MS^n Analysis With Fast Polarity Switching in the Agilent 1100 Series LC/MSD Trap SL

Application Note

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Introduction

During mass spectrometric analysis, many compounds respond better to a particular ionization mode and produce mostly positive or mostly negative ions. This creates an analytical challenge when a sample mixture contains both analytes that favor positive ionization and analytes that favor negative ionization.

Early liquid chromatography/mass spectrometer (LC/MS) systems could typically operate in only one ionization mode during a particular run. Sample mixtures sometimes required two runs, one in positive mode and one in negative mode, to ensure that all analytes were detected. Instrument improvements made it possible to devote separate chromatographic time segments during a single run to positive and negative ionization polarity. This solution is all that is needed if retention time and optimum polarity are know for each analyte.

Unknown mixtures obviously present a greater challenge. To meet this challenge, many modern mass spectrometers have the ability to switch polarities during the span of a single chromatographic peak. This ability is a tremendous time-saver because it enables a single run to detect unknown analytes in a mixture regardless of their preferred polarity. The faster a mass spectrometer can switch polarities, the more scans it can acquire from a particular chromatographic peak and the better the data quality will be.

One of the great advantages of modern quadrupole ion trap mass spectrometers is their ability to automatically perform multiple generations of data-dependent MS. This can provide both MS data to identify the mass of the analyte and MS^n data to provide structural information for positive identification. In general, the more MS and MS^n scans that can be performed over a chromatographic peak, the better the data will be and the more sensitive the analysis.

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Unfortunately, polarity switching can have a limiting effect on data-dependent MS^n. If polarity switching is not fast enough, an ion trap cannot collect enough scans over a chromatographic peak to perform multiple levels of MS with good results.

This application note demonstrates the ability of the Agilent 1100 Series LC/MSD Trap SL ion trap mass spectrometer to acquire positive and negative spectra and still perform automated, data-dependent MS^4 with excellent results thanks to extremely fast (≈ 0.5 seconds) polarity switching.

Experimental

All experiments were performed using an Agilent 1100 Series LC/MSD Trap SL equipped with a PhotoMate atmospheric pressure photoionization (APPI) source developed by Syagen Technology Inc. in cooperation with Agilent Technologies. The APPI source uses photons from a krypton lamp to initiate the ionization process. Negative mode APPI requires the addition of a source of thermal electrons, which in this example was provided by the acetone in the mobile phase.

A mixture of estradiol, diethylstilbestrol (DES), and progesterone was systematically analyzed by MS in positive mode, negative mode, and alternating positive/negative mode. Automated, data-dependent MS^n analyses were performed with positive and negative time segments and with alternating positive and negative scans.

Instrument Conditions

LC Conditions

Column:	ZORBAX SB-C18, 2.1 x 50 mm, 5 µm (860975-902)
Column temp.:	40°C
Flow rate:	0.4 ml/min
Injection volume:	3 µl
Sample:	estradiol (600 µg/ml); DES (300 µg/ml); and progesterone (100 µg/ml)
Mobile phase:	A = water, B= acetone
Gradient:	40% B at 0 min 70% B at 10 min

MS Conditions

Ionization mode:	Positive and negative APPI
Nebulizer:	60 psig
Drying gas flow:	5 l/min
Drying gas temp.:	250°C
Capillary voltage:	3000 V
Skimmer 1:	40 V
Capillary exit:	100 V
Trap drive:	42
Scan:	50–600 *m/z*
ICC on:	Target 20000; Maximum accumulation time 50 ms
Averages:	6 for MS, 4 for MS^n

Results and Discussion

Figure 1 shows results from the MS analyses in negative, positive, and alternating modes. All three compounds were ionized by APPI. Estradiol and DES ionized well in negative mode, while progesterone ionized well in positive mode. Polarity switching in the alternating positive/negative mode was fast enough to provide more-than-adequate sampling across chromatographic peaks.

A comparison of polarity switching using time segments versus alternating positive and negative scans is shown in Figure 2. Both of these analyses were performed successfully in combination with MS^n. Time segment analysis, however, would not have been possible without the previous analyses to determine retention times and optimum polarities for the three analytes.

