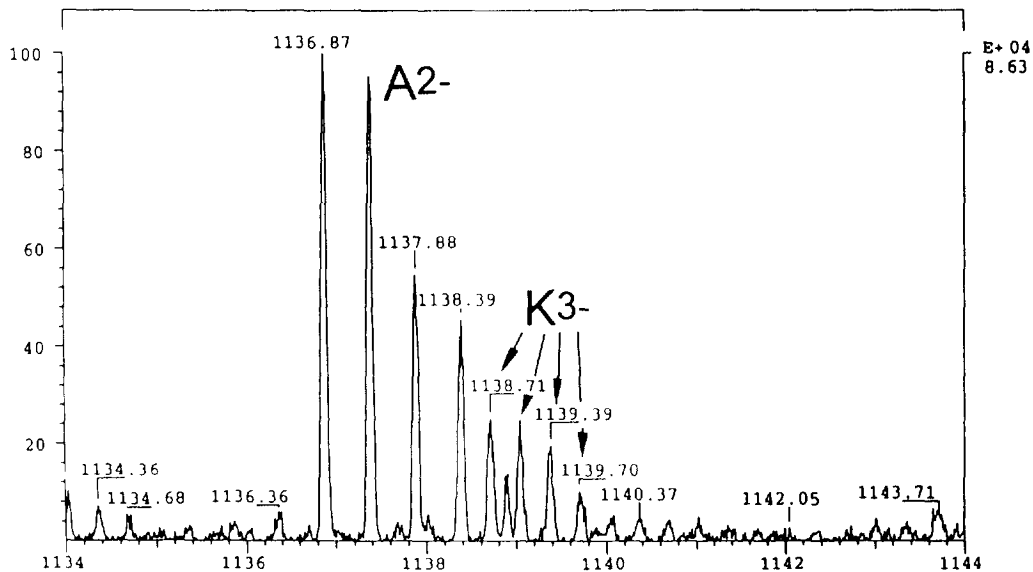

Fig. 9. Enlarged section of high-resolution negative ion ESI spectrum at $R = 10\,000$, showing resolved isotope clusters of components A and K.

structure (Fig. 6), hindering the unequivocal conformation of the proposed structure.

For analysis on a MAT 900 sector instrument, the sample was dissolved in H_2O at a concentration of 2 $\mu g/\mu l$ total and 20 μl of this solution was injected into the flow to the ESI-sprayer. The ESI-sprayer was operated in negative-ion mode at a flow-rate of 10 $\mu l/min$ of H_2O–CH_3CN (1:1) with 1% acetic acid.

A first measurement using the position and time resolved ion counting detector (PATRIC™ [29]) at a resolution of $R = 2500$, entirely separated two of the interfering peaks, I^{3-} and J^{3-}, but the other pair of peaks was still unresolved (Figs. 7 and 8).

Switching to the point detector (slit and secondary electron multiplier) and setting the resolution to $R = 10\,000$ helped to separate also the other pair of overlapping peaks, A^{2-} and K^{3-} (see Fig. 9), allowing verification of all of the 11 components of the proposed mixture.

3.3. Example 3

High specificity and high sensitivity for pesticides are required for the analysis of drinking water as well as residual analysis of food. Because these compounds are very labile and tend to fragment applying other ionization techniques [30,31], APCI allows the measurement of underivatized pesticides with good sensitivity. In

Table 2
List of pesticides used

A	Metoxuron	H_3C, Cl, NHCON – CH_3, OCH_3
B	Monuron	Cl, NHCON – CH_3, CH_3
C	Monolinuron	Cl, NHCON – CH_3, OCH_3
D	Chlortoluron	Cl, H_3C, NHCON – CH_3, CH_3
E	Metobromuron	Br, NHCON – CH_3, OCH_3
F	Metabenzthiazuron	S, N, CH_3, NCONH – CH_3
G	Isoproturon	$(CH_3)_2CH$, NHCON – CH_3, CH_3
H	Diuron	Cl, Cl, NHCON – CH_3, CH_3
I	Linuron	Cl, Cl, NHCON – CH_3, OCH_3

order to demonstrate the capabilities of a high-resolution mass spectrometer with this respect, the following analysis was performed on a MAT 900 sector instrument using the point detector at a resolution of $R = 5000$.

