

An LC/MS System Designed for Rapid High Throughput Analysis of Pharmaceutical Compounds

Application Note

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Abstract

The demands for high throughput in drug discovery have made the fast separations obtainable from sub-2 micron particle size columns very popular. These separations require detectors capable of high acquisition rates while still maintaining spectral fidelity. This Application Note describes a new quadrupole LC/MS system capable of scanning fast enough for the narrow peaks that result from these separations.

Introduction

In the development of new pharmaceutical products, there is a demand for shorter analysis times in order to analyze more samples per day on each LC/MS system. With little time for method

optimization, there is also a strong desire to get the right answer on the first injection without detailed knowledge of the sample concentration, optimum ionization technique or polarity of ions formed. Additionally, because of the rapid chromatographic methods used, a detector with fast acquisition rates is needed. A previous Application Note¹ describes using a Time-of-Flight (TOF) mass spectrometer for this application. However in many cases, the unit mass resolution delivered by less expensive single quad mass spectrometers is sufficient for the application. Previously, quadrupole mass spectrometers have had difficulty producing enough spectra across narrow peaks that may be less than a second wide. This Application Note describes the components of an LC/MS quadrupole system optimized for such high speed chromatography and universal detection.



Agilent Technologies

Experimental

The Agilent 1200 Series Rapid Resolution LC system was set up for alternating column regeneration (ACR)² as described in the previous note¹. The system consisted of the following modules:

- Two Agilent 1200 Series binary pump SL with micro degasser (x 2)
- Agilent 1200 Series high performance autosampler SL with thermostat.
- Agilent 1200 Series thermostated column compartment with 10-port valve for automatic column regeneration.
- Agilent 1200 Series diode array detector (DAD) SL with the capability to acquire data with a sampling rate up to 80 Hz.
- Agilent 6140 MSD Quadrupole with scan speed up to 10,000 u/sec equipped with multimode source
- Column: ZORBAX SB C18, 2.1 x 50 mm or 3 x 50 mm, 1.8- μ m particle size
- Software: ChemStation revision B.02.01 SR1

The samples used included the Agilent Electrospray LC Demo Sample (Agilent part number 59987-20033) containing 4 sulfa drugs. In addition, a range of pharmaceutical compounds were provided by customers to check spectral quality.

In this system the modules were stacked to place the UV detector in close proximity to the multimode source. For UV detection a 2- μ L flow cell was used in the DAD. The outgoing capillary is connected directly to the

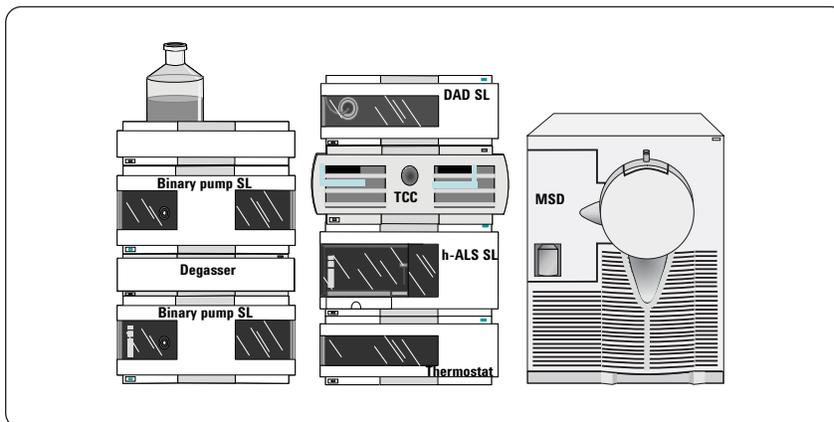


Figure 1
Agilent 1200 Series RRLC system with 6140 LC/MSD quadrupole.

nebulizer of the MSD. This instrument set-up is optimized to achieve the highest possible resolution. The system is shown in figure 1.

