A FAST SWITCHING APCI SOURCE

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OVERVIEW

Aim

- To characterize the performance of an APCI source that facilitates rapid corona current switching for LC/MS/MS sensitivity optimisation over a wide range of sample types.

Method

- An enhanced software algorithm and fast-transient corona power supply have been utilised to acquire multifunctional MRM data where the corona current is switched between scan functions during the interscan delay period.

Results

- The fast switching APCI source has been shown to enhance the signal of verapamil and reserpine by factors of 8.3 and 2.7, respectively, when compared to the conventional APCI acquisition mode.
- This technique has been shown to produce optimum sensitivity data whilst halving the total analysis time and halving the sample consumption.

INTRODUCTION

Atmospheric pressure chemical ionisation (APCI) is commonly used in the analysis of small organic compounds such as pharmaceuticals. These compounds can be broadly placed into two classes, namely highly polar or low to moderately polar analytes. Although electrospray ionisation can be used to analyse many of these compounds, there are cases when APCI ionisation is preferable[1]. However, it is found that the analyte types exhibit significant APCI tuning differences that are strongly dependent on factors such as source geometry, corona current and mobile phase composition[2,3]. The low to moderately polar analytes are typically found to require a high corona current (2-5µA) for efficient ionisation. On the other hand, highly polar analytes are believed to be preionized in the APCI heater tube and can be detrimentally retarded, deflected or dispersed by the field resulting from the corona pin voltage and/or the presence of a high concentration of reagent ions. Traditionally, this has hindered sensitivity optimisation for mixtures that contain both analyte types. Here, we present an experimental fast switching APCI source that eliminates the need to compromise LC/MS/MS sensitivity when analysing such mixtures.

METHODS

Samples and Liquid Chromatography

A sample solution was prepared by mixing the following standards in methanol (final concentrations are shown in brackets): verapamil (10pg/µL), reserpine (10pg/µL), corticosterone (100pg/µL) and hydroxyprogesterone (100pg/µL). 10µL of sample was injected on a Waters Symmetry column (C18, 3.5µm, 4.6 X 50mm) at a flow rate of 1mL/min. Mobile phase composition was A = 80/20 water/methanol and B = methanol. Both A and B contained 10mM ammonium acetate and 0.005% acetic acid. Analytes were eluted using the following gradient: from 35% B to 85% B in 6.5 min, to 90% B in 0.5 min, to 100% B in 0.1 min, held for 0.4 min, back to 35% B in 0.1 min and held for 12.4 min. Total run time was 20 min.

Mass Spectrometry

All data were obtained on a Waters Quattro Premier triple quadrupole MS fitted with a Waters Ion Sabre APCI probe. The Waters Masslynx software was modified to enable corona current switching during the interscan delay period. The APCI source was operated as follows: probe temperature = 650°C, source temperature = 140°C, desolvation gas flow = 350L/hr. All LC/MS/MS data were obtained in 4 channel MRM mode with an interscan delay (ISD) of 100ms, a dwell time of 100ms and an interchannel delay (ICD) of 50ms. The MRM details for the 4 analytes are given in Table 1.

Sample	Parent	Product	Cone volts (V)	Collision energy (V)	Gas cell pressure (mbar)
Reserpine	609.1	195.1	50.0	35.0	0.0023
Verapamil	455.1	165.1	50.0	30.0	0.0023
Corticosterone	347.1	329.1	35.0	15.0	0.0023
Hydroxyprog.	331.1	109.1	35.0	25.0	0.0023

Table 1. The MRM transitions and instrument parameters for the 4 test analytes.

RESULTS

Analyte Tuning

Figure 2 shows the APCI tuning characteristics obtained with the highly polar analyte verapamil for the two different probe positions shown schematically in Figure 1. These data were obtained by infusing a verapamil solution of 0.25ng/µL at 10µL/min into a mobile phase of 65/35 A/B (1mL/min). Figure 2 clearly shows that verapamil is optimised at a corona current of 0.1µA and moreover, a further doubling of the signal intensity is obtained by using the "near tune" probe position (Figure 1). The tuning characteristics for the the low to moderately polar analyte hydroxyprogesterone was determined by infusing a solution of 2.5ng/µL, as described above, and is shown in Figure 3. In contrast, hydroxyprogesterone signal intensity is shown to increase sharply with increasing corona current and optimises between 2-5µA. Only minor tuning differences are observed between the near and far tune positions for hydroxyprogesterone. From a comparison of Figures 2 and 3, it becomes apparent that the near tune probe position would result in the best compromise for all analytes. However, a LC/MS/MS analysis based on a single corona current value would result in significant loss of sensitivity for one or both analyte types.

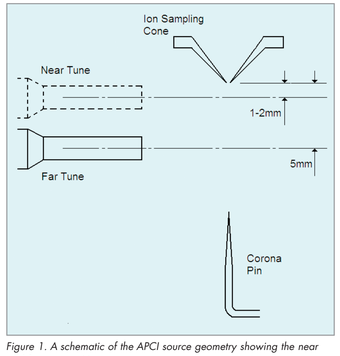

Figure 1. A schematic of the APCI source geometry showing the near and far tune positions.

