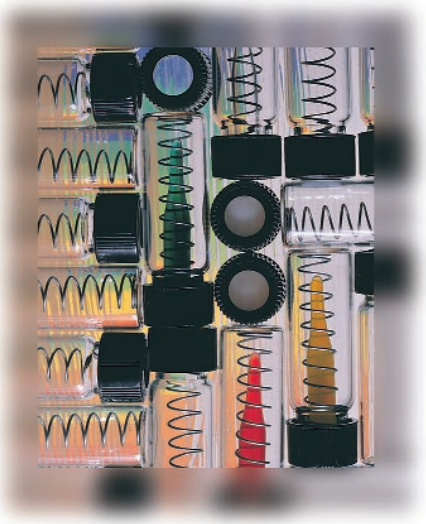
Comparison of Quadrupole, Time-of-Flight, and Fourier Transform Mass Analyzers for LC–MS Applications



The authors investigated the analytical capabilities of quadrupole, time-of-flight, and Fourier transform mass analyzers for liquid chromatography electrospray mass spectrometry (LC–MS) applications. They studied instrument parameters — such as sensitivity, mass range, and mass resolution — and issues related to sample preparation and separation for the characterization of low molecular weight compounds in complex environmental matrices. In this article, the authors discuss the effect of sample preparation on the performance of LC–MS analytical methods.

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In recent years, the combination of liquid chromatography with mass spectrometry (LC–MS) has become an established analytical technique for various fields of research (1,2). The value of LC–MS stems from its capability to perform selective separation on-line with sensitive, selective mass detection. This technique is highly effective for characterizing complex sample mixtures that would be difficult to analyze by conventional LC–UV analysis. Reaction products generated by combinatorial synthesis or samples derived from environmental and physiological matrices now can be characterized routinely by LC–MS for both high-throughput qualitative screening and trace-level quantification (2–4).

The on-line mass characterization of the LC effluent generally is accomplished using electrospray ionization MS. The electrospray ionization source has a number of features that make it ideal for LC–MS applications. The soft nature of the electrospray ionization source allows gentle ionization of nonvolatile, thermally labile, organic compounds with minimum fragmentation. Researchers have been able to characterize diverse classes of compounds as intact molecular ions with high sensitivity (5).

The electrospray ionization source can be interfaced successfully with several different mass analyzers. Currently, the quadrupole mass analyzer is the most widely used mass analyzer for LC–MS instrumentation. The quadrupole mass analyzer essentially is a mass filter that isolates ions of a selected mass-to-charge ratio (*m/z*) for detection (6). To acquire a complete mass spectrum, the mass analyzer must scan the mass-to-charge ratios selected for monitoring and individually detecting ions of different masses. Another mass analyzer, which relatively recently has become commercially available with the electrospray ionization source, is the time-of-flight (TOF) mass analyzer (7,8). Mass analysis for this instrument is performed by calculating the time required for accelerated ions of different masses to traverse a fixed distance. A less widely available LC–MS detector is the Fourier transform mass analyzer (9,10). The Fourier transform analyzer characterizes mass-to-charge ratios based on the current frequency generated when ions are trapped under the influence of a strong magnetic field. Unlike the quadrupole analyzer, both the TOF and Fourier transform analyzers characterize ions as batches or groups without scanning for different ions to acquire a complete mass spectrum. The electrospray ionization source also has been interfaced successfully with several other mass analyzers, including magnetic-sector and ion-trap analyzers (11,12).

Interfaced with an electrospray ionization source, the quadrupole, TOF, and Fourier transform mass analyzers each are capable of performing mass analysis for LC. However, each mass analyzer uses different principles to characterize the mass-to-charge ratios of ions generated by the electrospray ionization source. As a result, each mass analyzer has different strengths and limitations for various LC–MS applications.

