Enhancing the Productivity of Pharmaceutical Workflows using the Single Quadrupole Mass Spectrometer

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Introduction

Single quadrupole (SQ) mass spectrometers are routinely used for screening new chemical entities in drug discovery due to their specificity and ease of use. In this poster a versatile LC/MS method has been described for the unambiguous identification of nine active pharmaceutical ingredients within a run time of 2 min. The Agilent 1290 Infinity UHPLC system capable of delivering 1200 bars pressure for rapid gradient separations using methanol, and the Agilent 6150 SQ Mass Spectrometer capable of ultrafast scanning speeds (10,000 Da/s) and fast polarity switching (20 ms) were used in the study. The mass spectrometer was operated in ESI mode using Agilent Jet Stream Technology (AJS). The formulae and observed masses for the analytes used in this study are shown Table 1 below.

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

*Spironolactone is known to undergo a quantitative loss of the ethanethioic S-acid in the ESI source to form canrenone

Agilent 1290 Infinity Binary LC and Agilent 6150B Single Quadrupole MS

Experimental

LC conditions

Mobile phase: A = 0.005% formic acid in Water
B = Methanol

Flow rate: 1.0 mL/min

Gradient:

Time	%B
0.0	5
0.9	80
1.8	80
1.9	5
Method run time:	2.0 min
Post-run equilibration time:	1.0 min

Column: Agilent ZORBAX RRHD Eclipse Plus C18
2.1 x 100 mm, 1.8 µm (P.N. 959758-902)

Column temperature: 45 °C
Autosampler temperature: 6 °C
Injection volume: 5 µL

MS conditions

Ionization mode: AJS-ESI (+/–)
Drying Gas: 10 L/min @ 250 °C
Nebulizer pressure: 50 Psi,
Sheath gas: 10 L/min @ 300 °C,
Capillary voltage: 2000 V (+/ –)
Nozzle voltage: 2000 V (+/ –)

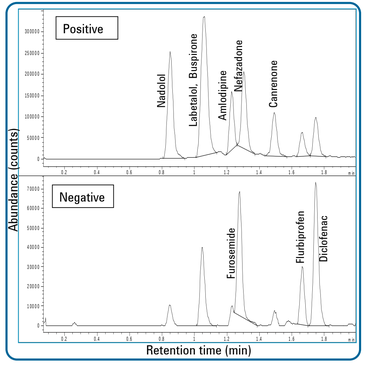
Ultra Fast Scan: 250–850 *m/z*
Polarity Switching Delay: 20 msec
Fragmentor: 110V
Peak width: 0.05 min
Threshold: 500
Step size 0.2 (fixed)
Gain 1.0

Sample Preparation

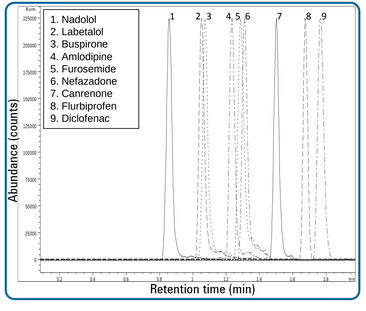
LC/MS grade formic acid and methanol were used for mobile phase preparation and sample dilution. Stock solutions of all the standards were prepared gravimetrically using methanol. Stock solutions were then combined and diluted with water to produce an aqueous mixture containing 1 µg/mL of each analyte, and a series of aqueous solutions containing 10, 50, 100, 500, 1000 and 5000 ng/mL each of buspirone and labetalol.

Results and Discussion

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 the following figure.



Good chromatographic peak shapes and separations were achieved for all the analytes, except for labetalol and buspirone which elute very closely and are not distinguishable in the TIC. However, by leveraging the high specificity of a mass spectrometer detector, these two analytes can easily be identified as two separate peaks in the overlaid extracted ion chromatograms (EIC) shown in the following figure.



