Advantages of Orthogonal Nebulizer Orientation in Electrospray for Quadrupole and Ion Trap Mass Spectrometers

Technical Note

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Introduction

With the increasing popularity of atmospheric pressure ionization (API) mass spectrometric techniques, electrospray ionization has become a widespread tool for quantitative determinations. When the sample matrix is complex, electrospray ionization can result in the formation of nondesolvated, charged residues as well as analyte ions (see Figure 1). These nondesolvated residues contaminate the ion optics, reducing signal and thus system performance. They also contribute to high background noise in mass spectra, again reducing system performance. In addition, desolvated residue ions reduce sensitivity, dynamic range, and performance of ion trap mass spectrometers through space charging.[1–3]

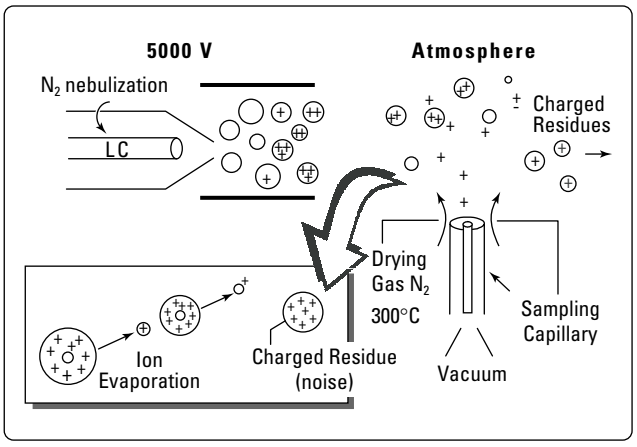

Figure 1. API-electrospray mechanism

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Optimal performance of the mass spectrometric system is required when performing quantitative analyses with numerous samples in complex matrices. Off-axis (orthogonal) nebulization reduces the negative effects of matrix contamination of the sampling orifice and ion optics compared to conventional on-axis nebulization.[4] Orthogonal nebulization also reduces the total number of charged particles that enter the sampling orifice (Figure 2). This is particularly important in maintaining ion trap precision, dynamic range and sensitivity.

Data from two different experiments are presented here. The first experiment is a long-term stability study involving decomposition of a potassium salt. It demonstrates the stability of a single-quadrupole mass spectrometer using orthoganol nebulizer orientation. The second experiment involves the detection of dihydroxyvitamin D_3 in plasma. It compares the results of on-axis and off-axis (orthoganol) nebulization in an ion trap mass spectrometer and contrasts those results with results from the same analysis performed using a triple-quadrupole mass spectrometer.

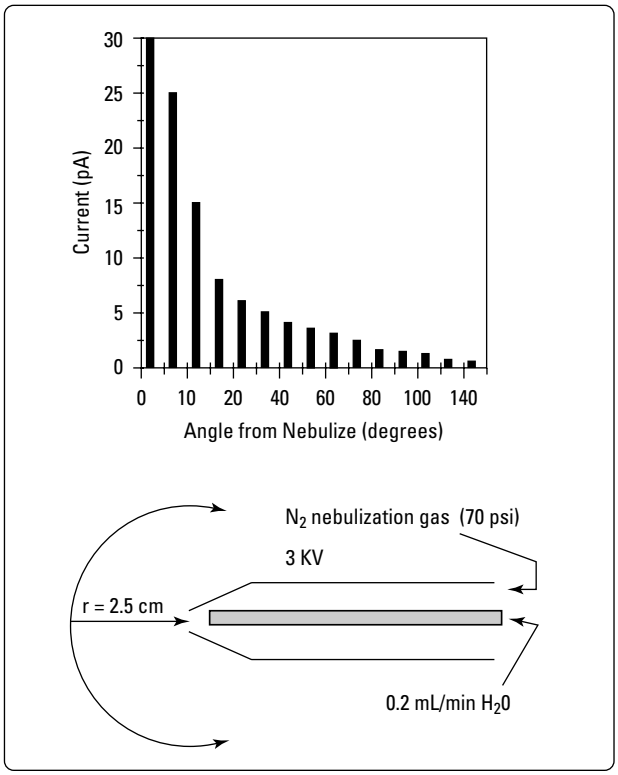


Figure 2. Spray currents measured at atmospheric pressure at various nebulizer tip angles

Experimental

In the first experiment, the kinetics of decomposition of a potassium salt (*m/z* 915) were studied through a series of hydrolysis experiments in which pH and temperature were varied. A dilute solution of potassium salt in buffer was incubated in a temperature-controlled environment with constant agitation for selected periods of time. A portion of the solution was then quenched with methanol. The resulting mixture was separated using an Agilent 1100 Series HPLC with a Keystone Betasil C_{18} reversed-phase HPLC column (100 mm × 2.0 mm, 5 µm particle size) and an ammonium acetate/methanol solvent gradient.