An artificial mixture of 9 pesticides in water (Table 2) at different concentrations (see Fig. 11) under the following LC conditions was used as the test mixture for the LC–MS experiments:

–HPLC: pump, LDC 4100 MS; flow-rate, 0.7 ml/min; solvent, water–methanol (1:1); column, Supelcosil RP-18; sample concentration, 1–1000 pg/μl per component; injector volume, 20 μl

–APCI: vaporizer temperature, 300°C; capillary temperature, 170°C; corona voltage, 3 kV.

–Reference: sample, PPG 400 (4 ng/μl); flow-rate, 30 μl/min (added postcolumn).

Poly propylene glycol (PPG) as an internal mass calibrant was added postcolumn. The aims of this experiment were sample identification (step 1) and sample quantitation (step 2).

For step 1 the mass spectrometer was operated in full-scan mode, using electrical scanning in the mass range 185 to 270.

For the reconstructed ion chromatogram (RIC) in Fig. 10 a sample concentration of 1 ng/μl per component (20 ng injected) was applied.

Averaged spectra taken from the peak regions were mass calibrated using the PPG masses (M_r 193 or M_r 251). For all calibrated spectra the elemental compositions of the used pesticides were found with an average mass deviation of 1 to 2 milli mass units (mmu) (Table 3).

In step 2 the mass spectrometer was operated in the multiple-ion detection (MID) mode in order to find the detection limits for the different pesticides. Five time windows have been defined to get the utmost sensitivity by a maximum dwell time. The sum of all MID windows for three separate MID runs (Fig. 11) demonstrates that the detection limits for the individual pesticides are between 0.5 and 5 pg/μl (10 and 100 pg).

In additional experiments the response curves

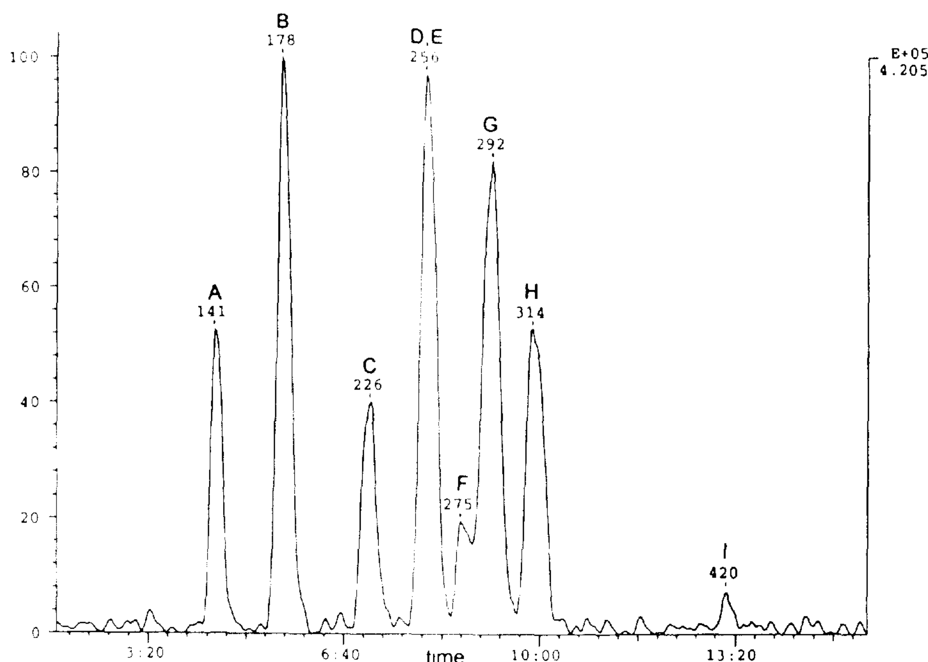

Fig. 10. Background subtracted RIC trace for HPLC separation of pesticide mixture, measured at $R = 5000$.