In this article, we compare the analytical capabilities of the quadrupole, TOF, and Fourier transform mass analyzers for LC–MS applications for the characterization of low molecular weight compounds in complex matrices. This article is not intended to provide a detailed review of all electrospray MS instrumentation. The focus of this study was to investigate the general attributes of mass analyzers — such as mass resolution and scanning and nonscanning modes — and issues relevant to quantitative and qualitative LC–MS analyses of complex mixtures. In addition, we also examine the importance of sample preparation and cleanup procedures for instrument sensitivity and mass resolution.

Experimental

Materials: We obtained fenbuconazole, the active ingredient in Indar; tebufenozide, the active ingredient in Confirm and Mimic; and wheat hay and corn matrices from Rohm and Haas Co. (Philadelphia, Pennsylvania). Figure 1 shows the structures of these analytes. We used high performance liquid chromatography (HPLC)–grade solvents in the extraction and cleanup procedure obtained from Fisher Scientific (Pittsburgh, Pennsylvania).

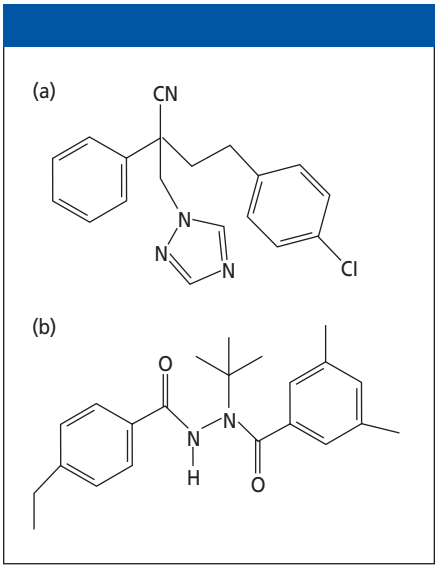

Figure 1: Structures of analytes (a) fenbuconazole (MW 336) and (b) tebufenozide (MW 352).

Sample cleanup procedures: The complete comprehensive cleanup procedure involved extraction and partition, open silica column, and solid-phase extraction (SPE) cleanup.

Extraction: We mixed 2 g of wheat hay with 150 mL of extraction solvent (90:10 [v/v] methanol–0.10 N hydrochloric acid) and shook them for approximately 30 min. The extract was separated by vacuum filtration, and the filter cake was rinsed with 50 mL of extraction solvent.

Partition cleanup: We transferred the filtrate to a 500-mL separatory funnel and performed liquid–liquid extraction with hexane and methylene chloride. Hexane partition extraction involved the addition of 20 mL of 20% aqueous sodium chloride solution followed by the addition of 50 mL of 100% *n*-hexane. We saved the aqueous phase for methylene chloride partition extraction. We added 100 mL of 20% aqueous sodium chloride followed by 100 mL of methylene chloride. Then we collected the organic phase and performed a second 100-mL methylene chloride partition extraction. Both fractions of the organic phase were combined, collected, and dried with a rotary evaporator.

Open silica column cleanup: We slurry packed 30 mL of activated silica (63–200 mm d_p) with 100% *n*-hexane into a 25 cm $\times$ 19 mm column. The sample, dissolved in 25 mL of 5% ethyl acetate–hexane, was added to the column. We rinsed the sample container with 10 mL of the 5% ethyl acetate–hexane solution and then with 50 mL of 10% ethyl acetate–hexane solution. The sample was eluted with 100 mL of 50% ethyl acetate–hexane. Solution fractions were dried by rotary evaporation.

SPE cleanup: We preconditioned 500-mg phenyl cartridges (Supelco, Bellefonte, Pennsylvania) with 5 mL of 5% acetonitrile–water solution. Samples were dissolved in 5% acetonitrile–water and added to the column. Columns were rinsed with 5 mL of the 5% acetonitrile–water solution and subsequently washed with 5 mL of 10% acetonitrile–water. The sample was eluted with 15 mL of 35% acetonitrile–water, and the extract was collected and dried by rotary evaporation. We redissolved the sample in 4 mL of 1:1 acetonitrile–water, fortified it with analyte, and analyzed it by LC–MS.

