Fast LC analysis with quadrupole MS detection – without loss of spectral quality

Combining the Agilent 6140 quadrupole MS with the Agilent 1200 Series Rapid Resolution LC system

Application Note

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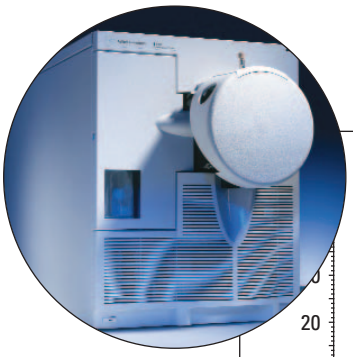

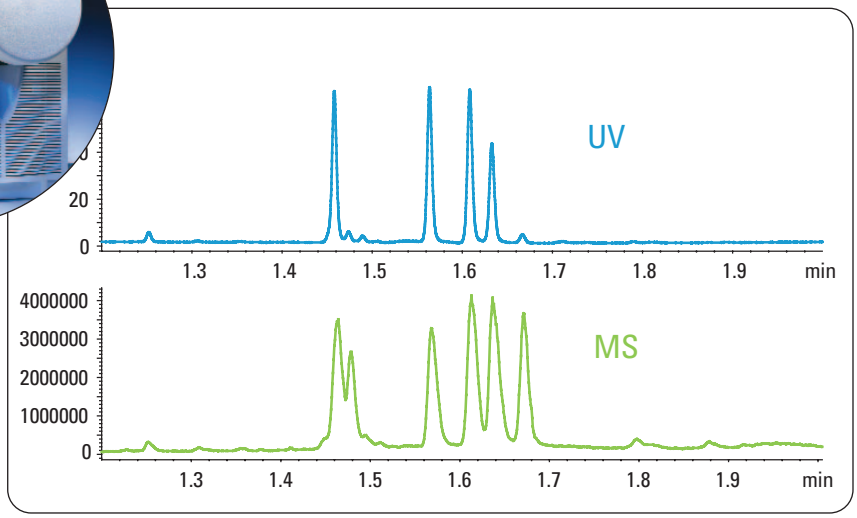

Abstract

In this Application Note the Agilent 6140 quadrupole LC/MS and Agilent 1200 Series Rapid Resolution LC systems are combined to demonstrate:

- the need for an fast-scanning, quadrupole MS detector
- fast positive/negative switching
- uncompromised MS spectra quality in ultra-fast-scanning mode

Further, the source of peak broadening of the MS peaks is explained and several application examples are given.

Agilent Equipment

- 1200 Series Rapid Resolution LC system
- 6140 Quadrupole MS detector

Application Area

- High throughput MS analysis in drug discovery

Agilent Technologies

Introduction

With the introduction of sub-2-micron particle columns and ultra-fast HPLC systems such as the Agilent 1200 Series Rapid Resolution LC (RRLC) system[1], MS instruments are required, which are able to keep pace with the fast chromatography. Ideally a TOF instrument would be used together with the RRLC system for optimum performance. However high mass accuracy is not always required. Furthermore, the price of a TOF instrument is higher than for a single quadrupole instrument. Therefore, Agilent developed the 6100 quadrupole LC/MS systems Series[2], which includes the 6140 model that is especially designed for ultra-fast scan speeds with 10000 amu/second. In this Application Note the need for such a high scan speed is demonstrated and results from fast-scanning experiments including fast positive/negative switching are presented. The high-quality spectral data obtained from chromatograms measured using ultra-fast scanning is of key importance here.