The separated species were detected using an Agilent 1100 Series LC/MSD single-quadrupole mass spectrometer operated in selected ion monitoring (SIM) mode using orthogonal, negative-ion electrospray. Figure 3 shows the orthogonal nebulization configuration of the LC/MS system.

For the second experiment, rat plasma was spiked with dihydroxyvitamin D_3 and mixed with an equal volume of chlorobutane. The dihydroxyvitamin D_3 was partitioned into the chlorobutane phase, which was removed and evaporated to dryness. The extract was reconstituted with 70% acetonitrile in water.

Separation was performed using an Agilent 1090 HPLC with a Zorbax XDB C_{18} reversed-phase HPLC column (50 mm × 2.1 mm) under isocratic conditions of 70% acetonitrile with 1% acetic acid. Mass detection was performed using an experimental ion trap that included both off-axis and on-axis nebulization configurations with an ion trap mass analyzer from Finnigan MAT.[4] Figure 4 shows the schematic of this HPLC/ion trap system. For comparative purposes, identical dihydroxyvitamin D_3 plasma extracts were analyzed under the same LC conditions using an Agilent 1100 Series HPLC system and a Micromass Quattro II triple-quadrupole mass spectrometer.

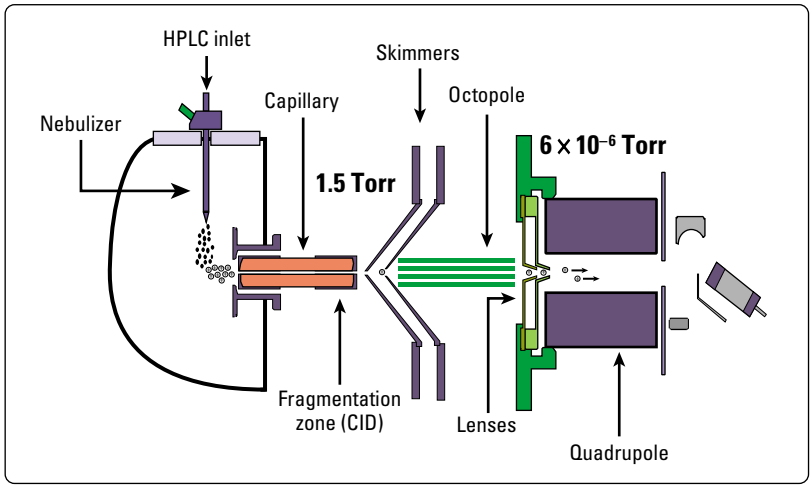
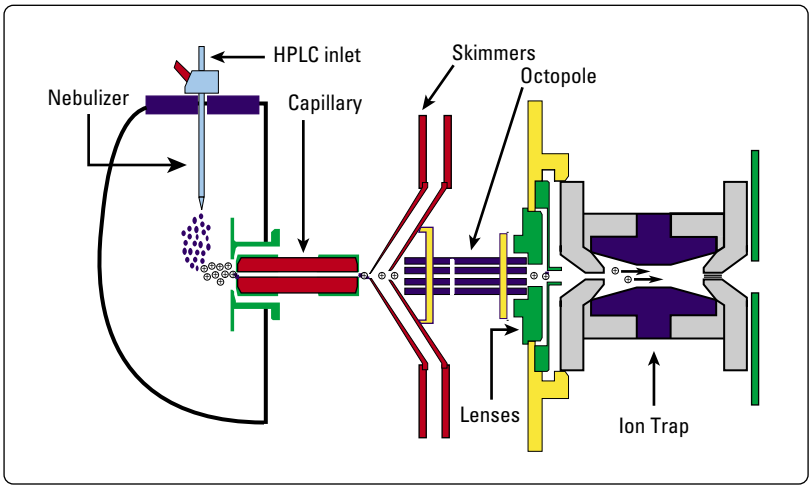


Figure 3. Schematic of the LC/MSD system with electrospray ion source

Figure 4. Schematic of the ion trap LC/MS system



Results and Discussion

Orthogonal nebulization spray using the single-quadrupole LC/MSD

The results from the analysis of 800 hydrolysis samples run over 15 days shows the ruggedness of the orthogonal spray nebulization configuration. The average standard deviation (Table 1) was found to be only 2.6%. This is significantly better than the 5–8% that is typical for mass spectrometric detection. Figure 5 shows the potassium salt chromatograms from samples 1, 200, and 750. Over the course of this experiment the system exhibited a constant response, preserving the precision and accuracy necessary for quantitation of the analyte.

