Optimizing LC/MSD Acquisition Parameters for Positive/Negative Mode Switching

Technical Note

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Introduction

The demand for higher LC sample throughput (number of samples per unit time) has led to constant improvements in LC equipment and methods. These improvements have resulted in chromatographic peak widths as narrow as 0.1–0.2 minutes. Such narrow peaks can present a challenge for mass spectrometric detection, especially when advanced techniques such as positive/negative switching are employed to increase the amount of information collected from each sample.

The Agilent 1100 Series LC/MSD is a highly sensitive detector, capable of acquiring excellent data from very narrow chromatographic peaks for a wide variety of analytes. Instrument parameters, however, need to be adjusted carefully when the LC/MSD is used in positive/negative switching mode or signal loss may occur. This note explores what parameters settings to use to obtain the best data and avoid signal loss.

Experimental

All experiments were performed using an Agilent 1100 Series LC/MSD system comprised of a binary pump, vacuum degasser, autosampler, thermostatted column compartment with column switching valve, diode array detector (DAD), and LC/MSD SL quadrupole mass spectrometer. The sample was the four-component electrospray LC/MS demonstration sample (Agilent part number 59987-20033) containing sulfamethizole (MW = 278), sulfamethazine (MW = 270), sulfachloropyridazine (MW = 284), and sulfadimethoxine (MW = 310) at the concentration of 100 ng/µl each. The LC/MSD was tuned in both positive and negative modes using the Autotune program with default parameters.

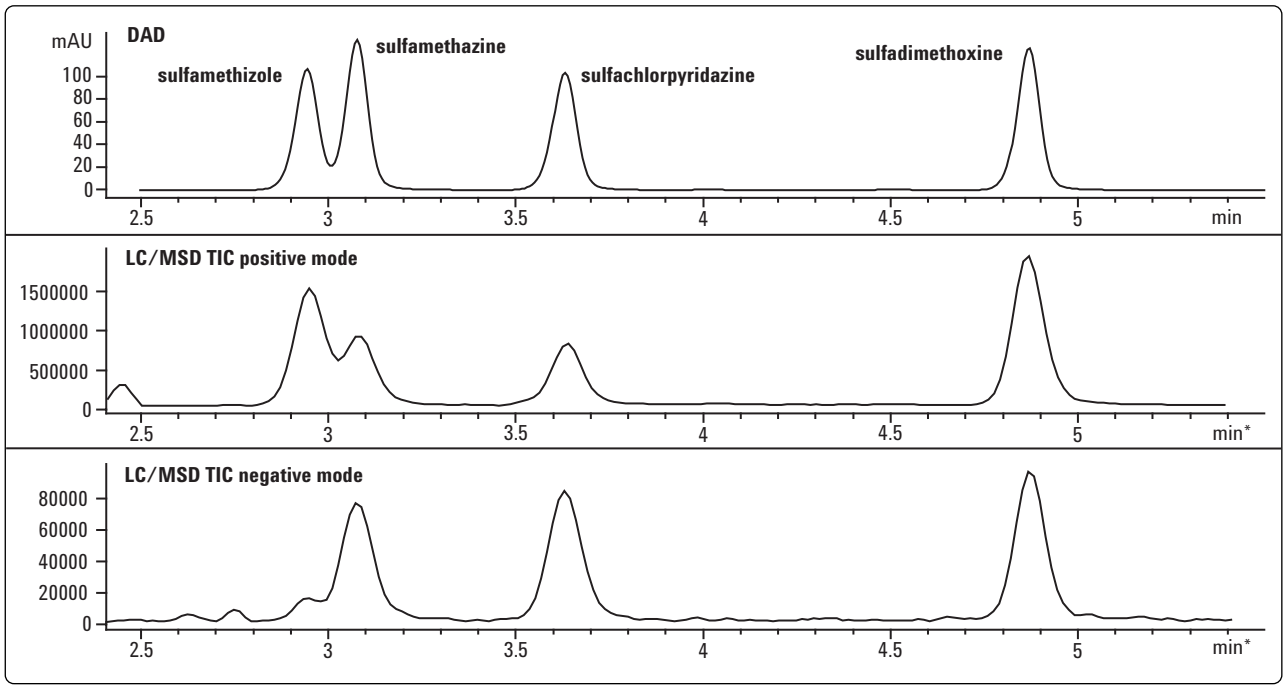

Figure 1. DAD and LC/MSD operated with typical parameters

Results and Discussion

Figure 1 shows the UV and MS chromatograms for the sulfa drugs. These results were obtained using typical LC/MSD acquisition parameter settings except for the scan range (*m/z* 150–350).