Experimental

Equipment

The Agilent 1200 Series Rapid Resolution LC/MS system included the following modules:

- Agilent 1200 Series binary pump SL
- Agilent 1200 Series high-performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series diode array detector SL (micro flow cell: 2 µL, 3 mm path length)
- Agilent 6140 quadrupole LC/MS with ESI source

The system was controlled using the Agilent ChemStation (rev. B.03.01. SR1). The new capillary that provides fast positive/negative switching is standard on Agilent 6100 Series instruments shipped after August 2007. Earlier instruments require the following in addition to the B.03.01.SR1 or later software:

- one resistive capillary, (Agilent part number G1960-80060)
- one narrow-bore capillary, (Agilent part number G1960-20310)

Results and discussion

The need for a fast scanning quadrupole MS detector

With the introduction of the Agilent 1200 Series Rapid Resolution LC system run times of 2 minutes and less can easily be achieved. To ensure separation and detection of all peaks in the UV chromatogram diode-array detectors with data rates up to 80 Hz were developed. Until recently only a TOF MS instrument was fast enough to keep pace with chromatography leading to peak widths of less than one second. However, with the introduction of the Agilent 6140 quadrupole MS instrument, an MS detector is now available that can not only be used for such fast runs but also can deliver MS spectra with excellent data quality.

Since the quadrupole MS is a scanning instrument, different parameters such as mass range, step size and applied signal filtering have an influence on the actual scan speed. Figure 1 shows how the scan speed can be increased by modifying these

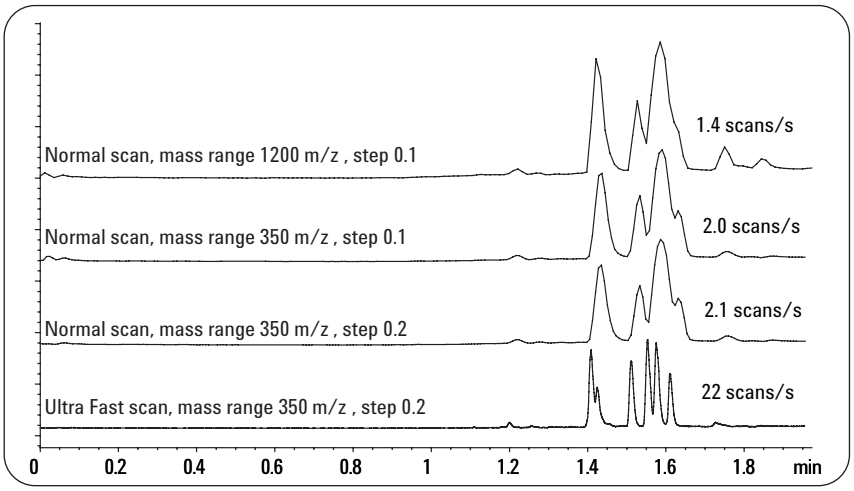

Figure 1
Fast chromatographic runs with different SQ scan speeds.

parameters, however, only to a certain degree. Without changing to the ultra-fast scan mode of the 6140 quadrupole MS the maximum scan speed is about 2 scans/s, in ultra-fast scan mode about 20 scans/s can be achieved.

In Figure 2 the area between 1.2 and 1.8 minutes is enlarged and the four peaks MS2 – MS5 are labeled with their respective peak widths of 0.60 – 0.70 s. To achieve the usually required ten scan points across the chromatographic peaks a scan speed of about 5000 amu/s (fast scan speed of the 6130 model) would have been sufficient (table 1), but only because of the rather small mass range of 350 amu. Doubling the mass range to 700 amu (e.g. from 150 – 850 amu) results in about 5 scans across the peak for a scan speed of 5000 amu/s but does not reach the desired 10 scans across the peak that is available from a scan speed of 10000 amu/s.

		Scans across peak		
Peak	Peak-width	2500 amu/s	5250 amu/s	10000 amu/s
MS2	0.70 s	5	10	20
MS3	0.65 s	5	10	19
MS4	0.68 s	5	10	19
MS5	0.60 s	4	9	17

Table 1
Scans across the peak width for different scan speeds.

