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Short communication

Application of liquid chromatography–atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer

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Abstract

Combined liquid chromatography–mass spectrometry (LC–MS) is increasingly used as a powerful technique in research and quality control. Among thermospray, FAB and electrospray, atmospheric pressure chemical ionization (APCI) is a powerful technique for high flow-rate LC–MS. APCI has primarily been used with a quadrupole mass spectrometer, where only low mass resolution experiments are possible. In this article we describe a newly designed APCI source for high-accelerating-voltage and high-resolution double focusing mass spectrometers. Detection in the pg range is possible.

1. Introduction

Atmospheric pressure chemical ionization (APCI) is a powerful technique for high flow-rate liquid chromatography–mass spectrometry (LC–MS). However, APCI has primarily been used with quadrupole mass spectrometer, and LC–MS analysis was made under low mass resolutions.

We designed and constructed a new APCI source for high-ion-accelerating voltage and high-resolution double focusing mass spectrometers.

2. Experimental

All data shown were obtained with a JEOL JMS-SX102A reversed geometry (BE) mass spectrometer operating at an accelerating voltage of 5 kV. Flow injection and HPLC analysis were carried out by using an HP 1090 LC system. The flow-rate was 1 ml/min into the APCI source. The absorbance was measured by placing a UV detector between the column and the APCI interface. The APCI ion source is constructed with a heated nebulizer, a discharge electrode and skimmers (Fig. 1). Skimmer 1 has an aperture of 300 μm in diameter and skimmer 2 of 400 μm. Skimmers and lenses are all on axis to obtain high transmission. No contamination of

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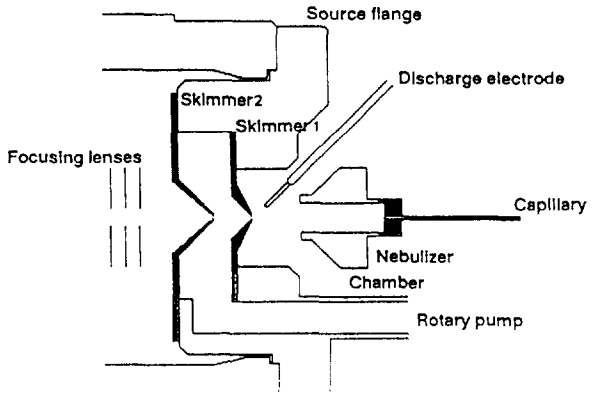

Fig. 1. Schematic block diagram of the APCI source.

the analyzer by neutral fragments is to be expected since we are using a sector mass spectrometer. Two experiments were performed. In the reserpine experiment a Develosil ODS-5 150 mm × 4.6 mm I.D. column was used with a flow-rate of 1 ml/min, a mobile phase consisting of water–methanol (30:70), an internal standard of 100 ppm PEG300–methanol, and a resolution of 1000. The nebulizer temperature was 300°C, the ion source chamber temperature was 400°C, and the accelerating voltage was +5 kV. No nebulizer gas was used. A calibration curve of reserpine between 10 pg and 1 ng was made to test the linearity.

In the second experiment we used four different hormones (cortisone, hydrocortisone, corticosterone, progesterone). We injected 10 μl of a mixture of 100 ppm each on a Develosil ODS-5 150 mm × 4.6 mm I.D. column at a flow-rate of 1 ml/min, a mobile phase of water (A)–methanol (B), with a linear gradient of B = 50–90% in 15 min, an internal standard of 100 ppm PEG300–methanol, and a resolution of 3000. The nebulizer temperature was 290°C, the ion source chamber temperature was 400°C and the accelerating voltage was +5 kV. No nebulizer gas was used.

3. Results and discussion

Fig. 2 shows a typical positive APCI mass spectrum of reserpine (M_r = 608) which gave an intense protonated molecular ion. No characteristic fragment peaks of reserpine were detected. The small ion peaks present are due to not fully subtracted background noise. Fig. 3 shows the selected-ion monitoring (SIM) chromatogram of reserpine from 10 pg to 1 ng. Fig. 4 presents a calibration curve of reserpine for the same concentration range as in Fig. 3. Excellent linearity is obtained. This result provides significantly better sensitivity on sector MS, also shown in Fig. 3.

The use of high-resolution accurate mass measurements in APCI LC–MS allows the determination of elemental compositions of the ions