Figure 2 shows the extracted ion chromatogram (EIC) responses for the last peak, sulfadimethoxine (MW = 310), which forms an $[M+H]^+$ ion as the base peak in positive mode and an $[M-H]^-$ ion as the base peak in negative mode.

For all injections done during this study, the DAD response varied little (%RSD for sulfadimethoxine = 1.3%, eight injections). Thus, the injection system performance was reproducible, and all variations reported below were due to the changes in the LC/MSD acquisition parameters.

The effect of different LC/MSD acquisition parameters on the response for sulfadimethoxine was examined by varying the scan range, stepsize, time filter, and peak width (Table 1). The response

ANALYSIS METHOD

Chromatographic Conditions

Column:	50 mm x 2.1 mm ZORBAX SB-C18 5 µm Rapid Resolution cartridge (Agilent PN 873700-902)
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Gradient:	Start with 5% B at 5 min 40% B at 6 min stop run Post run 3 min
Flow rate:	0.4 ml/min
Column temperature:	35°C
Injection volume:	1 µl
Diode-array detector:	Signal 272, 16 nm; reference 380, 20 nm

MS Conditions

Source:	Electrospray (ESI)
Drying gas flow:	10 l/min
Nebulizer:	30 psig
Drying gas temperature:	350°C
Vcap:	4000 V (positive and negative)
Stepsize:	0.10 u
Chromatographic peak width:	0.1 minutes
Time filter:	Yes
Scan range:	*m/z* 150–350
Fragmentor:	110 V (positive mode) 130 V (negative mode)

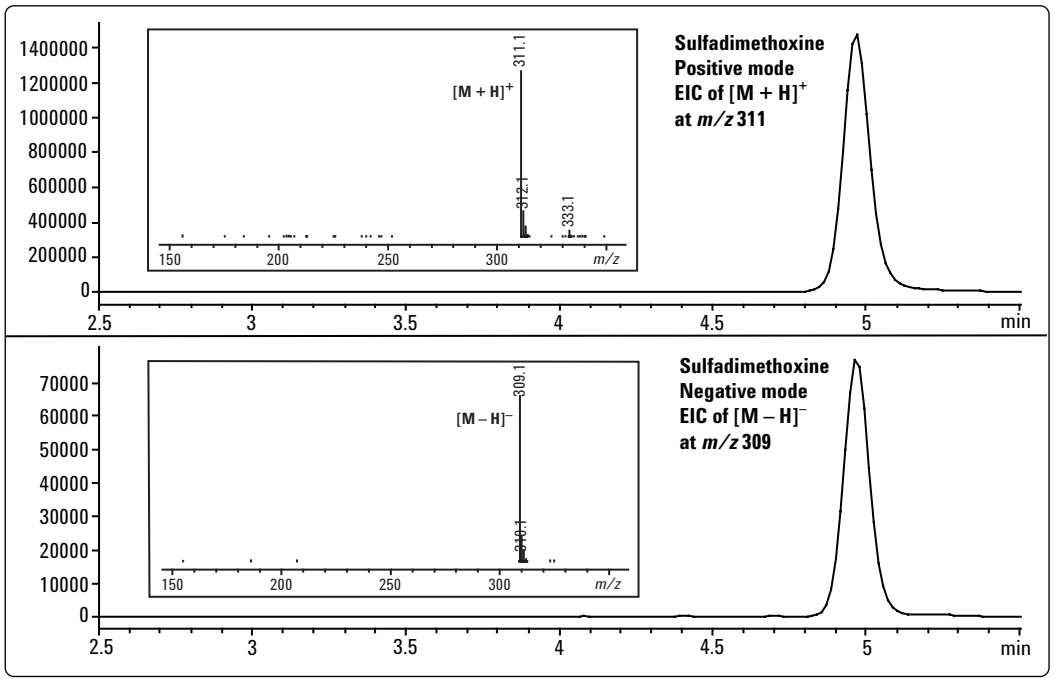

Figure 2. Extracted ion chromatograms and spectra for sulfadimethoxine, positive- and negative-mode electrospray

of the other drugs in the sample varied in a similar fashion, so only the sulfadimethoxine data are presented. Also, the LC/MSD response for sulfadimethoxine in negative mode exactly paralleled the response in positive mode, so only the positive-mode data are presented.

The data show that increasing the stepsize causes a decrease in area, but disabling the time filter has essentially no effect. Increasing the scan range to *m/z* 150–1150, a range more typical for analyses, required using the scan speed override feature in the software. There was a dramatic drop in signal abundance to 15 percent of the values obtained when using the typical conditions. However, the signal was recovered completely by increasing the chromatographic peak-width parameter from 0.1 minutes to 0.3 minutes. To assure that the LC/MSD faithfully reproduced the shape of a chromatographic peak when using 0.3 minute peak widths, the time filter was disabled.