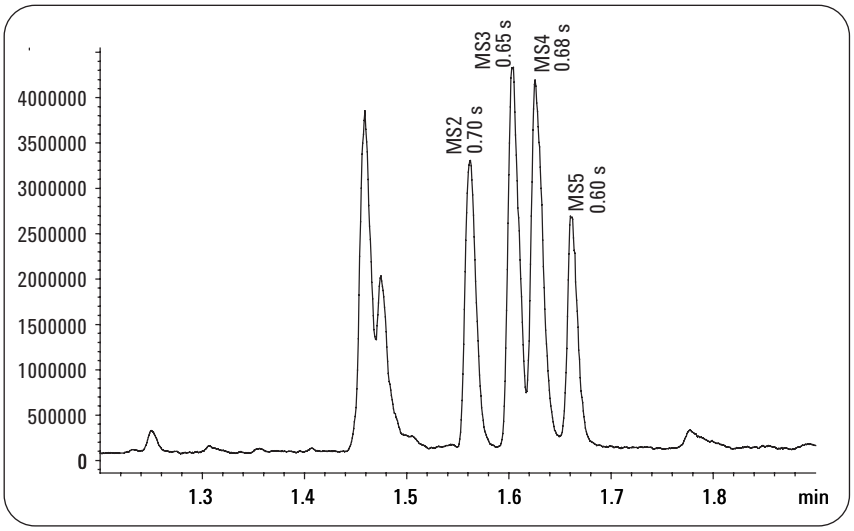

Figure 2
Peak widths of peaks MS2 – MS5.

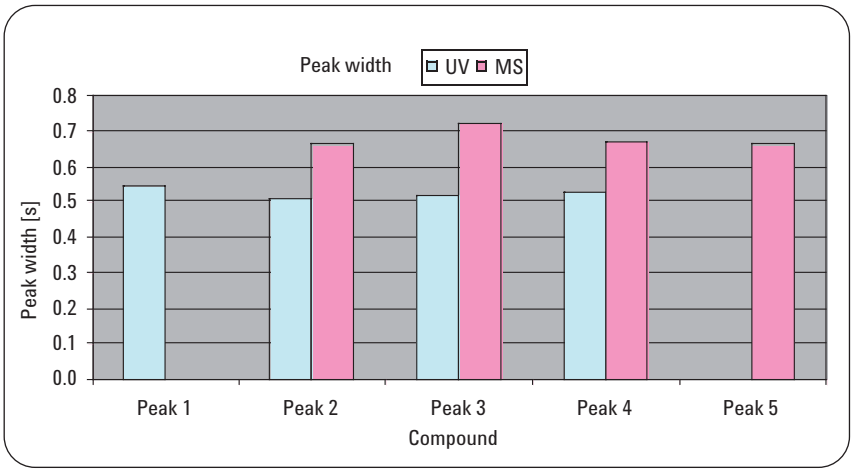

Figure 3
Peak widths of peaks MS2 – MS5.

MS peak width in comparison with UV peak width

When comparing the peak widths of UV and MS peaks the MS peaks are always wider than the UV peaks when the highest data rate of the UV detector is applied. In the chromatograms shown on the cover page the MS peaks from a positive scan experiment are about twice as wide as the UV peaks.

However, in the system setup the flow coming from the column goes through the UV detector cell and through a piece of tubing into the MS sprayer. These additional volumes add some peak broadening to the MS signal due to dispersion. To measure the influence of these volumes two experiments were performed. In the first experiment the peak widths of the UV and MS peaks were compared when they were connected directly to the column using the same piece of tubing. This eliminates the influence of the peak broadening due to dispersion of the UV detector cell. As shown in figure 3 the corresponding MS peaks were only about 30 % wider than the UV peaks.

To measure the influence of the dispersion due to the UV flow cell and the tubing, an additional, identical UV flow cell and the tubing used to connect the UV detector to the MSD were placed in the flow path between the column and the UV detector flow cell. As shown in figure 4 the UV peak width increased by about 70 %. This means the 100 % peak width increase discussed above is the sum of peak broadening by the MS itself (30 %) and the broadening due to dispersion caused by the UV flow cell and tubing (70 %).