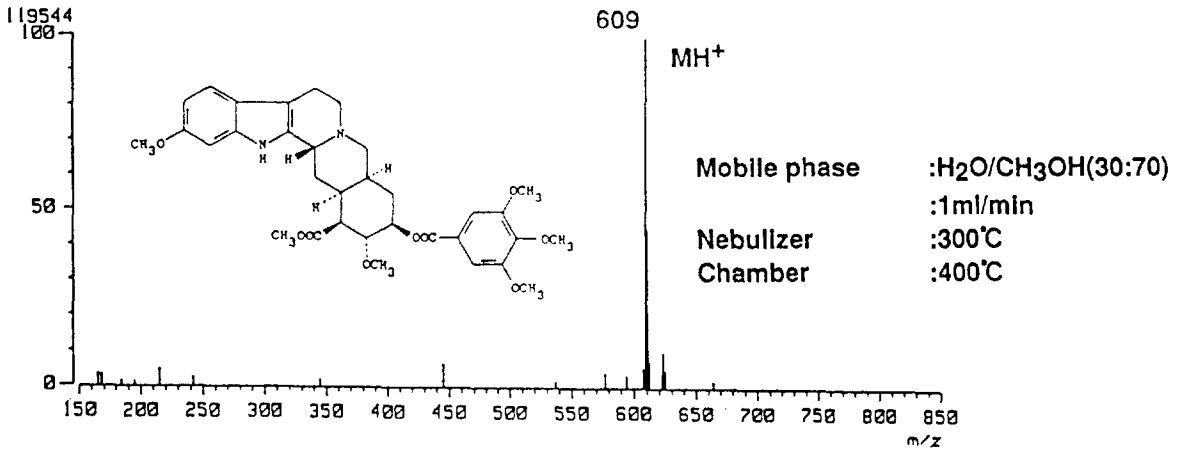

Fig. 2. APCI spectrum of reserpine.

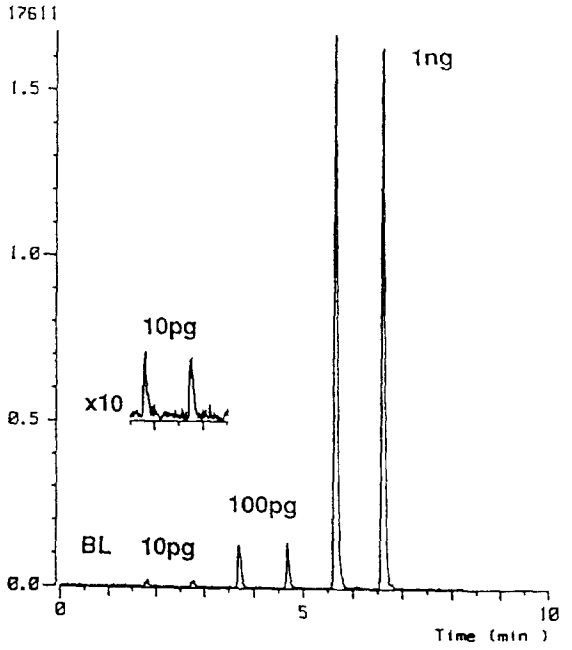

Fig. 3. Selected-ion monitoring (SIM) chromatogram of reserpine (10 pg to 1 ng).

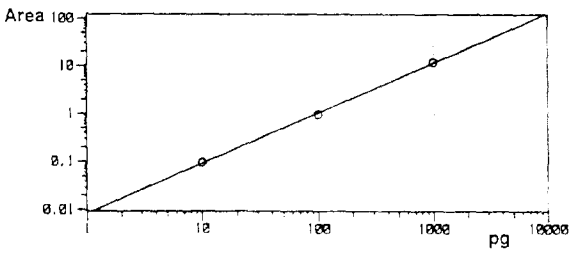

Fig. 4. Calibration curve of reserpine (10 pg to 1 ng).

from the component represented in the LC peaks. Fig. 5 and Table 1 presents the accurate mass measurements of protonated molecules of cortisone, hydrocortisone, corticosterone and progesterone. The observed mass values are typically within 2.6 mmu of the theoretical values

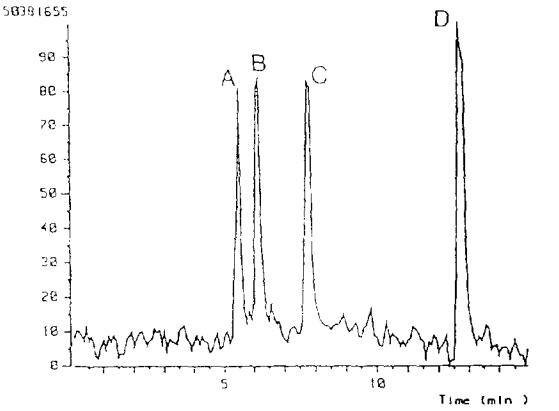

Fig. 5. Total ion current (TIC) of APCI LC–MS measurement.

Table 1
High-resolution accurate mass measurement with APCI LC–MS

Sample	Observed	Theoretical	Error (mmu)
(A) Cortisone	361.2014	361.2015	−0.1
(B) Hydrocortisone	363.2162	363.2172	−1.0
(C) Corticosterone	347.2248	347.2222	+2.6
(D) Progesterone	315.2332	315.2324	+0.8

and the root mean square (rms) error is within 4 mmu (three consecutive injections).

4. Conclusion

APCI LC–MS on sector MS is well-suited for the quantification of reserpine at concentration levels higher than a few picograms.

The selectivity of the detection can be increased by using high-resolution mass spectrometry which also allows accurate mass measurements of the components represented in the LC peaks.