Enhancing the Productivity of Pharmaceutical Workflows Using the Agilent 6150 Single Quadrupole Mass Spectrometer

Application Note

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Abstract

Simple, generic methods are commonly used for the routine screening of new molecular entities in a drug discovery environment. Here we describe a versatile LC/MS method that was developed using an Agilent 6150 Single Quadrupole (SQ) Mass Spectrometer coupled to an Agilent 1290 Infinity LC System that is suitable for molecular weight confirmation or impurity analysis. The UHPLC system is capable of delivering 1200 bars of pressure for rapid gradient separations using methanol, while the ultrafast scanning capability of the mass spectrometer provided unambiguous identification of nine active pharmaceutical ingredients within a run time of 2 minutes. The use of methanol as the organic solvent, instead of acetonitrile, is a greener method that reduces solvent costs by almost 75 %. The fast polarity switching feature of the MS system enabled simultaneous analysis of compounds in both positive and negative ion modes, effectively doubling productivity while reducing solvent consumption and waste generation. The high reproducibility of the method was demonstrated using replicate injections. Two co-eluting analytes, buspirone and labetalol, were identified based on their *m/z* values and were quantified using the peak areas of the extracted ion chromatograms. The peak areas were linear over 2.5 orders of magnitude. This method can also be applied in a multi-user environment for drug discovery or development laboratories using Agilent Easy Access software.

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Introduction

Single quadrupole mass spectrometers are routinely used for screening new chemical entities in drug discovery due to their specificity and ease of use. The 6150 Single Quadrupole Mass Spectrometer delivers ultrafast scanning speeds (10,000 Da/s) and fast polarity switching (20 ms), making it an ideal detector for the high-throughput analysis of pharmaceutical compounds. A high-throughput generic LC/MS method for the analysis of pharmaceutical compounds in positive ionization mode using an Agilent 6140 Single Quadrupole LC/MS system has been described earlier.[1]

This application note demonstrates the rapid separation and detection of nine pharmaceutical compounds with a generic polarity switching LC/MS method using the 6150 Single Quadrupole Mass Spectrometer coupled to a 1290 Infinity LC System equipped with Agilent ZORBAX RRHD series columns. A mixture of pharmaceutical compounds preferentially forming either positive or negative ions was selected for analysis. The formulae and the observed masses of the analytes used in this study are shown in Table 1.

Experimental

Preparation of Standards

Stock solutions of all the standards (Sigma-Aldrich, Bangalore) were prepared in LC/MS grade methanol. They were then diluted with distilled water to produce an aqueous mixture containing 1 µg/mL of each analyte. In addition, a dilution series was prepared containing 10, 50, 100, 500, 1000, and 5000 ng/mL each of buspirone and labetalol in water. The analytes were chromatographically separated and analyzed using the fast polarity switching LC/MS conditions shown in Table 2.

Table 1. Analyte characteristics: ion polarity, formulae, and observed *m/z*.

Compound	Ion polarity	Formula	Observed *m/z*
Amlodipine	+	$C_{20}H_{25}ClN_2O_5$	409.0
Buspirone	+	$C_{21}H_{31}N_5O_2$	386.2
Canrenone/Spironolactone	+	$C_{22}H_{28}O_3$ / $C_{24}H_{32}O_4S$	341.2
Diclofenac	-	$C_{14}H_{11}Cl_2NO_2$	293.8
Flurbiprofen	-	$C_{15}H_{13}FO_2$	487.2
Furosemide	-	$C_{12}H_{11}ClN_2O_5S$	328.8
Labetalol	+	$C_{19}H_{24}N_2O_3$	329.0
Nadolol	+	$C_{17}H_{27}NO_4$	310.0
Nefazadone	+	$C_{25}H_{32}ClN_5O_2$	470.2

Table 2. LC/MS conditions.