During simplified sample preparation, we bypassed various steps of the comprehensive method (for example, the open column, methylene chloride liquid–liquid partition,

or SPE step). For methods involving only hexane liquid–liquid partition extraction, we subjected the matrix extract to liquid–liquid partition extraction with 100 mL of 100% *n*-hexane (without the sodium chloride addition). We collected the polar phase, dried it under nitrogen, and subsequently fortified it for analysis or continued to clean it up using SPE.

LC–MS analysis: LC was performed using an Agilent 1100 HPLC system (Agilent Technologies, Wilmington, Delaware); the injection volume was 50 µL. Fenbuconazole was chromatographed using a 15 cm × 3 mm, 5-µm d_p Supelcosil LC-18-DB column (Supelco). The solvent gradient was water (solvent A) and acetonitrile (solvent B). The flow rate of the mobile phase was 0.8 mL/min. The initial mobile-phase composition of 70% A was maintained for 1 min and decreased to 50% at 5 min. The percentage of solvent A was decreased to 40% at 8 min and maintained for an additional 3 min. Solvent A then was returned to 70% at 12 min.

For the fast analysis of tebufenozide, we used a 5 cm × 2.1 mm, 3.5-µm d_p Symmetry C18 column (Waters, Milford, Massachusetts). The solvent gradient was water (solvent A) and acetonitrile (solvent B). The flow rate of the mobile phase was 0.8 mL/min. The initial gradient was 60% A and was decreased to 10% at 4 min and maintained for 2 min. Solvent A then was returned to 60% at 6.5 min. Effluent from the liquid chromatography was split to allow a 10–25 µL/min flow rate to the ion source.

LC–MS analysis was performed using an API-365 triple quadrupole electrospray ionization mass analyzer (PerkinElmer, Foster City, California), a Mariner TOF electrospray ionization mass analyzer (PerSeptive Biosystems, Framingham, Massachusetts), and an Apex Fourier transform mass analyzer (Bruker Daltonics, Billerica, Massachusetts). We acquired the quadrupole MS data using spray, orifice, and ring potentials of 4500 V, 30 V, and 200 V, respectively. The TOF electrospray ionization mass analyzer was operated with the spray tip, nozzle, and skimmer potentials applied at 3200 V, 80 V, and 12 V, respectively. The Fourier transform mass analyzer used a 4000-V spray source and a 7-T superconducting magnet with a total analysis time of 2.5 s and a data-acquisition time of 0.256 s. The extracted ion chromatograms were generated using the protonated analyte signal. Data-acquisition time for the quadrupole and TOF mass analyzers was 2.0 s.

Results and Discussion

LC–MS analytical applications can be classified into two general categories: quantitative and qualitative. Quantitative LC–MS applications such as trace analysis typically demand the absolutely lowest detection limits. In addition, the target analyte is generally known, which makes the acquisition of a complete mass spectrum unnecessary. Qualitative LC–MS applications such as product or metabolite identification typically involve the detection and identification of unknown target compounds or side-reaction products. As a result, this type of analysis requires the acquisition of a complete mass spectrum for each chromatographic data point. We examined the effect of mass range, mass resolution, and sample preparation on the sensitivity of the TOF, quadrupole, and Fourier transform mass analyzers for both qualitative and quantitative LC–MS applications.

Mass range: Quadrupole mass analyzers are scanning instruments. As with any scanning detector, there is a significant drawback to acquiring data across a large number of channels; that is, throughout a wide mass range. Figure 2 shows a series of LC–MS extracted ion chromatograms of fenbuconazole acquired by a quadrupole mass analyzer operating under selected-ion monitoring and scanning modes. We obtained the maximum signal-to-noise (*S/N*) response for the analyte when data were acquired by selected-ion monitoring (Figure 2a). The *S/N* response was significantly lower when a mass spectrum was acquired for each chromatographic data point (in scanning mode) with a mass range of 10 *m/z* (Figure 2b). We observed additional loss in the *S/N* response results when the mass range scanned was increased to 40 *m/z* and 100 *m/z* (Figures 2c and 2d).