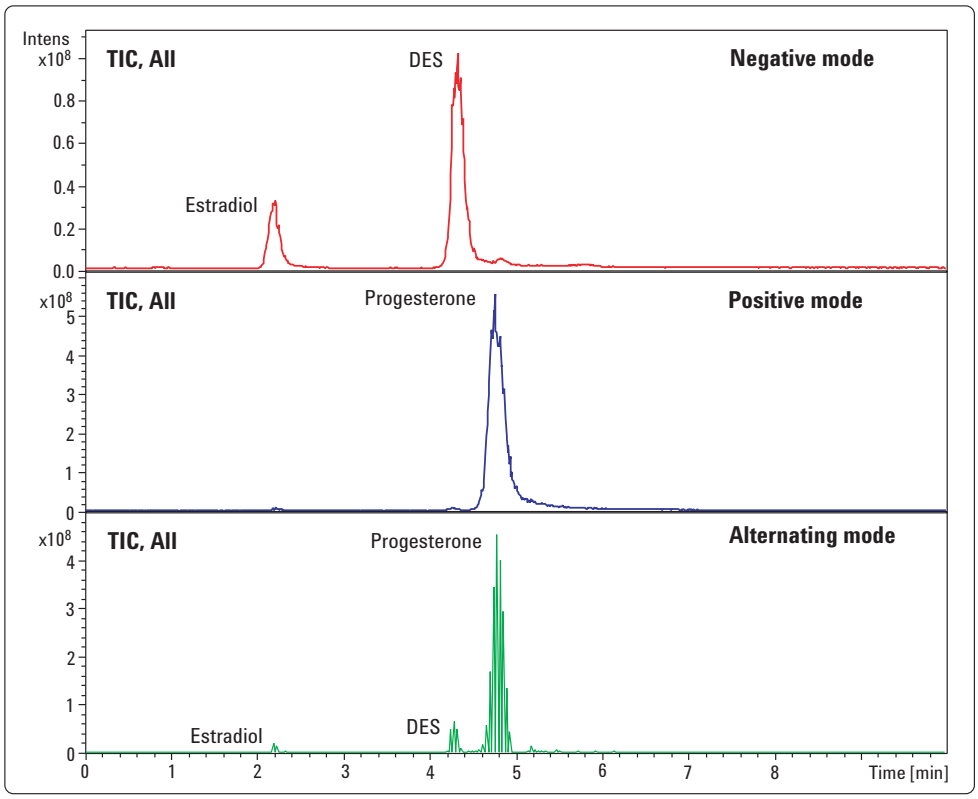


Figure 1. Comparison of positive, negative, and alternating modes of detection

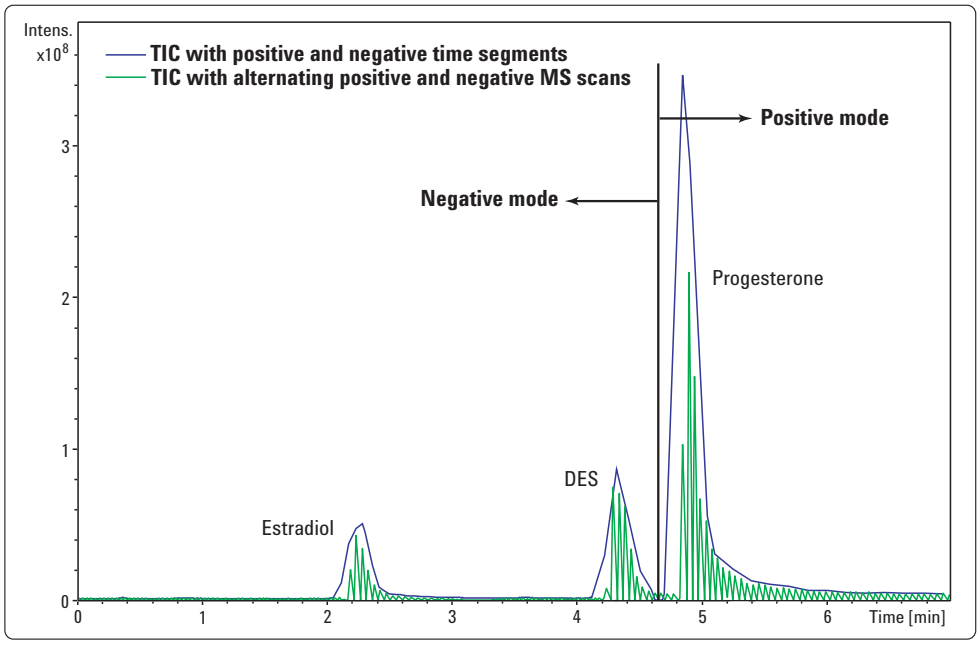
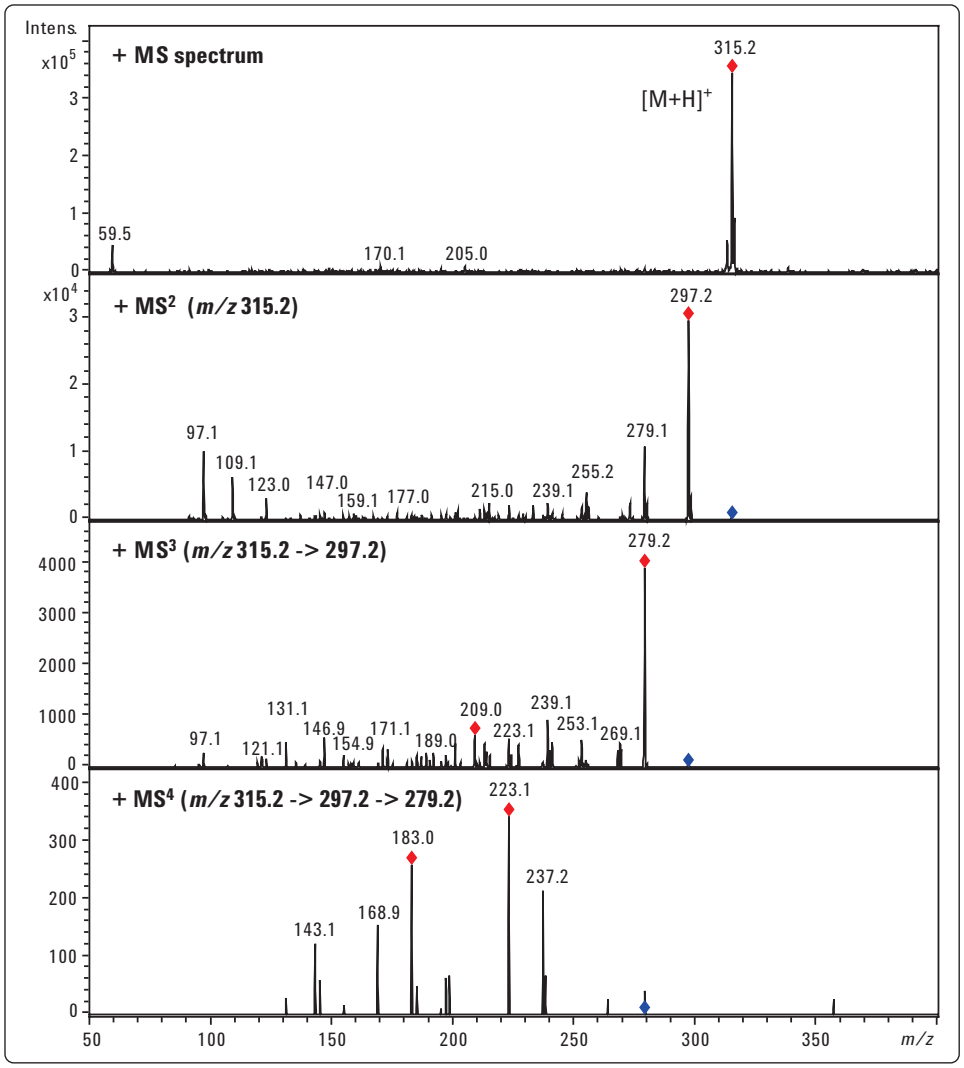


Figure 2. MS^n analysis using positive/negative time segments versus alternating positive/negative scans

Figure 3 shows MS^4 spectra obtained for progesterone in the alternating positive/negative scan mode. These data demonstrate that alternating positive/negative analyses can be accomplished on the LC/MSD Trap SL in the chromatographic time scale, without sacrificing MS^n data.

Fast polarity switching is complemented by other time-saving LC/MSD Trap features for screening unknowns. These include fully automated MS^n and data mining. The LC/MSD Trap SL can perform up to five stages of fully automated, data-dependent MS^n. The instrument applies a variety of user-set criteria to select precursor ions on the fly and then performs MS^n analyses. Once a multistage MS data file has been acquired, the data can be mined using the powerful Find Compounds data analysis feature. This function identifies all unique MS^n experiments, generates total ion chromatograms for precursor ions, and generates and organizes averaged mass spectra for related MS and MS^n experiments. This combination of sophisticated LC/MSD Trap features maximizes useful analytical information while minimizing analysis time.

Figure 3. Positive ion MS^4 spectra for progesterone, obtained while acquiring alternating positive and negative scans



Conclusions

Thanks to its fast polarity switching, the Agilent 1100 Series Trap SL can generate useable MS^n data in combination with polarity switching—in the chromatographic time scale. This makes it easier to perform MS^n analyses on unknown samples that may strongly favor one ionization mode or the other. The combination of polarity switching, automated data-dependent MS^n, and automated data mining features on the LC/MSD Trap SL can greatly accelerate the analysis of unknowns.

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Printed in the U.S.A. October 7, 2002
5988-7784EN

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