LC conditions	
Column/cartridge	Agilent ZORBAX RRHD Eclipse Plus – C18, 2.1 × 100 mm, 1.8 µm (p/n: 959758-902)
Column temperature	45 °C
Flow rate	1.0 mL/min
Mobile phase A	0.005 % formic acid in ultrapure water
Mobile phase B	Methanol
Gradient	5 % B initially; ramp up to 80 % B over 0.9 minutes; hold at 80 % B for 0.9 minutes; bring to 5 % B in 0.1 minutes; stop data collection at 2.0 minutes and hold for a post time of 1.0 minutes
Autosampler temperature	6 °C
Injection volume	5 µL
MS conditions	
Ionization mode	AJS-ESI positive and negative
Drying gas	10 L/min at 250 °C
Nebulizer pressure	50 psi
Sheath gas	10 L/min at 300 °C
Capillary voltage	2000 V (±)
Ultrafast scan	250–850 *m/z*
Fragmentor	110 V
Peak width	0.05 minutes
Threshold	500
Step size	0.2 (fixed)
Gain	1.0

System Configuration

The Agilent LC/MS System consisted of an Agilent 6100 Series Single Quadrupole LC/MS equipped with an Agilent Jet Stream source and the following LC modules:

- 1290 Infinity Binary Pump
- 1290 Infinity Autosampler
- 1290 Infinity Thermostat
- 1290 Infinity Thermostatted Column Compartment

The system was controlled using Agilent ChemStation software (B.04.03).

Results and Discussion

This application note demonstrates the suitability of the 6150 Single Quadrupole Mass Spectrometer for the analyses of positive and negative ions in a single run using the Agilent Jet Stream source combined with the 1290 Infinity LC System. The ultrafast scanning capabilities (10,000 Da/s) and fast polarity switching (20 ms) of the 6150 Single Quadrupole MS system enabled the detection of nine pharmaceutical compounds, using both positive and negative ionization modes, in a run time of 2 minutes.

The positive and negative total ion chromatograms (TIC) of a 1 µg/mL aqueous mixture of each standard obtained using the generic LC/MS polarity switching method are shown in Figure 1. An overlaid extracted ion chromatogram (EIC) is shown in Figure 2. Spironolactone is known to undergo a quantitative loss of the ethanethioic S-acid in the ESI source to form canrenone (*m/z* 341.2).[2] Good chromatographic peak shapes and separations were achieved for all the analytes, except for labetalol and buspirone which elute very close together and are not distinguishable in the TIC. However, by leveraging the high specificity of mass spectrometry, these two analytes can easily be identified as two separate peaks from extracted ion chromatograms.

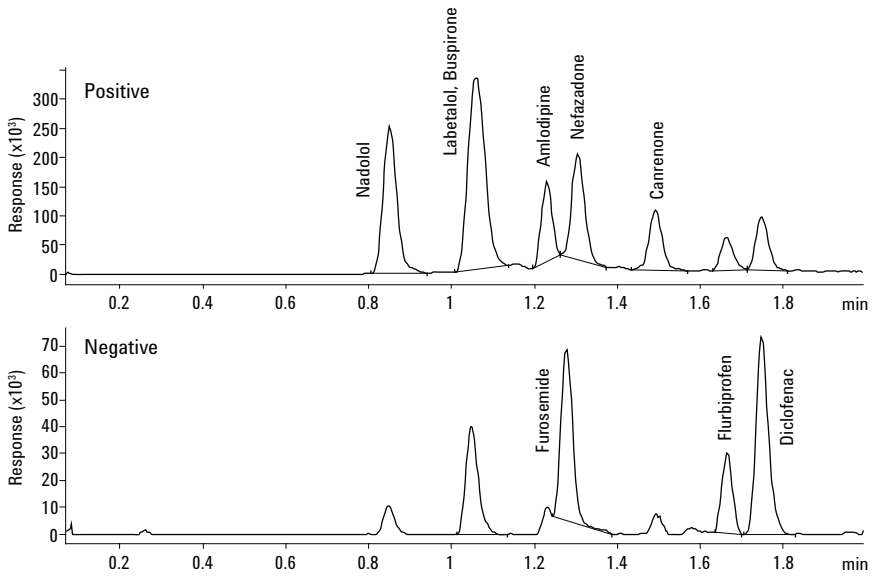

Figure 1. Positive and negative TIC (1 µg/mL aqueous mixture) of each standard obtained using the generic LC/MS polarity switching method.