The impact of polarity switching on analyte response was evaluated using six replicate injections of the 1 µg/mL mixture of standards made with and without polarity switching. Table 2 (below) shows that the average peak areas for the two sets of data differed by less than 15% for all compounds. This demonstrates that fast polarity switching during data collection has a negligible impact on analyte responses.

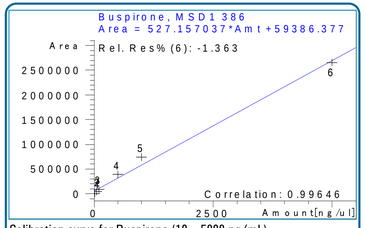
Compound/ Ion Polarity	Peak Area (Switching)	Peak Area (Non-switching)	% Difference
Nadolol/+	992056.67	1016325.67	–2.39
Labetalol/+	741015.07	706120.12	4.94
Buspirone/+	1316795.05	1531507.03	–14.02
Amlodipine/+	549113.50	507910.78	8.11
Furosemide/–	109279.07	115073.28	–5.04
Nefazadone/+	1222094.78	1409209.62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

Method reproducibility with polarity switching was investigated by making 50 replicate injections of the 1 µg/mL mixture of standards. The RSDs of the retention times and peak areas were calculated for each analyte and summarized in Table 3 (below). The data shows excellent retention time reproducibility with RSD values less than 0.2% for all analytes. Peak area reproducibility is also good with RSD values less than 12% for all analytes except flurbiprofen, which is prone to dimer formation in solution.

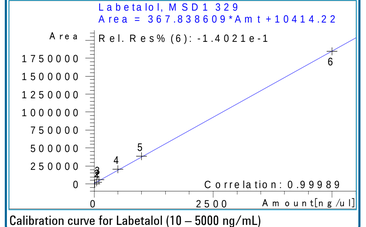
Compound	Ion Polarity	RSD (Ret. Time)	RSD (Peak Area)
Nadolol	+	0.15	9.51
Labetalol	+	0.15	10.72
Buspirone	+	0.15	8.70
Amlodipine	+	0.15	9.52
Furosemide	–	0.13	9.28
Nefazadone	+	0.14	6.81
Canrenone	+	0.13	9.80
Flurbiprofen	–	0.14	16.98
Diclofenac	–	0.17	6.80

Results and Discussion

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



Calibration curve for Buspirone (10 – 5000 ng/mL)

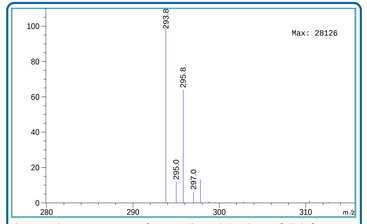


Calibration curve for Labetalol (10 – 5000 ng/mL)

To confirm that isotopic fidelity is maintained at ultrafast scan speeds, the ChemStation "Tabulate Mass Spectrum" function was used to obtain relative isotopic abundances from the diclofenac mass spectrum. These values were then compared with the theoretical isotopic distribution values in Table 4. The correlation between theoretical and observed experimental values for the five most abundant isotopes are within 20%, which is generally sufficient for unambiguous identification of diclofenac.

Peak #	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

Table 4: Experimental vs. theoretical isotope distribution for diclofenac



Averaged mass spectrum from replicate injections of diclofenac

Conclusions

- The fast chromatographic performance of the UHPLC system and ultrafast scanning capability of the Agilent 6150 mass spectrometer enabled the detection of all nine analytes within a run time of 2 min.
- By utilizing fast polarity switching with the Agilent 6150 mass spectrometer, compounds preferentially ionizing in either positive or negative ionization modes were detected in a single analytical run.
- The experimentally obtained isotopic abundances of diclofenac mass peaks compared well with the theoretically predicted abundance values demonstrating the utility of ultrafast scan speeds for compound confirmation.
- The LC/MS method is amenable to both identification and quantitation of co-eluting compounds such as labetalol and buspirone. Response linearity from 10 to 5000 ng/mL was achieved for both buspirone and labetalol.
- The use of methanol instead of acetonitrile as the organic mobile phase reduces solvent operating costs by approximately 74% at current prices.