Table 1. Variation in sulfadimethoxine response as a function of the LC/MSD acquisition parameters: scan range, stepsize, time filter, and peak width

Scan range (*m/z*)	Stepsize (u)	Time Filter	Peak Width (min)	Peak Area	Area Relative to First Analysis (%)
150–350	0.10	Yes	0.10	9350167	100
150–350	0.25	Yes	0.10	5483010	59
150–350	0.10	No	0.10	10853544	116
150–350	0.25	No	0.10	5362623	57
150–1150	0.10	Yes	0.10	1420274	15
150–1150	0.10	Yes	0.30	12532220	134
150–1150	0.10	No	0.10	810428	9
150–1150	0.10	No	0.30	13457996	144

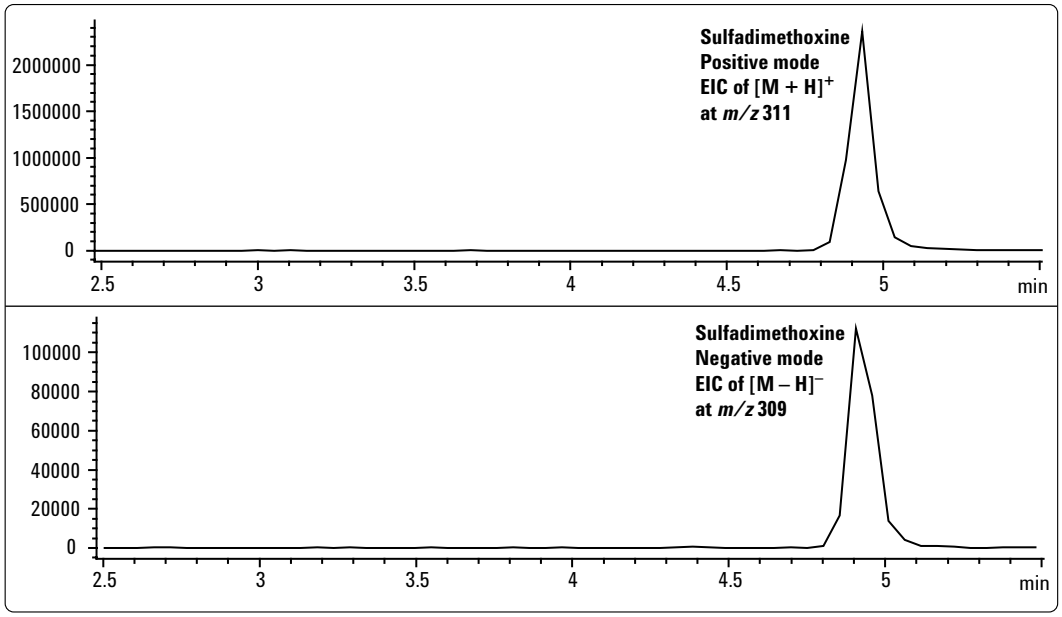

Figure 3. Sulfadimethoxine response when using optimized acquisition parameters; six to nine scans acquired across the peak

The eluting peak must be sampled a minimum number of times for chromatographic fidelity. The empirical rule is that at least six to nine data points, or scans, need to be acquired across the peak in order for the response to be within 10–20 percent of the true value. The data were checked by inspection of the list of the chromatographic window contents or by inspection of a magnified display of the peak.

Conclusions

For cases where a narrow scan range can be used, the default parameters used for LC/MSD acquisition are acceptable. The scan range may be time programmed during the analysis for increased flexibility, if desired. However, if a wide scan range is desired in conjunction with positive/negative switching, there will be a substantial loss in signal abundance if the default parameters are used. The software displays a warning to the user under these conditions and it is generally not recommended to use scan speed override unless a reduced response is acceptable. The recommended adjustment is to increase the chromatographic peakwidth parameter (from 0.1 to 0.3 minutes, for example) while also disabling the time filter. The sampling rate should be verified to assure the fidelity of the data. Increasing the scan stepsize is not recommended when analyzing small molecules unless a decrease in response is acceptable.

As with all cases of operating an instrument near the limits of its performance, there are trade-offs. Disabling the time filter in the software decreases the signal/noise of the LC/MSD, and increasing the chromatographic peak width reduces the quantitative reproducibility (increases the percent RSD of the response) of the system. In essence, some sensitivity and some reproducibility are sacrificed in order to obtain positive/negative mode data over a wide mass range in one run with high response.

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