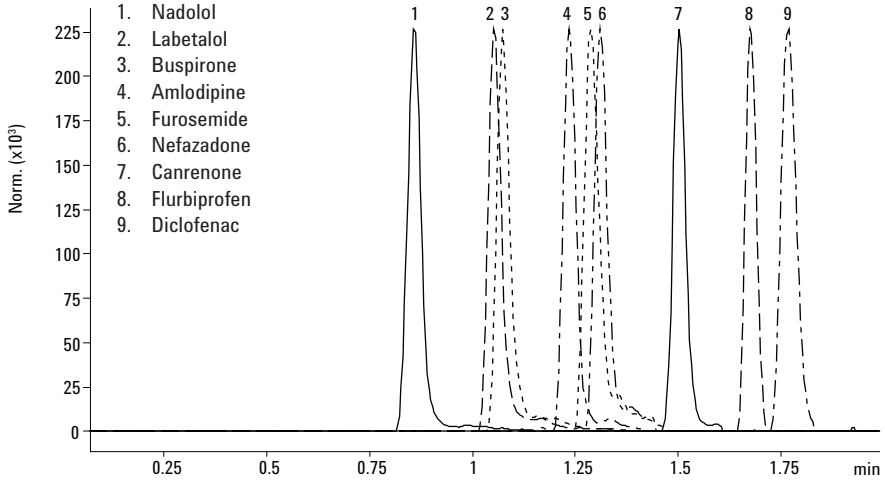

Figure 2. Overlaid Extracted Ion Chromatograms (EIC) of all nine compounds.

During method development, setting the nozzle voltage at a value that is typically used in the positive ionization mode (0 – 500 V) enhanced the responses of the compounds ionizing in the positive ion mode. However, under fast polarity switching conditions, the ionization of negative ion mode compounds is unfavorably impacted. Hence, it was beneficial to adjust the setting of MS source parameters by reducing capillary voltage and optimizing nozzle voltage with the AJS source when developing methods using fast polarity switching.

Six replicate injections of the 1 µg/mL aqueous mixture of standards were made and the data were collected with and without polarity switching. Table 3 shows that the percentage difference in the average peak areas of all the compounds are less than 15 %, demonstrating that polarity switching during data collection has a negligible impact on the analyte responses.

To test the reproducibility of this method, 50 replicate injections of the 1 µg/mL aqueous mixtures of the standards were made, and the RSDs of the retention times and peak areas were calculated for each analyte. The data summarized in Table 4 show that overall the reproducibility of the retention time for all the peaks is excellent, with RSD values less than 0.2 %. Peak area reproducibility is also good with RSD values less than 12 %, except for flurbiprofen, which is likely due to unstable dimer formation. Overall, the results demonstrate excellent assay reproducibility.

Table 3. Average peak areas and percent difference of compounds with and without polarity switching.

Compound	Ion polarity	Peak area (Switching)	Peak area (Non-switching)	% Difference
Nadolol	+	992056.67	1016325.67	-2.39
Labetalol	+	741015.07	706120.12	4.94
Buspirone	+	1316795.05	1531507.03	-14.02
Amlodipine	+	549113.50	507910.78	8.11
Furosemide	-	109279.07	115073.28	-5.04
Nefazadone	+	1222094.78	1409209.62	-13.28
Canreone	+	210334.37	236842.42	-11.19
Flurbiprofen	-	70619.67	70627.37	-0.01
Diclofenac	-	79196.48	78276.83	1.17

Table 4. Retention time and peak area reproducibility.

Compound	Ion polarity	RSD (Retention time)	RSD (Peak area)
Nadolol	+	0.15	9.51
Labetalol	+	0.15	10.72
Buspirone	+	0.15	8.70
Amlodipine	+	0.15	9.52
Furosemide	-	0.13	9.28
Nefazadone	+	0.14	6.81
Canrenone	+	0.13	9.80
Flurbiprofen	-	0.14	16.98
Diclofenac	-	0.17	6.80

In addition to the unambiguous identification of individual analytes, mass spectral detection allows quantitation of closely eluting and co-eluting analytes. Although the present scan method was developed primarily for qualitative nontargeted analysis, we were able to determine the response linearity of two very closely eluting analytes, buspirone and labetalol, based on their EIC signals. Figures 3A and 3B show the calibration curves of buspirone and labetalol, over 2.5 orders of linear dynamic range (10 – 5000 ng/mL) with R^2 values greater than 0.99 for both compounds. This is sufficient for estimating yields by synthetic chemists in the drug discovery phase.

