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ELECTROSPRAY IONIZATION-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION SOURCE FOR USE IN OPEN ACCESS AND HIGH THROUGHPUT LC-MS APPLICATIONS

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INTRODUCTION

Fast and accurate analytical methods are essential to keep pace with sample libraries produced from combinational chemistry and high throughput biological screening. The samples under investigation may span large mass ranges and have considerable differences in chemical structure. Many laboratories now use a combination of ionization techniques for the characterization of these samples including atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and photoionization (PI). Traditionally a change in ionization mode would require a change in instrument ion source and re-optimization of source parameters, resulting in lost analysis time.

In this work we have developed a new combined electrospray ionization – atmospheric pressure chemical ionization source (ESCi™ Multi-Mode Ionization Source*) for use in on-line HPLC applications. The combined source allows alternate on-line electrospray and APCI scans within a single analysis with polarity switches.

METHODS

The new combined Waters® Micromass® ESCi Multi-Mode Ionization Source has been designed to be a simple replacement for the existing mass spectrometer interfaces. This ESCi Multi-Mode Ionization Source uses the existing Waters ESI source hardware with the addition of the APCI discharge needle and a modified high voltage power unit to supply it. During operation the high voltage power supply alternatively switches from the electrospray capillary to the APCI discharge needle with good fidelity at very fast interscan delay times. In these experiments, this was set to 0.1 or 0.2 seconds.

A 96-well plate containing a variety of samples covering molecular weights from 150 to 500 amu were analyzed using the short gradient LC/UV/MS method outlined. Existing APCI and ESI sources were evaluated and then the plate was reanalyzed on the same instrument (Waters Micromass ZQ™ Mass Detector) using the combined system.

*Patent pending

LC Method

Instrument: Waters Alliance® HT
Column: Waters XTerra® MS 2.1 mm x 30 mm @ 30 °C
Mobile Phases: A=water, B=acetonitrile, C=50/50 water/acetonitrile with 1% formic acid

Gradient Conditions:

Time	Flow	%A	%B	%C
0.00	0.35	95	0	5
1.50	0.70	0	95	5
2.50	0.70	0	95	5
2.51	0.70	95	0	5
3.00	0.70	95	0	5

Injection Volume: 1.0 mL in methoxyethanol

UV Method

Waters 2996 Photodiode Array Detector scanning 225 to 320 nm.

MS Method

Each ionization method was optimized independently using separate source tuning parameters (capillary voltage or APCI pin voltage, sample cone and extraction cone). Common tuning parameters include all gas flows and interface temperatures.

Instrument: Waters Micromass ZQ Mass Detector
Ionization: ESI, APCI and ESCi
Scan: 150 to 800 amu, 0.2 sec/scan, 0.2 sec interscan time
Cone Voltage: 20 volts throughout
Total Run Time: 3 minutes
Scan Mode: ESI source, alternate +/- switching
APCI source, alternate +/- switching
ESCi Multi-Mode Ionization Source, +/- switching and ionization mode switching (ESI+/ESI-/APCI/APCI+)

MICROMASS® MS TECHNOLOGIES

RESULTS

ESCi Multi-Mode Ionization Source Optimum Flow Rate Evaluation

Diphenhydramine ionizes readily by both ESI and APCI. Repeat injections of diphenhydramine at various eluant flow rates from 50 to 1000 mL/min were performed on the ESCi Multi-Mode Ionization Source in single mode operation. Figure 1 shows the relationship between ion signal intensity versus flow rate for each ionization method.
This data is comparable for standard ESI and APCI systems showing optimal performance is not significantly compromised at extremes of flow rate.

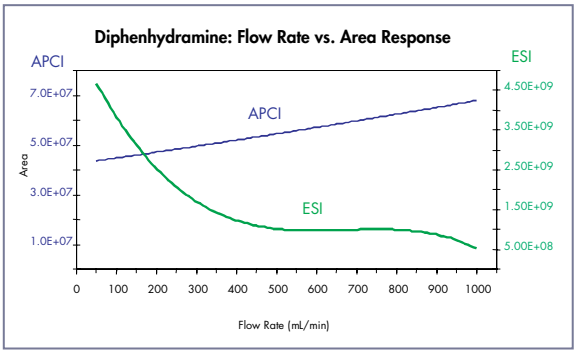

Figure 1: Optimum flow rate evaluation for the ESCi Multi-Mode Ionization Source

Compound Collection

The driving force behind the development of this new ESCi Multi-Mode Ionization Source is for use in the analysis of the AstraZeneca Alderley Park solid compound collection. This requires the characterization of some 500,000 samples. Identification criteria require that samples pass the three following requirements:
(1) a UV peak greater than a determined height (to prove sample injection),
(2) correct molecular weight determined from the positive and/or negative mass spectrum,
(3) purity of greater than 85% determined from the UV

Analytical results to date show that the majority of samples in this collection will ionize by ESI. However, past analysis required a change from the ESI source to the APCI source at the end of a plate run to analyze a further 5-10%. A 96-well plate containing a variety of samples covering molecular weights from 150 to 500 amu were analyzed using a short gradient HPLC method. Existing APCI and ESI sources were evaluated and then the plate was reanalyzed on the same instrument (Waters Micromass ZQ Mass Detector) using the combined source. Figures 2a, 2b and 2c show example data obtained from the compound collection samples.