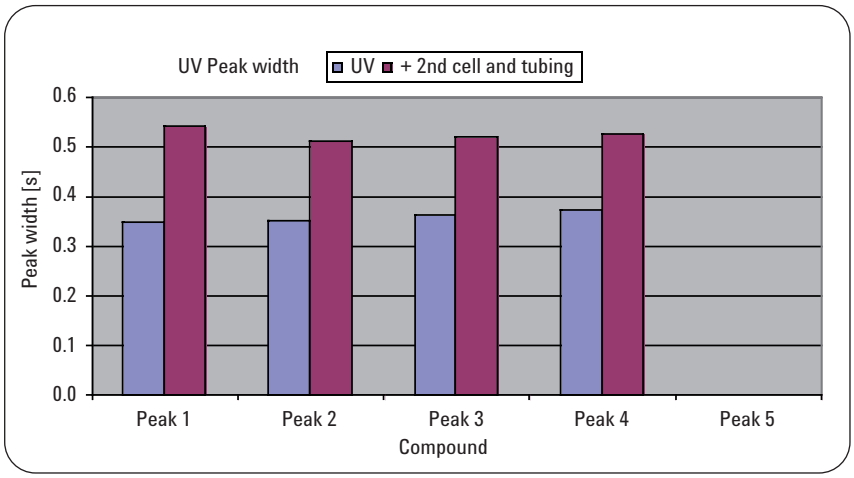

Figure 4
Peak broadening caused by dispersion of the UV flow cell and the tubing.

Fast positive/negative switching

For new target compounds with unknown ionization characteristics it can be advantageous to analyze them in positive and in negative ionization mode to ensure they are detected by the MS detector. While this could be done in two separate runs the ultra-fast scanning possibility of the 6140 SQ allows positive/negative switching in a single run with sufficient data points across the peak even for fast runs. In the upper part of figure 5 the UV trace and the MS scan from an experiment in positive ionization mode are shown. With the ultra-fast scanning mode of the 6140 quadrupole MS 23 scans per second could be measured. The lower trace shows the positive trace from a positive/negative switching experiment. Since the instrument has to do the measurements sequentially only 50 % of the scan time can be used for either mode. Due to the delay required for the polarity switching (20 ms) the actual scan rate is even slightly lower than 50 %. Figure 5 shows that the scan rate dropped from 23 scans/s to 9 scans/s for the positive trace from positive/negative switching but this

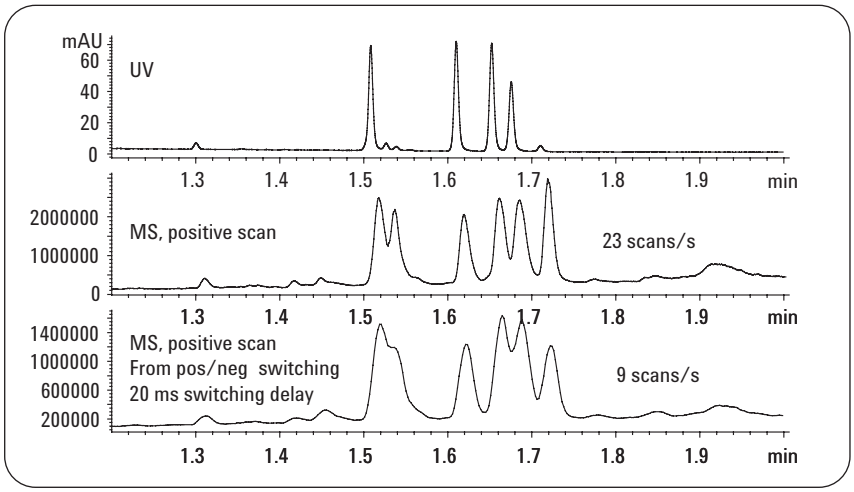

Figure 5
Comparison of MS traces from positive only and positive/negative switching experiments.

data rate is sufficient for most applications to provide very good positive/negative data in a single run.

Spectral quality

When doing fast or ultra-fast experiments using a fast scanning quadrupole MS instrument it is not only important to keep pace with the chromatography, but also not to compromise spectral quality. To test the spectra quality of the 6140 quadrupole MS in ultra fast scan mode the following experiments were done:

In the first experiment the MS spectra of the compounds were compared, acquired from a normal run (run time 10 minutes) in normal scan mode and from a fast run (run time 2 minutes, e.g. figure 3) in ultra-fast scan mode. For the five compounds MS1 to MS5 the spectra

were identical whether acquired in normal scan mode in the long run or ultra-fast scan mode in the fast run. Figure 6 shows the mass spectra of compound MS3 as an example. With a molecular mass of 418.17 amu the $[M+H]^+$ adduct as well as the $[M+Na]^+$ adduct and the same fragments can be seen in both spectra.