Method conditions

Solvent A:	1 mM ammonium acetate in water with 2 % methanol;
Solvent B:	1 mM ammonium acetate in methanol.
Flow	1.3 mL/min for 2.1-mm column, 2-mL/min for 3-mm column.
Gradient:	8 % B to 60 %B in 0.5 min.
Stop time:	0.8 min

The autosampler was used in overlapped mode with automatic delay volume reduction. The column compartment operated at 80 °C The diode array detector operated at 40 Hz, wavelength 272 nm/16, ref 360/100, spectra acquired across all peaks 210–400 nm.

MS conditions

Multimode source combined with ESI-APCI mode	
Scan range:	m/z 100 - 600,
Vaporizer:	200 °C
Dry gas:	6 L/min at 265 °C,
Nebulizer:	35 psi,
Fragmentor:	150 V,
VCap:	3000 V
Corona current:	4 μ A,
Charging volt.:	2000 V

Results and discussion

By using 1.8 micron particle size columns at elevated temperatures and high linear velocities, fast separations with excellent resolution were possible. At flow rates of 1.2 to 1.4 mL/min with 2.1 mm ID columns or 2 mL/min with 3 mm ID columns, peak widths of less than 1 second were obtained. In Drug Discovery applications, the desire is for a mass spectrometer that will ionize the broadest range of samples and acquire quality spectra at scan speeds fast enough to match the chromatographic performance.

Previous work had determined that using water methanol gradients with ammonium acetate as a modifier and the multimode source in dual mode resulted in ion formation for the broadest range of compounds. Since this source can handle flow rates of up to 2 mL/min without splitting, it is a good match for a high resolution system.

A study was performed to determine the impact of scan speed on apparent resolution. Using the same chromatographic conditions, the MS data was acquired at 1, 2, 4, 6 and 20 spectra/sec. The resulting chromatograms are shown in figure 2.

As can be seen in figure 2, the traditional scan speeds of 1 to 2 spectra per second are inadequate to deal with the chromatographic resolution of the system. It is only a data rates of 6 spectra/second or better that the MS peak widths begin to approach those obtained from the UV data. Previous work indicated that complaints of peak dispersion in the MS source were often due to undersampling by the MS detector. This can be shown better if you look at the peak width at half maximum (PWHM) for the third peak which is sulfamethazine. The peak widths are shown in table 1.

Detector	Data Rate (Hz)	PWHM (msec)
UV	40	373
MS	6	804
MS	20	546

Table 1
Peak width for different data rates for sulfamethazine.

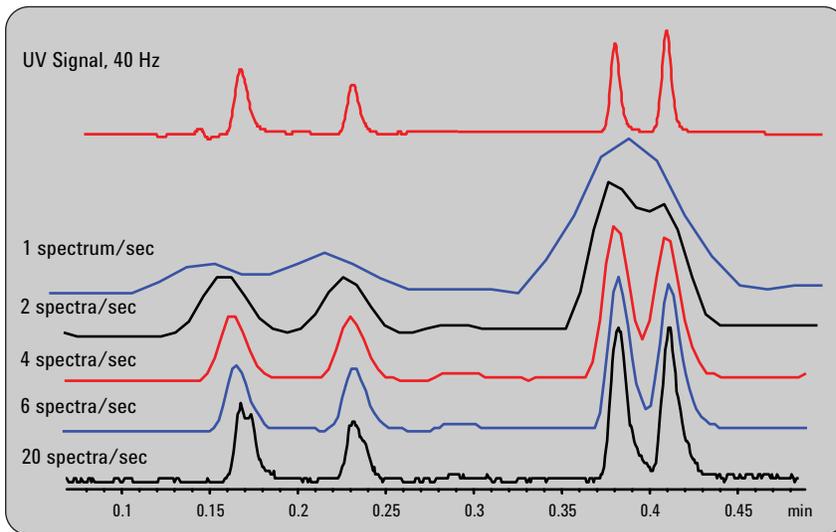


Figure 2
Effect of scan speed on resolution.