	Peak Area			
APCI Mode	Verapamil	Corticosterone	Hydroxyprog.	Reserpine
Near Tune, 0.1 µA	57186	396	1704	5228
Near Tune, 5.0µA	8375	3455	9243	2087
Near Tune, fast switching 0.1 and 5.0µA	69746	3312	11200	5726

Table 2. Collated mass chromatogram peak areas for the data shown in Figures 4 and 6.

Conventional Analysis

The tuning characteristics of the highly polar and low to moderately polar analytes imply that conventional LC/MS/MS analysis of the 4 test analytes can only be optimised by acquiring two separate chromatograms, i.e. the first at 0.1µA and the second at 5µA. This type of analysis is shown in Figure 4, where the TICs from two separate MRM acquisitions have been overlaid to aid comparison. As expected, Figure 4 clearly shows that corticosterone and hydroxyprogesterone are optimised at 5µA whilst verapamil and reserpine are optimised at 0.1µA. Table 2 collates the mass chromatogram peak areas obtained for the 4 analytes in both the 0.1 and 5µA acquisitions (rows 1 and 2).

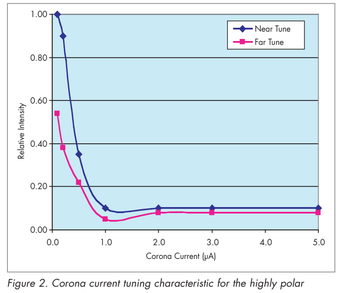

Figure 2. Corona current tuning characteristic for the highly polar analyte verapamil.

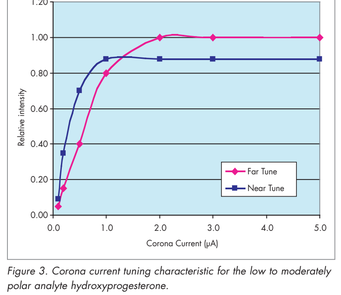

Figure 3. Corona current tuning characteristic for the low to moderately polar analyte hydroxyprogesterone.

Fast Switching Analysis

From a practical viewpoint, it would be desirable to obtain the optimised sensitivity data of Figure 4 in a single LC/MS/MS acquisition, i.e. halving the analysis time and halving the sample consumption. Figure 5 illustrates the fast corona current switching method used in the present study to achieve this objective. This shows how data is acquired by alternately switching between two identical scan functions whilst switching the corona current between 0.1 and 5µA during each interscan delay (ISD). The corona power supply used in this study has a switching characteristic, t_s, of approximately 20ms, which is compatible with typical ISD values for LC/MS (20-100ms). Figure 6 shows the resulting TIC chromatograms obtained from a single acquisition using the fast switching method described above. From a comparison of Figures 4 and 6, it is clear that optimum APCI sensitivity can be obtained from a single acquisition with the concomitant analysis time and sample consumption benefits. Table 2 (row 3) shows the mass chromatogram peak areas obtained for the data in Figure 6, which indicate that no loss in sensitivity is observed with fast corona switching. Furthermore, the peak areas suggest an improvement of factors 8.3 and 2.7 for verapamil and reserpine, respectively, when comparing the fast switching data with data obtained by the conventional practice of operating with a fixed corona current of 5µA (row 2). Finally, the data can also provide information regarding the degree of polarity of each analyte.

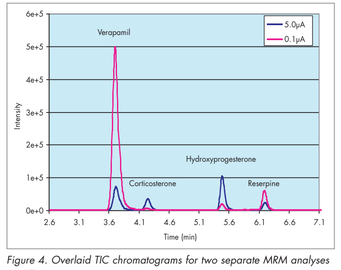

Figure 4. Overlaid TIC chromatograms for two separate MRM analyses at different corona currents.

CONCLUSIONS

- Fast corona current switching can be used to obtain optimum APCI sensitivity for samples containing a mixture of highly polar and low to moderately polar analytes.
- 8-fold signal intensity increases have been observed for verapamil when comparing the fast switching APCI to conventional APCI conducted at corona currents of 5µA.
- Future studies will investigate optimum switching times, limits of quantitation and the linear dynamic range of this technique.

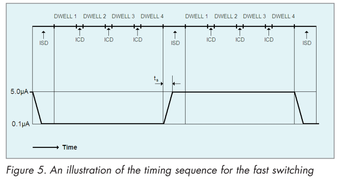

Figure 5. An illustration of the timing sequence for the fast switching APCI source.

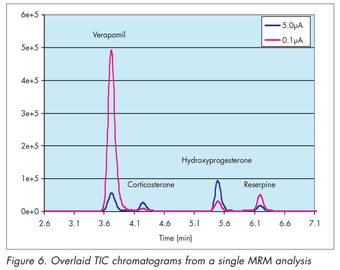

Figure 6. Overlaid TIC chromatograms from a single MRM analysis obtained by rapidly switching the corona current.

REFERENCES

1. Matuszewski BK, Constanzer ML and Chavez-Eng CM, Anal. Chem., 70, 882-889, 1998.
2. DR Doerge, MI Churchwell, LG Rushing and S Bajic, Rapid Comm. Mass Spectrom., 10, 1479-1484, 1996.
3. S Cristoni, L Rossi Bernardi, I Biunno, M Tubaro and F Guidugli, Rapid Comm. Mass Spectrom., 17, 1973-1981, 2003.

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