Table 1. Ruggedness of the LC/MSD using orthogonal nebulization

Hydrolysis Sample #	Internal Standard Area
1	2.965×10^6
70	2.984×10^6
150	2.932×10^6
300	2.935×10^6
500	2.926×10^6
800	2.901×10^6

Average internal standard area: 2.992×10^6

Standard deviation (n = 800): 2.6%

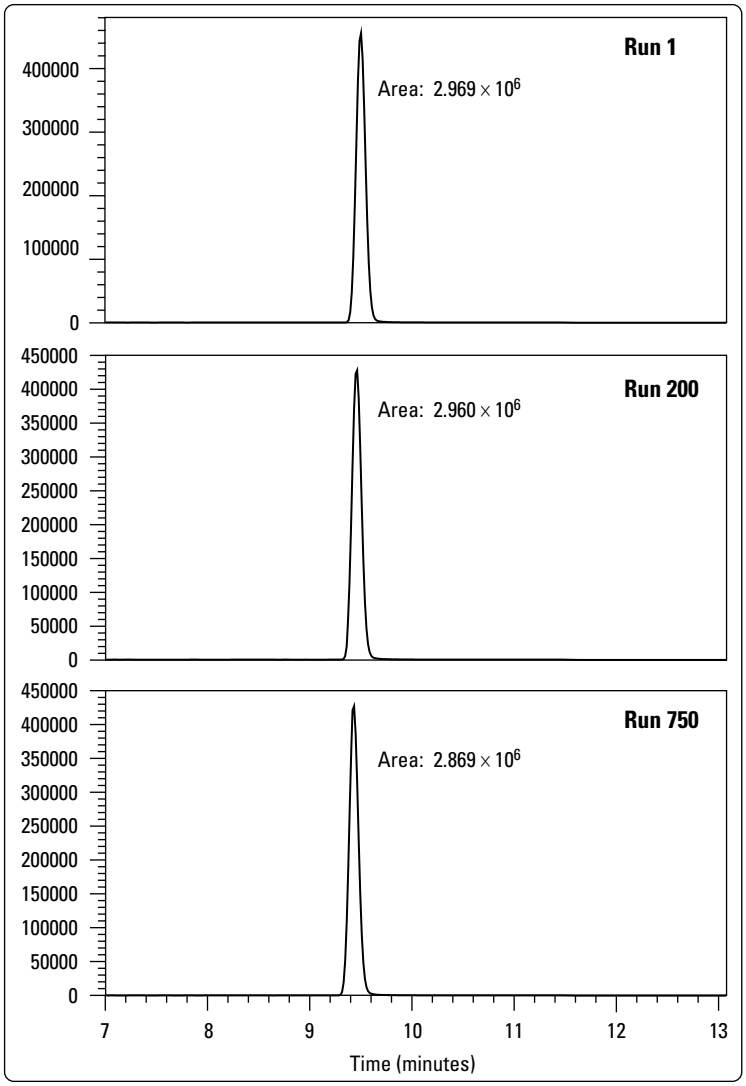
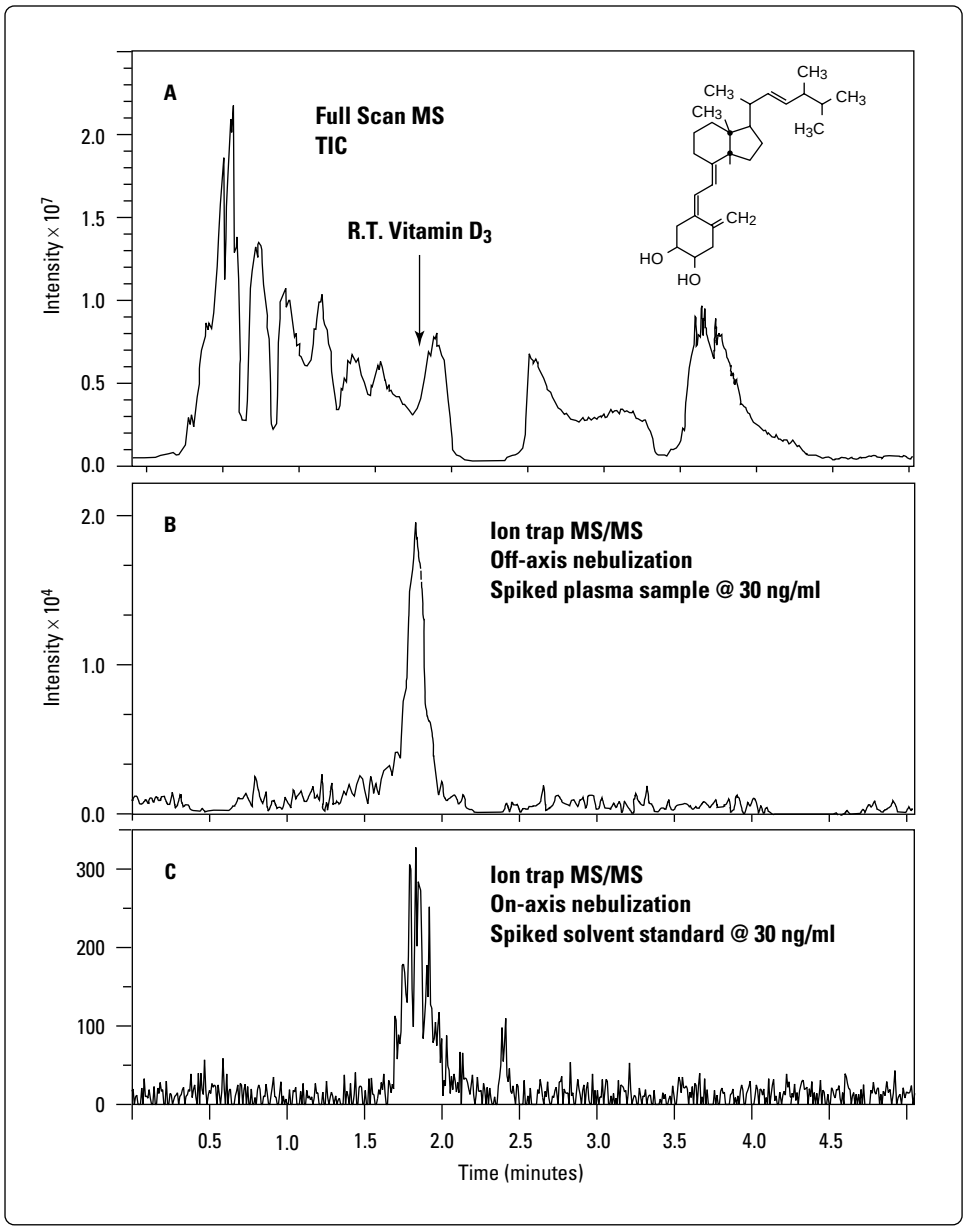