Figure 3 shows the quantitative relationship between relative signal response and mass range acquired using the quadrupole and TOF mass analyzers. As the mass range increased from the selected-ion monitoring mode to 10 *m/z*, the *S/N* from the extracted ion chromatograms acquired by the quadrupole mass analyzer decreased by approximately 80% relative to the selected-ion monitoring mode. We observed a greater than 90% decrease of *S/N* response as the acquired mass range increased to 100 *m/z*. In contrast, the *S/N* responses obtained from TOF and Fourier transform analyzers were constant when acquired in the same mass ranges.

The decrease in signal-to-noise with respect to increasing acquired mass range is

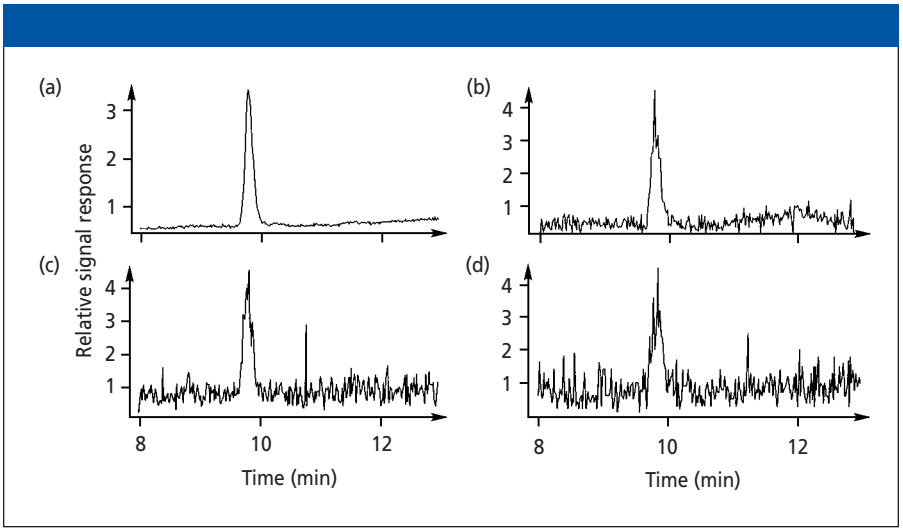

Figure 2: Quadrupole LC–MS extracted ion chromatograms of 0.02-µg/mL fenbuconazole obtained using (a) selected-ion monitoring and scanning a mass range of (b) 10, (c) 40, and (d) 100 *m/z*.

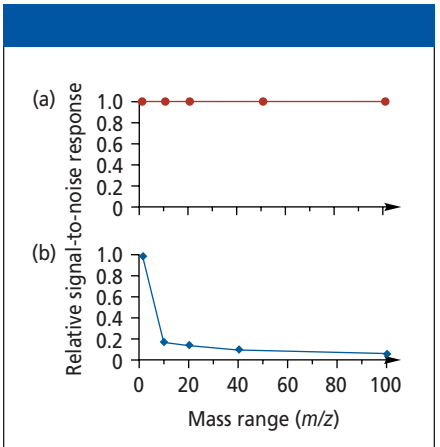

Figure 3: *S/N* response of 0.02-µg/mL fenbuconazole as a function of mass range. Data acquired for each chromatographic point using (a) time-of-flight and (b) quadrupole LC–MS.

caused by the quadrupole mass analyzer's limitation in monitoring a single mass-to-charge ratio at any given acquisition time. To generate a complete mass spectrum, the quadrupole mass analyzer must scan the mass range to be acquired and detect ions of different mass-to-charge ratios individually. Instead of using the entire acquisition time to monitor a single ion (selected-ion monitoring mode), scanning requires the allocation of a fraction of the total acquisition time to each monitored mass-to-charge ratio. Increasing the mass range scanned during a given time can be accomplished only by decreasing the time allocated to monitor any given mass-to-charge ratio. Because the *S/N* response is a function of acquisition time, increasing the mass range scanned decreases the *S/N* response of the extracted ion chromatograms.