Using this generic method, we were able to successfully match experimentally obtained isotopic abundances with the theoretically predicted isotopic distribution to confirm the mass spectrum of diclofenac. Ten replicate injections of 1 µg/mL aqueous mixture containing all the standards were made. The mass spectral threshold value was lowered to 200 during data collection for this experiment to ensure that the less abundant isotopes of carbon (^{13}C) and chlorine (^{37}Cl) were accurately captured. From each run, the mass spectrum of diclofenac was generated by averaging the spectra across the peak. The relative isotopic abundances were obtained using the ChemStation **Tabulate Mass Spectrum** function. These experimentally determined isotopic abundances were then compared with the theoretical isotopic distribution calculated using the PNNL molecular weight calculator.[3] The average % errors in Table 5 are less than 20 % indicating successful correlation between theoretical and observed experimental values, which is generally sufficient for unambiguous identification of diclofenac.

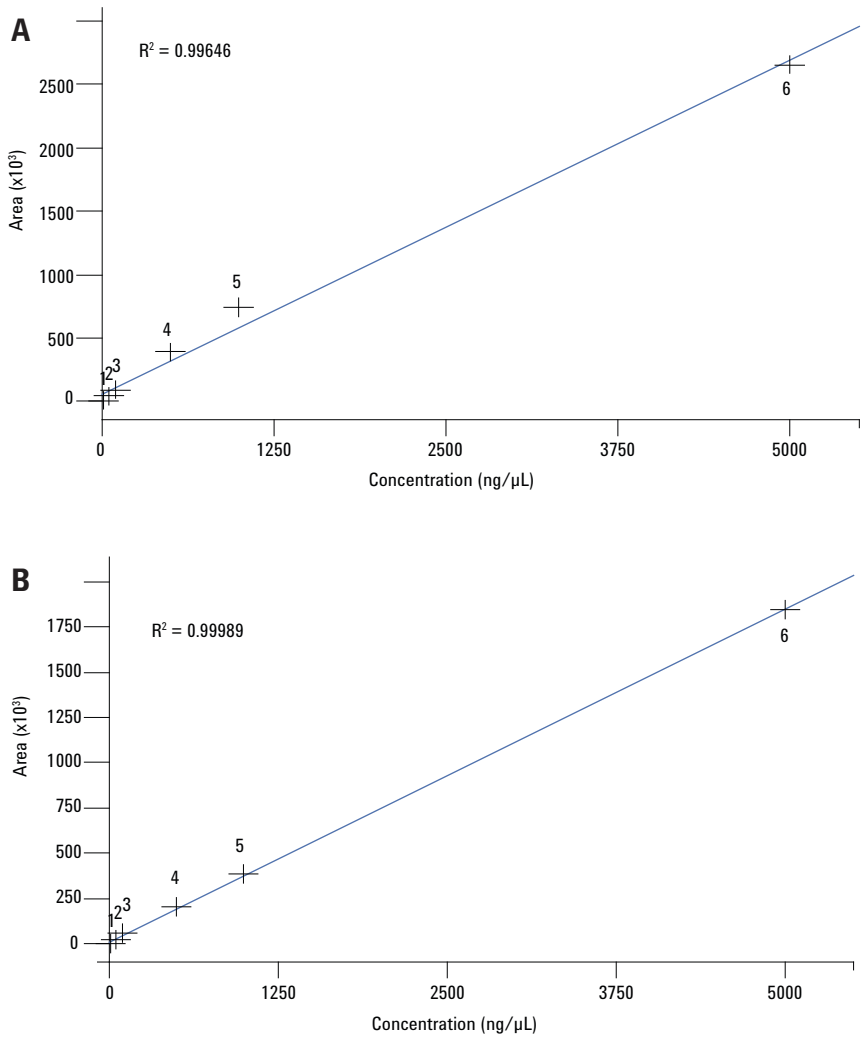

Figure 3. Standard curves for buspirone (A) and labetalol (B).

Table 5. Experimental versus theoretical isotopic distribution for diclofenac.