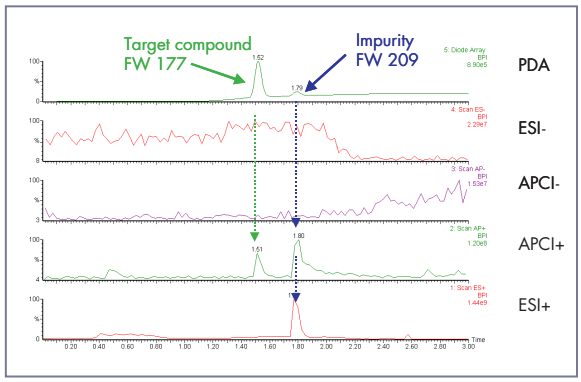

Figure 2a: Example data from the Compound Collection_1: Major Peak (RT 1.52 min) is ionized in APCI+ mode only. Minor impurity (RT 1.79 min) ionizes in both APCI+ and ESI+ modes

In Figure 2b a comparison of the data obtained by the ESCi Multi-Mode Ionization Source to the single mode sources is shown for a compound of molecular weight 319 amu that ionizes in all four modes. In this example the protonated molecular ion, adduct ions and major fragment ions are equivalent in the ESI source and the ESCi Multi-Mode Ionization Source operating in ESI mode for both polarities (top four spectra in Figure 2b). Some additional fragment ions are produced in the ESI source for both ionization modes. The APCI data obtained does show significant differences. The major

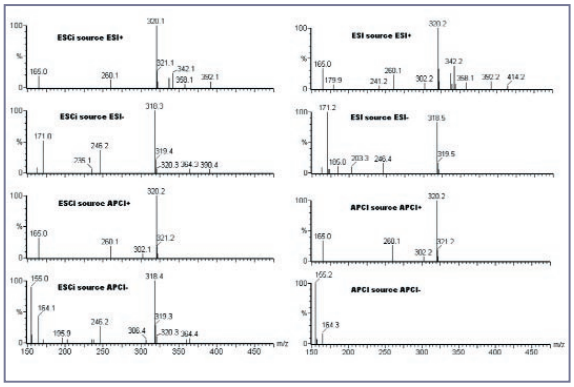

Figure 2b: Example data from the Compound Collection_2: Comparison of mass spectral data obtained for a compound of 319 amu analyzed by ESCi multi-mode ionization source (left column), ESI source (upper right) and APCI source (lower right).

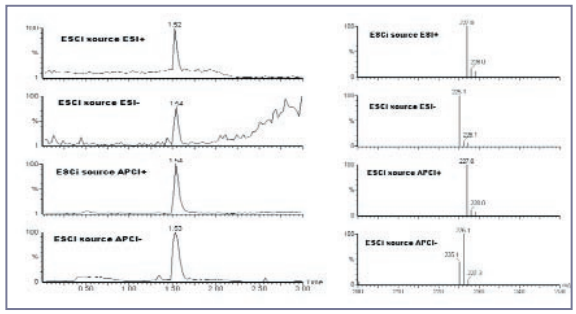

Figure 2c: Example data from the Compound Collection_3: This example demonstrates a compound of molecular weight 226 amu. Under negative ion APCI conditions a deprotonated and a radical cation are produced.

peaks in positive ion APCI mode are similar for both sources. In negative ion mode the APCI source produced only fragment ions (m/z 155 and 164) compared to the ESCi Multi-Mode Ionization Source, which produced a deprotonated molecular ion (m/z 318 [M-H]-) as the base peak. This is most likely due to the standard APCI source producing more thermal degradation, which is a disadvantage in this application where a pseudo molecular ion is desired for initial identification purposes.

The full results obtained for the plate analysis are as follows:

Plate Summary

	ID	Tentative	Reject
APCI	72	10	14
ESI	71	6	19
ESCi	80	5	11

- Project = 500,000 compounds
- ESCi improvement 10%
- Results in 50,000 more 'found' using the single ESCi technique that would otherwise have to be re-analyzed

CONCLUSIONS

A 96-well plate containing a variety of samples covering molecular weights from 150 to 500 amu were analyzed using a short gradient HPLC method.

- Existing APCI and ESI sources were evaluated and then the plate was reanalyzed on the same instrument using the combined system.
- The instrument electronics can readily switch between the two different methods and polarities within a 100 millisecond interscan time period.
- The qualitative performance of the combined source has been compared to the existing electrospray and APCI interfaces and found to be equivalent.

The new ESCi Multi-Mode Ionization Source has greatly reduced the analysis time of sample plates by eliminating the need for repeat analysis. The ESCi Multi-Mode Ionization Source is to be introduced onto open access systems.

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