In contrast to the quadrupole mass analyzer, the TOF and Fourier transform mass analyzers are not scanning instruments. Both instruments acquire ions from the electrospray ionization source in batches and simultaneously characterize the masses of all ions present in each individual batch. For this reason, the sensitivity of the TOF and Fourier transform analyzers is not limited by the acquired mass range. However, during the time in which the instrument is performing mass analysis, ions produced by the electrospray ionization source are left unmonitored during the duty cycle. Because no such dead time is associated with the quadrupole analyzer when operating in the selected-ion monitoring mode, the application of a quadrupole instrument for LC–MS analysis potentially can provide greater sensitivity than TOF or Fourier transform analyzers. In our study, we found the sensitivity of the quadrupole mass analyzers to be approximately eightfold greater than that of TOF or Fourier transform analyzers. This comparison should be interpreted as a relative value. Because of variations in the electrospray spray source, ion transmission efficiency, and general instrument design, we were unable to make a direct comparison of absolute detection limits of the different mass analyzers.

Mass resolution: Mass resolution, defined as the ratio of peak mass to the peak width at half maximum intensity ($m/\Delta m$), reflects the general selectivity of the mass analyzer. Higher mass resolution spectra will provide more-selective data for extracted ion chromatograms. Figure 4 shows the mass spectra for fenbuconazole obtained from the quadrupole, TOF, and Fourier transform mass analyzers. For optimum *S/N* response, the quadrupole mass analyzer generally is configured to acquire spectra with mass resolution of approximately 400–600. The TOF and Fourier transform mass analyzers can routinely acquire spectra with resolutions of approximately 2800 and 50,000, respectively. The latest TOF and Fourier transform instruments can achieve mass resolutions of 10,000 and 1 million, respectively.

Quadrupole analyzers can be tuned to acquire mass spectra with resolution approaching that of TOF analyzers; however, they do so at the expense of overall signal response. Figure 5 illustrates the effect of increasing mass resolution of our quadrupole mass analyzer on its *S/N* response. The *S/N* response obtained with a resolution of approximately 1000 was roughly eightfold less than the signal acquired with a resolution of approximately 120. As mass resolution increased, the transmission of ions through the analyzer and the ion detector decreased, resulting in the loss of sensitivity.

To investigate the importance of high-resolution MS for the characterization of complex matrix samples, we compared extracted ion chromatograms from different mass analyzers. Figures 6a and 6b show LC–MS extracted ion chromatograms acquired from a wheat hay matrix sample fortified with tebufenozide using the quadrupole analyzer, configured to acquire signals with resolutions of approximately 1000 and 120, respectively. We processed matrix samples using only hexane liquid–liquid partition. The extracted ion chromatogram, acquired with a resolution of approximately 1000 (Figure 6a), shows one peak characteristic of the analyte. The signal-to-noise ratio was comparable to that of extracted ion chromatograms obtained

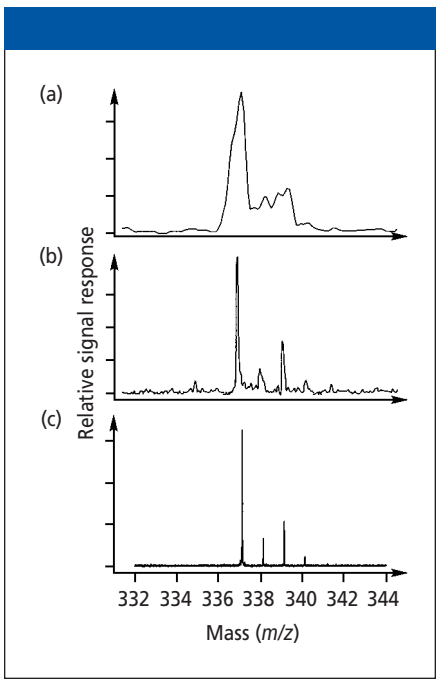

Figure 4: Mass spectra of 0.2-μg/mL fenbuconazole obtained by (a) quadrupole, (b) TOF, and (c) Fourier transform mass analyzers.