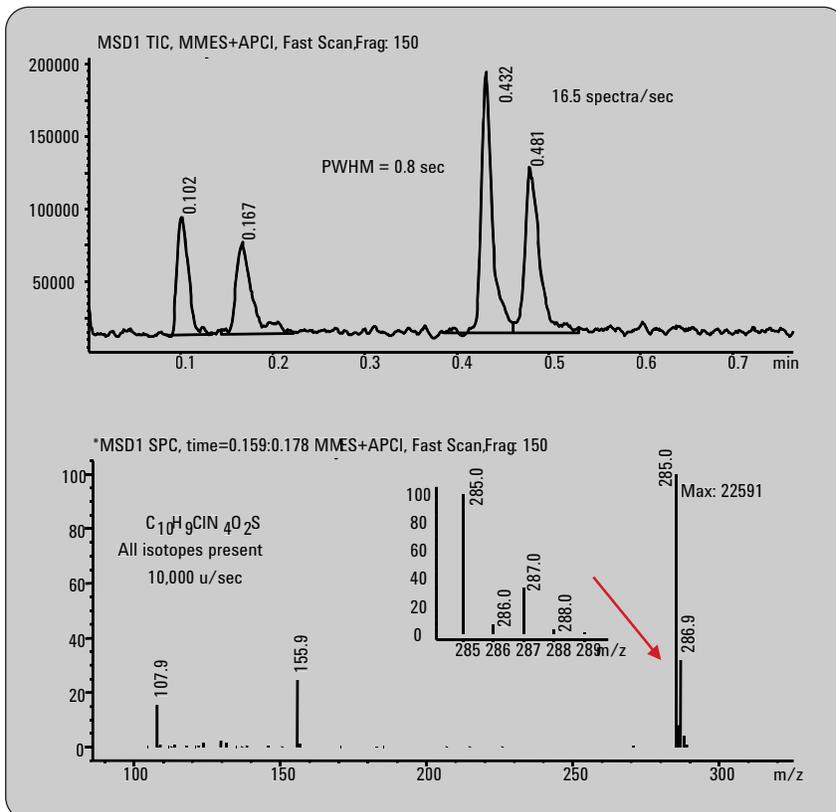


Figure 2
TIC of ES Demo mix and spectrum of sulfachloropyridazine, scanning at 10,000 u/sec.

With modern electronics it is possible to drive the quadrupole fast enough to get scan speeds of 5,000 to 12,000 u/sec. However, the loss in sensitivity and spectral quality render the data useless. This is because the mass filter is changing faster than the ion is moving through to the detector. For example, scanning at 600 u/sec, an ion at m/z 500 will experience a shift of 0.1 u in the quadrupole setting. At 10,000 u/sec, this shift can be greater than 1 u. At faster scan speeds and higher masses, this lag has a significant effect on ion transmission. Trying to overcome this by increasing ion energy, results in fewer rf cycles and resolution suffers. Reducing quadrupole resolution improves transmission but isotopic information is lost.

The Agilent 6140 MSD quadrupole operates at 1.4 MHz instead of the standard 1 MHz. This provides for more rf cycles to offset the faster scanning of the mass filter. In normal scan mode, the quadrupole stepped in 0.1 u steps. The detector signal was measured each 20 µsec and 2 or more samples are averaged. In ultra fast scan mode, the quadrupole stepped in 0.2 u steps and only one 20 µsec sample taken. This provided a scan speed of 10,000 u/sec. Because of the greater step size, the spectra were filtered in the mass domain with a different digital filter than was used for the normal scanning speeds. Scanning the range of m/z 100 - 600, the system gave 16.6 spectra/sec. Figure 3 shows results for the demo sample. The mass spectrum of the second

peak, sulfachloropyridazine, is shown below the TIC. Similar results were obtained with the pharmaceutical library samples.

Conclusions

The Agilent 6140 MSD quadrupole can acquire good quality mass spectra at the scan speeds needed to match the narrow peaks obtained from 1.8-µm columns operated at high flow rates. This MS, in combination with the Agilent 1200 RRHT HPLC system is capable of meeting the high throughput needs of drug discovery laboratories.

References

1. Michael Frank "Agilent 1200 Series Rapid Resolution LC System and the Agilent 6210 TOF MS- Highest Data Content with Highest Throughput" *Agilent Application note, publication number 5989-4505EN, 2006.*
2. Udo Huber "High Throughput HPLC - Alternating Column Regeneration with the Agilent 1100 Series Valve Solutions" *Agilent Application note, publication number 5988-7831EN, 2002.*

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