Table 3
Elemental compositions related to mass spectra taken from peaks A–I of Fig. 10, calculated and observed mass of the most abundant isotope peak

Peak	Elemental composition	Mass calculated	Mass observed
A	$C_{10}H_{14}N_2O_2Cl$	229.0744	229.0747
B	$C_9H_{12}N_2O_1Cl_1$	199.0639	199.0655
C	$C_9H_{12}N_2O_2Cl_1$	215.0587	215.0569
D	$C_{10}H_{14}N_2O_1Cl$	213.0794	213.0800
E	$C_9H_{12}N_2Br_1O_2$	259.0082	259.0103
F	$C_{10}H_{12}N_3O_1S_1$	222.0701	222.0696
G	$C_{12}H_{19}N_2O_1$	207.1498	207.1506
H	$C_9H_{11}N_2O_1Cl_2$	233.0248	233.0251
I	$C_9H_{11}N_2O_2Cl_2$	249.0198	249.0233

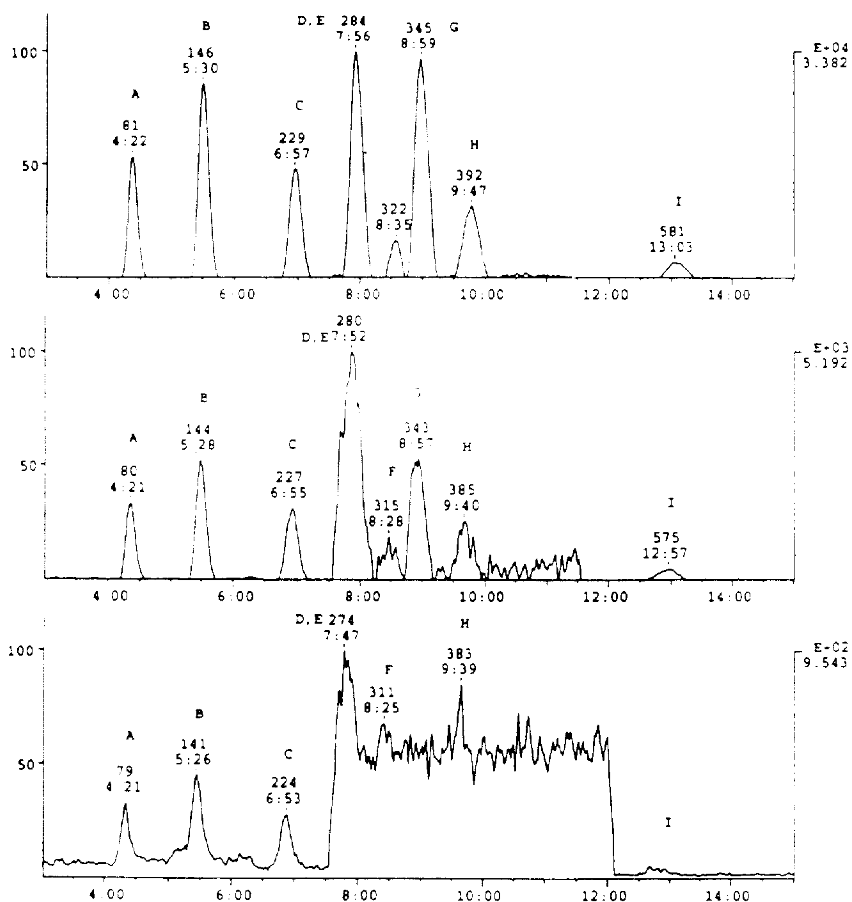

Fig. 11. ID traces for different dilutions of pesticide mixture: upper, 100 pg/μl (2 ng); middle, 10 pg/μl (200 pg); lower, 1 pg/μl (20 pg); measured at $R = 5000$.

for the 9 pesticides have been determined by performing double injections of the pesticide mixture at four different concentrations between 1 and 1000 pg/μl onto the HPLC column. The response curves are all absolutely linear and typically a standard deviation of better than 2% was achieved.

4. Conclusions

High-resolution mass spectrometry is complementing the capabilities of modern API sources by providing additional information, which is not available on quadrupole instruments, such as the unequivocal determination of the charge state of multiple charged ions and a more exact mass value, both making sequence elucidation of peptides much more straightforward.

Complex sample mixtures can be analyzed by high-resolution ESI–MS without prior chromatographic separation, which might be the only choice in cases where chromatographic separation is not possible.

The new API source equipped with the APCI sprayer allows to perform LC–MS measurements of underivatized pesticides in the low picomol range with good reproducibility and accuracy. Detection limits in MID mode range from 10 to 100 pg.

API on sector instruments has become a routine ionization technique, which is replacing more and more the former LC–MS methods like TSP, PBI and CFFAB.

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