Figure 5. Selected ion chromatograms for the internal standard demonstrate virtually no performance dropoff even after 750 injections

Orthogonal and nonorthogonal nebulization in an ion trap

The conditions chosen to analyze the dihydroxyvitamin D_3 in plasma represent high throughput (retention time = 1.9 minutes) but poor separation of the analyte from interferences (matrix ions and charged particles). Figure 6 shows the comparison of (A) the full scan ion trap analysis (off-axis spray) of a plasma sample; (B) the MS/MS ion trap analysis (off-axis spray) of a spiked plasma sample @ 30 ng/ml; (C) the MS/MS ion trap analysis (on-axis spray) of a spiked solvent standard @ 30 ng/ml.

Figure 6. LC/ion trap electrospray of dihydroxyvitamin D_3 in plasma comparing off-axis and on-axis nebulization



The sensitivity using the off-axis nebulization is also shown in Figure 7, which compares the chromatograms of a 3 ng/ml sample of dihydroxyvitamin D_3 in plasma using the (A) off-axis ion trap (CID: *m/z* 399 —> 381) with the (B) triple-quadrupole system (CID: *m/z* 399 —> 135).

Off-axis nebulization also offers improved precision compared to the on-axis configuration (see Table 2) and comparable precision to the triple quadrupole.

Ruggedness is shown in Figure 8, which compare signal response for a constant level of dihydroxyvitamin D_3 between off-axis and on-axis nebulization for the analysis of 60 plasma extracts. The off-axis results show less than 10% variance in measured peak area over the 60 samples. The on-axis results show a loss in signal by a factor of 3–4. This loss is due to the buildup of residues on the capillary orifice and end plate, reducing sampling efficiency.