Peak no.	*m/z*	Observed	Theory	% Error
1	293.8	100	100	0
2	294.85	13.54	15.72	-13.84
3	295.8	68.58	65.49	4.72
4	296.8	8.37	10.17	-17.74
5	297.8	10.87	11.22	-3.14

Solvent Cost Calculations and Considerations

While it is possible to obtain a rough estimate of the methanol consumption for this method using the average of the starting and ending percentage of the organic phase, times the length of the gradient run,[4] a more accurate estimate of 1.20 mL was obtained using the Agilent Solvent Consumption Calculator.[5] Since acetonitrile/water mixtures have slightly higher eluotropic strengths than corresponding methanol/water mixtures, the following gradient conditions were used to estimate the consumption of acetonitrile at 1.07 mL per run:

Gradient: 3 % B initially; ramp up to 73 % B over 0.9 minutes; hold at 73 % B for 0.9 minutes; bring to 3 % B in 0.1 minutes; stop data collection at 2.0 minutes and hold for a post time of 1.0 minutes.

Although the consumption of acetonitrile is lower, its higher purchase price and disposal costs offset the difference. At the time of this publication, the average per-liter price from three domestic suppliers was approximately $ 130 for acetonitrile versus $ 30 for methanol. Therefore, the use of methanol instead of acetonitrile lowers the cost-per-analysis by approximately 75 %. In addition, using polarity switching to investigate multiple analytes in a single run lowers solvent consumption and waste generation by 50 % while doubling productivity to provide additional cost savings.

Another advantage of replacing acetonitrile with methanol is that methanol is considered to be a greener solvent, making this method more environmentally friendly.[6] This method can also be applied in a multi-user environment for drug discovery or development laboratories using Agilent Easy Access software.[7]

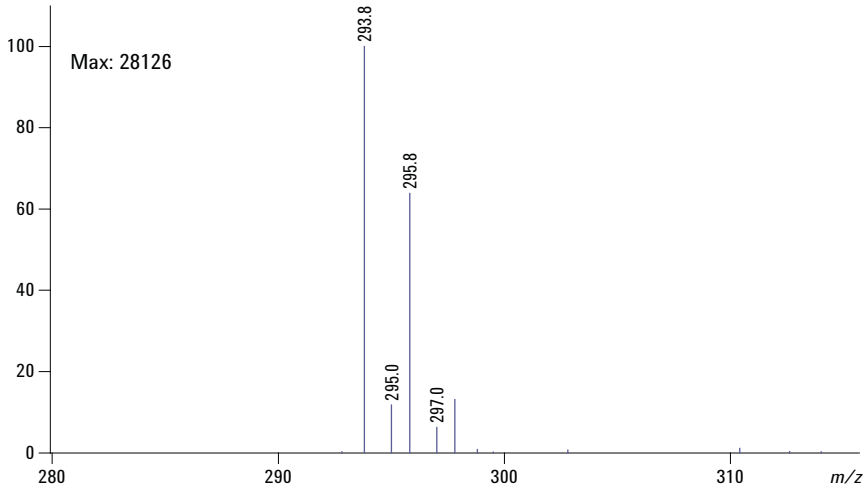

Figure 4. A representative averaged mass spectrum from replicate injections of diclofenac.

Conclusions

This application note describes a simple, generic LC/MS method for the detection of nine pharmaceutical compounds using a 1290 Infinity LC System coupled to a 6150 Single Quadrupole Mass Spectrometer equipped with the Agilent Jet Stream ESI source. The fast chromatographic separation of the UHPLC system and ultrafast scanning capability of the mass spectrometer enabled the detection of all nine analytes within a run time of 2 minutes. By leveraging the fast polarity switching feature of the 6150 Single Quadrupole Mass Spectrometer, compounds ionizing in both positive and negative ionization modes were detected in a single analytical run.

This LC/MS method is amenable to both identification and quantitation of co-eluting compounds such as labetalol and buspirone. Response linearity from 10 – 5000 ng/mL was achieved for both buspirone and labetalol. In addition, the experimentally obtained isotopic distribution of diclofenac mass peaks compared well with the theoretically predicted isotope ratios, demonstrating the ability of the instrumentation and method for compound confirmation.

Finally, by using methanol instead of acetonitrile as the organic modifier solvent for this method, operating costs were reduced by almost 75 % at current prices and a more environmentally-friendly result was achieved.

References

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