In a second set of experiments 16 pharmaceutical compounds were injected using a 2 minute gradient and the measured spectra were analyzed. Figure 7 shows the actually measure ratio of the $[M+1]^+$ and the $[M+2]^+$ ions (^{13}C isotope) compared to the expected ratio.

For most compounds the difference between measured and calculated ration is less than 5 %, only for two compounds (UH_TS 4 and UH_TS16) the difference was higher (approximately 10 %). Three compounds from the sample set contained chlorine and therefore also the isotope ratio ^{35}Cl and ^{37}Cl was measured and calculated. The results are shown in figure 8.

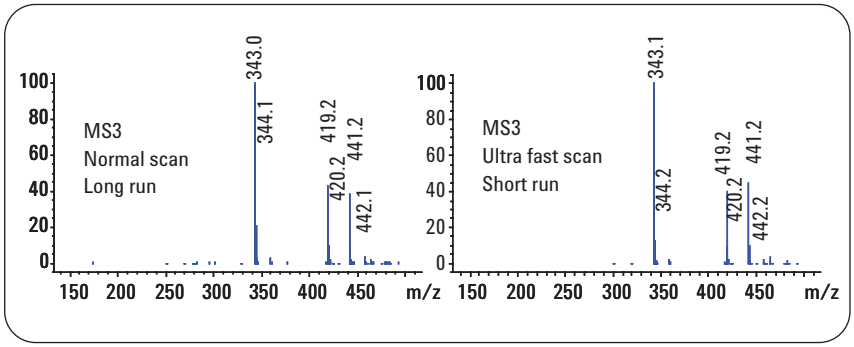

Figure 6
Comparison of the MS spectra of compound MS3 in normal and ultra fast scan mode.

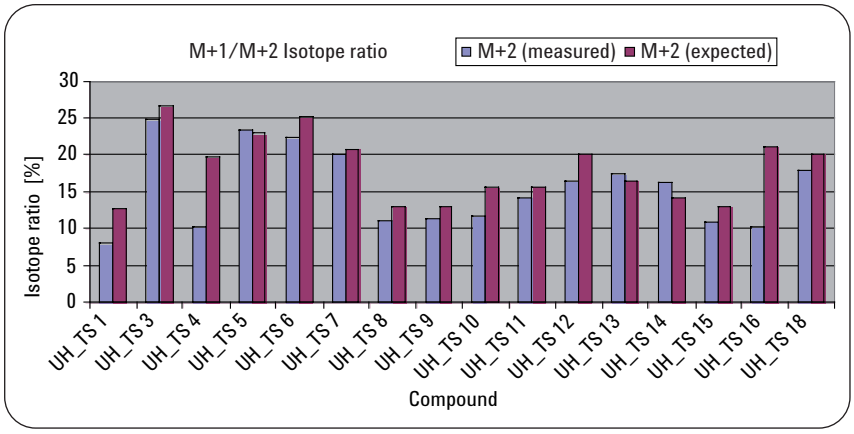

Figure 7
Measured and calculated ration of the M+1/M+2 isotope ratio.

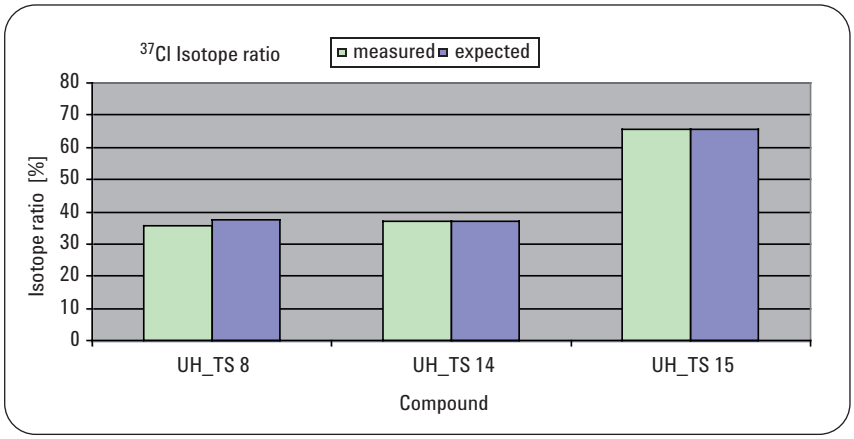

Figure 8
Measured and calculated chlorine isotope ratio.