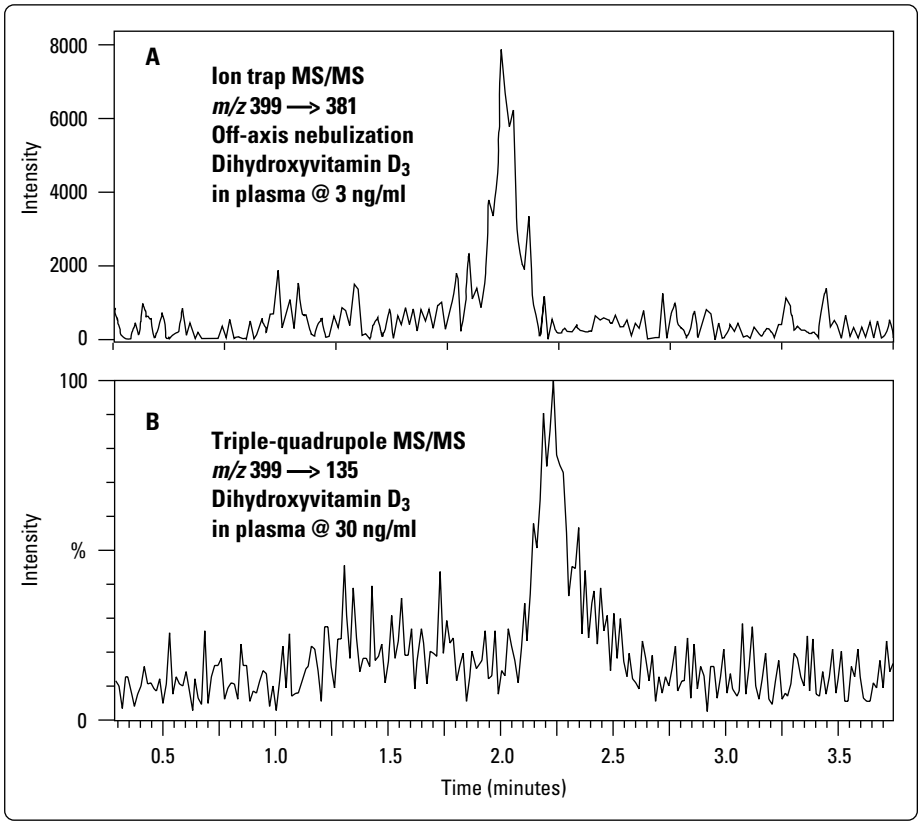


Figure 7. LC/MS/MS CID of dihydroxyvitamin D_3 in plasma comparing ion trap (off-axis nebulization) with a triple quadrupole

Table 2. Comparison of precision for the determination of dihydroxyvitamin D_3 in plasma using off-axis and on-axis nebulization electrospray on an ion trap mass spectrometer

Instrument Configuration	% Relative Standard Deviation	
	@ 3 ng/ml	@ 30 ng/ml
MS/MS Ion trap (on-axis)	ND	14.1
MS/MS Ion trap (off-axis)	7.1	5.6

ND = not detected

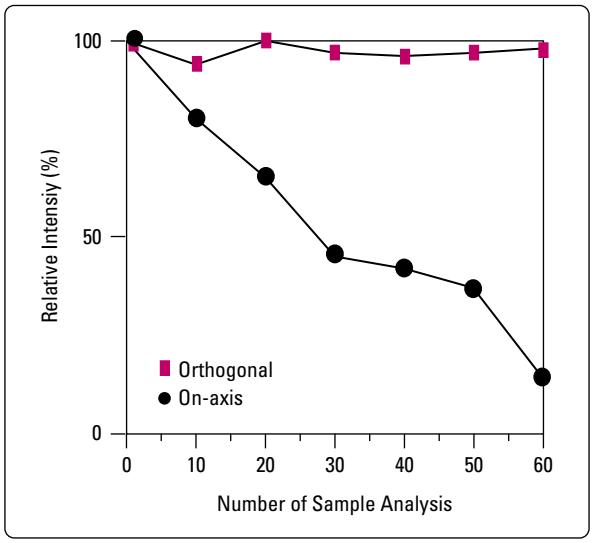


Figure 8. Comparison of ruggedness of on-axis and off-axis nebulization

Conclusion

Nondesolvated, charged residues created during the electrospray process, negatively affect the performance of mass spectrometers. Compared to on-axis nebulization, off-axis nebulization reduces the number of nondesolvated residues transported into the mass analyzer and reduces the contamination of the sampling orifice and mass analyzer. Thus, sensitivity, precision, accuracy, and ruggedness are improved. The performance of the system remains nearly constant, as required for quantitative analyses.

References

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