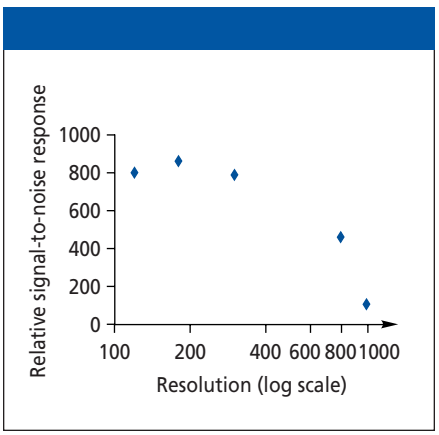

Figure 5: *S/N* response for 0.02-μg/mL fenbuconazole acquired by quadrupole LC–MS with various mass resolutions.

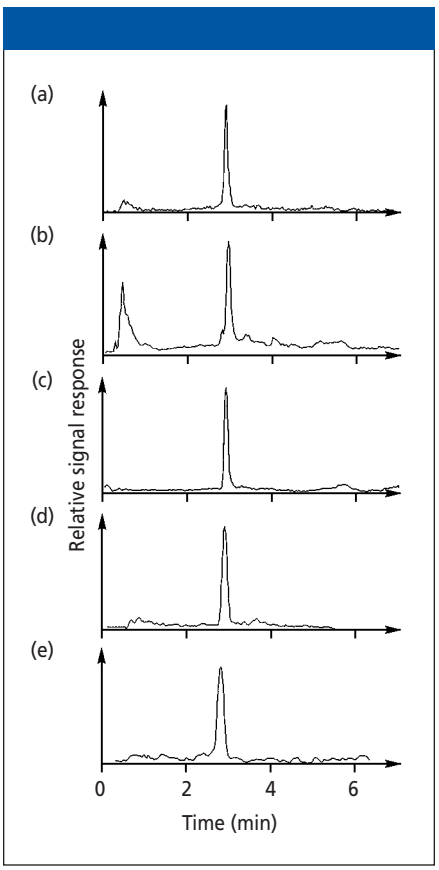

Figure 6: LC–MS selected-ion chromatograms of 0.02-μg/mL tebufenozide obtained using (a) quadrupole MS with resolution approximately 1000, (b) quadrupole MS with resolution approximately 120, (c) quadrupole MS with resolution approximately 120 after comprehensive cleanup, (d) TOF LC–MS, and (e) Fourier transform LC–MS.

from the TOF and Fourier transform analyzers (Figures 6d and 6e). However, signal acquisition with a resolution of approximately 120 resulted in a poorer quality chromatogram (Figure 6b) that had an additional peak appearing early in the analysis.

These results demonstrated that higher mass resolution can improve the quality of the extracted ion chromatograms of complex matrix samples. Higher mass resolution instrumentation allows more-selective signal acquisition, thereby reducing the noise introduced by coeluting matrix components. However, extremely high mass resolution does not necessarily provide better data. The extracted ion chromatogram acquired by the Fourier transform mass analyzer (Figure 6e) had a resolution of approximately 50,000 and was of comparable quality to the extracted ion chromatogram of the TOF analyzer (Figure 6d), which had a resolution of approximately 2800, or of the quadrupole analyzer (Figure 6a), which had a resolution of approximately 1000.

LC separation: The traditional approach to eliminating signal interference and ambiguity in LC using UV detection involves comprehensive sample preparation and cleanup procedures. Although mass analyzers are significantly more selective than conventional UV detectors, they still require careful sample handling and cleanup.