One compound (UH_TS 15, chloramphenicol) contained two chlorine atoms. The spectrum and the calculated and measured isotope ratio for the different ions are shown in figure 9. The difference between the measured and the calculated isotope ratio is about 1 % or less for all ions (table 2).

Mass	Expected [%]	Measured [%]
323	100.0	100.0
324	13.0	13.6
325	65.7	64.5
326	8.4	7.1
327	11.4	11.9
328	1.4	1.0

Table 2
Expected and measured isotope ratios

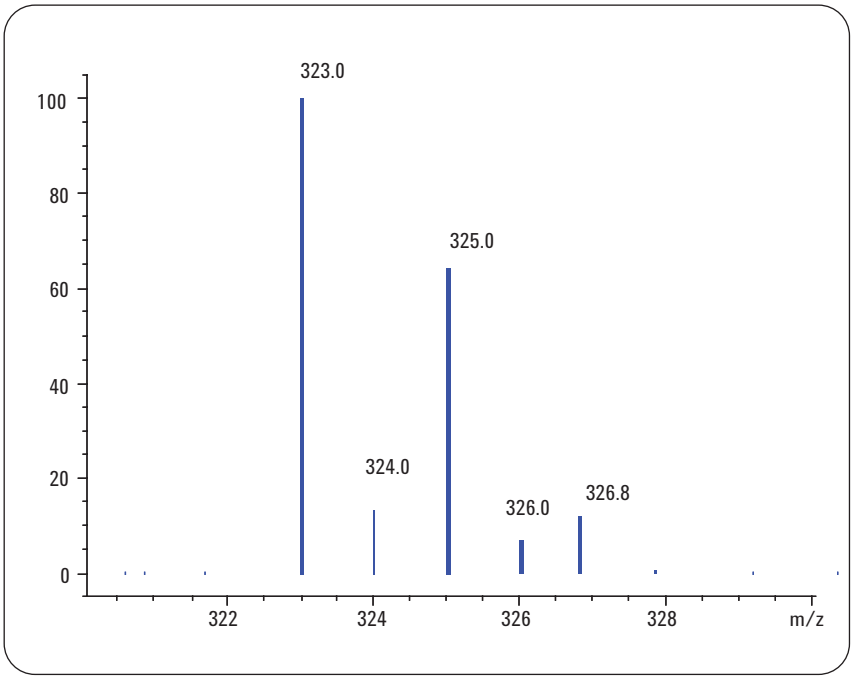

Figure 9
Spectrum and isotope ration for chloramphenicol.

Conclusion

In this Application Note the need for a fast-scanning quadrupole MS instrument for fast RRLC runs was demonstrated, especially if positive/negative switching is required. Further, the different sources for peak broadening in the quadrupole MS were explained and it could be shown that 70 % of the peak broadening is caused by dispersion in the UV detector flow cell and the tubing used to connect the MS to the UV detector. Only 30 % came from the MS itself. Finally, the excellent spectra quality for data acquired under ultrafast scanning conditions was shown. As a result it could be demonstrated that the Agilent 6140 quadrupole MS is the ideal partner of the Agilent 1200 Series Rapid Resolution LC system for fast chromatographic runs.

References

1.
"Agilent 1200 Series Rapid Resolution LC System", *Agilent Technologies Brochure, publication number 5989-4340EN*, **2006.**

2.
"Agilent 6100 Series Quadrupole LC/MS Systems", *Agilent Technologies Brochure, publication number 5989-5037EN*, **2006.**

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