Figure 7 shows high-resolution Fourier transform MS spectra acquired from the direct infusion of a corn matrix extract fortified with 0.02 μg/mL of fenbuconazole into an electrospray mass spectrometer without prior LC separation. The sample, processed using only an SPE cleanup procedure, shows the signal characteristic of the target analyte surrounded with peaks contributed by matrix components (Figure 7a). Figure 7b shows the mass spectrum of the same matrix sample, but this time we used a comprehensive sample preparation procedure involving liquid–liquid partition extraction, open-column separation, and SPE. The background signals contributed by matrix components were noticeably reduced, but the signal response of the analyte was approximately fivefold greater.

Figure 8a shows the TOF mass spectrum of tebufenozide in wheat hay extract that we acquired after LC separation without any preliminary cleanup procedure. Although this approach to characterizing samples is less complex and labor intensive, the mass spectrum shows that LC alone is incapable of removing all matrix components from the analyte. By applying a short cleanup procedure involving hexane liquid–liquid extraction, SPE, and LC, we were able to dramatically reduce the background signal contributed by the matrix and to improve signal sensitivity by a factor of approximately 3 (Figure 8b). After this cleanup, the extracted ion chromatograms we obtained using the quadruple mass analyzer with a resolution of approximately 120 (Figure 6c) were more comparable to those of unprocessed matrix samples acquired by the TOF and Fourier transform instruments (Figures 6d and 6e).

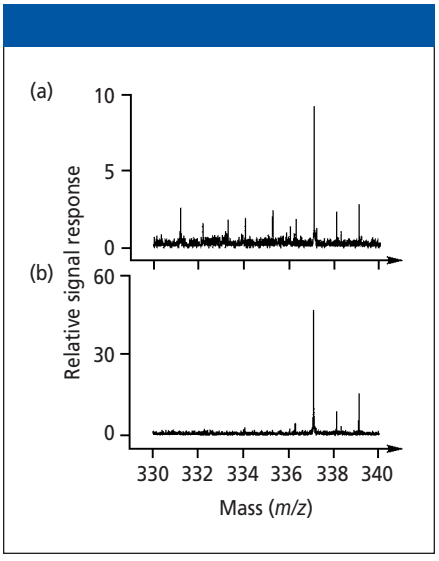

Figure 7: Fourier transform mass spectra of 0.2-μg/mL fenbuconazole (resolution of 50,000) in a corn extract after (a) an SPE cleanup only and (b) a comprehensive sample cleanup.

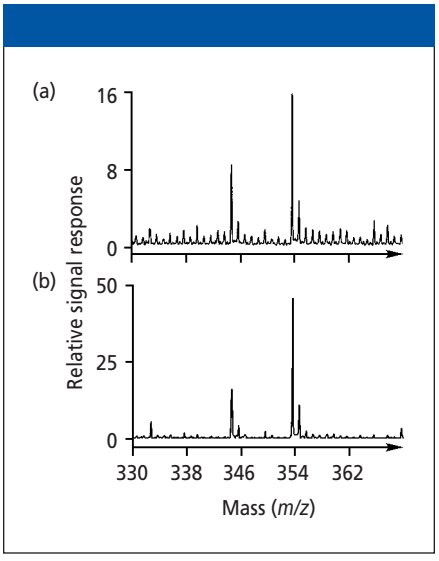

Figure 8: LC–TOF–MS spectra of tebufenozide (0.02 μg/mL) (a) in a wheat hay matrix and (b) after simplified sample cleanup.

The drawback to cleaning up matrix samples is that the procedures tend to be highly labor intensive, so they impede the overall throughput of an analytical method. The high resolution of a Fourier transform mass analyzer can effectively resolve the matrix component peaks from the target analyte. As a result, sample preparation procedures involving raw sample products or matrix extracts can be minimal for higher mass resolution LC–MS instruments. However, one issue associated with characterizing complex mixtures cannot be addressed by high-resolution mass spectrometry: The electrospray ionization source is highly susceptible to ion-suppression effects (13,14).

The efficiency of target analyte ion formation can be severely affected when a target analyte is simultaneously ionized with matrix components. This effect accounts for the significant differences in signal response shown in Figures 7 and 8. To obtain maximum sensitivity and reproducibility, analysts must clean the sample sufficiently to prevent coelution of matrix and analyte components. Sample cleanup may be unnecessary to address signal interference between coeluting analyte and matrix components. However, it generally is required to ensure accurate and reproducible quantitative data when characterizing complex matrix extracts.

Appropriate sample preparation procedures can reduce the extent of signal suppression and eliminate much of the signal interference at the same time. For this reason, low-resolution mass analyzers used with an efficient separation procedure can acquire extracted ion chromatograms from complex matrix samples with signal quality similar to that of a high-resolution instrument.

Qualitative and quantitative applications: Quantitative applications generally involve measuring specific, known compounds. A complete mass spectrum is unnecessary for this type of analysis. The acquisition of a complete mass spectrum is essential for qualitative applications, in which the sample composition may not be entirely known. The quadrupole mass analyzer operating under selected-ion monitoring is well suited for quantitative applications. Operating the analyzers with a mass resolution of approximately 500, we generally achieved lower detection limits with the quadrupole analyzer under selected-ion monitoring than with the TOF or Fourier transform analyzers.

In addition, our results have shown that operating the quadrupole mass analyzer at lower resolution does not preclude it from being highly effective in characterizing samples from complex matrices. Because the LC–MS characterization of complex sample mixtures increases the probability of encountering mass or signal interference, we might argue that high resolution is essential for this type of analysis. In cases that involve trace analysis of matrix samples, significant signal suppression effects generally are present. Analysts can compensate for signal suppression by using internal standards (15,16) or mobile-phase additives (17); however, the best sensitivity will require sample cleanup. Those cleanup procedures typically eliminate chromatographic noise associated with mass interference by the matrix components. As a result, analytical methods that require comprehensive sample cleanup need not rely on high mass resolution instrumentation to address mass interference.

Although capable of obtaining reliable quantitative information, TOF and Fourier transform mass analyzers are better suited for qualitative LC–MS applications. Both of these mass analyzers can acquire data throughout a larger mass range with lower

detection limits than the quadrupole analyzer. The higher mass resolution capabilities can effectively filter chromatographic noise contributed by matrix components and help target confirmation through higher mass accuracy.

The limited mass range sensitivity and resolution of the quadrupole mass analyzer does not preclude it from high-throughput qualitative applications. The product yield of many microscale combinatorial synthesis experiments is in the 10 μg–10 mg range. Wide mass range scanning experiments (approximately 500 *m/z*) can be performed easily with sample concentrations of roughly 1 μg/mL. Mass resolution of approximately 300–700 still can provide acceptable spectra for many qualitative applications that involve drug or compound discovery research. However, the application of TOF or Fourier transform instrumentation is preferred for high-throughput analysis methods that require minimum cleanup for complex samples such as physiological matrices and polymers.

Conclusion

In this article, we compared the analytical capabilities of quadrupole, TOF, and Fourier transform mass analyzers for LC–MS applications. The TOF and Fourier transform mass analyzers can obtain high-resolution mass spectra throughout a broader mass range with greater sensitivity than the quadrupole mass analyzer. We obtained the greatest sensitivity from the quadrupole instrument operating in the selected-ion monitoring mode. The high mass resolution offered by TOF and Fourier transform analyzers can significantly improve the overall quality of extracted ion chromatograms acquired from complex sample mixtures such as matrix samples and extracts. When dealing with complex sample mixtures and applying or designing suitable LC–MS analytical methods, analysts should consider the sample preparation procedure to obtain the optimum performance from any mass analyzer.

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