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Highly sensitive multiplexed analysis of Levosalbutamol from plasma using LC/MS/MS

ASMS 2016 WP 716

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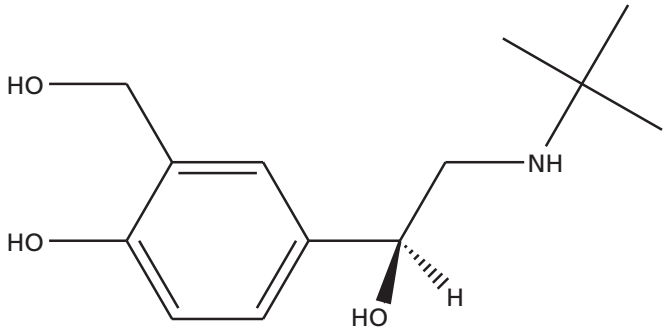
Introduction

Salbutamol is the most widely used short-acting β2-agonist in the symptomatic relief of asthma and Chronic Obstructive Pulmonary Disease (COPD). In all formulations, salbutamol consists of a racemic mixture of equal amounts (50:50) of (R)- and (S)-isomers. Although these isomers are chemically identical, they differ in conformation, being exact non-superimposable (mirror) images of one another, or stereoisomers. (R)-salbutamol has been shown to have a 2-fold greater binding affinity than racemic salbutamol and a 100-fold greater binding affinity than (S)-salbutamol for the β2-adrenergic receptor. As a result, the bronchodilator property of racemic (R,S)-salbutamol is attributed entirely to (R)-salbutamol which is also known as 'Levosalbutamol'[1]. There is an increased demand by pharmaceutical organizations to quantitatively estimate levosalbutamol at lower levels due to reduced dosages for paediatric formulations. With this increase in demand, there is a necessity to increase the throughput of highly sensitive levosalbutamol analysis.



Molecular formula - C13H21NO3

Figure 1. Structure of levosalbutamol

At lower concentrations, levosalbutamol tends to show interference from plasma. Inevitably run time will be longer for achieving chromatographic resolution, hence leading to lower analytical throughput.
Nexera MX with Dual Stream Technology (DST) system was designed to improve the operation rates of LC/MS/MS and throughput. This system switches two flow lines for analysis using the flow line switching valve. While one flow line is analyzing, the other one washes and equilibrates column. This parallel processing reduces the cycle time for sample analysis.
Therefore, a method on Nexera MX with LCMS-8060, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan has been developed to increase the throughput for highly sensitive quantitation of levosalbutamol from plasma.

Method of analysis

Sample preparation

Preparation of aqueous calibration levels and quality control samples

Levosalbutamol (Sigma Aldrich) calibration standards at concentration levels of 0.1 pg/mL, 0.2 pg/mL, 0.5 pg/mL, 1.5 pg/mL, 4 pg/mL, 18 pg/mL, 30 pg/mL, 50 pg/mL, 70 pg/mL and 80 pg/mL and quality control samples - LQC (0.5 pg/mL), M1QC (5 pg/mL), M2QC (35 pg/mL) and HQC (75 pg/mL) were prepared in water : acetonitrile (40:60 v/v).

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Preparation of matrix matched calibration levels and quality control samples

300 µL of plasma was taken in 15 mL tarson tubes to which 300 µL of 1 M potassium dihydrogen phosphate buffer was added and vortexed to ensure complete mixing of contents. To above buffered plasma, 3 mL of methyl tertiary butyl ether (MTBE) was added and vortexed for 5 minutes. It was then centrifuged at 4000 rpm for 10 minutes. Supernatant was separated and evaporated at 40 °C for 15 minutes in low pressure nitrogen evaporator. The residue was reconstituted in 1000 µL water : acetonitrile (20:80 v/v). This solution was then used as a diluent to prepare matrix matched calibration levels from 0.1 pg/mL to 80 pg/mL and quality control samples[2].

LC/MS/MS analysis

Levosalbutamol was analyzed using Nexera MX coupled with LCMS-8060 triple quadrupole system from Shimadzu Corporation, Japan.

The Nexera MX offers twice the sample processing capacity for LC/MS/MS analysis. In regular LC/MS/MS analysis, due to the various processes required, such as washing and equilibrating the column with an initial mobile phase concentration and operating the autosampler to load the next sample, the time between injecting samples cannot be fully utilized for acquiring data. Therefore, efforts to increase LCMS throughput involved reducing the time required for the above processes. In contrast, the Nexera MX maximizes the LC/MS/MS data acquisition time by alternately using two streams to inject samples into the LC/MS/MS system (shown in Figure 2).

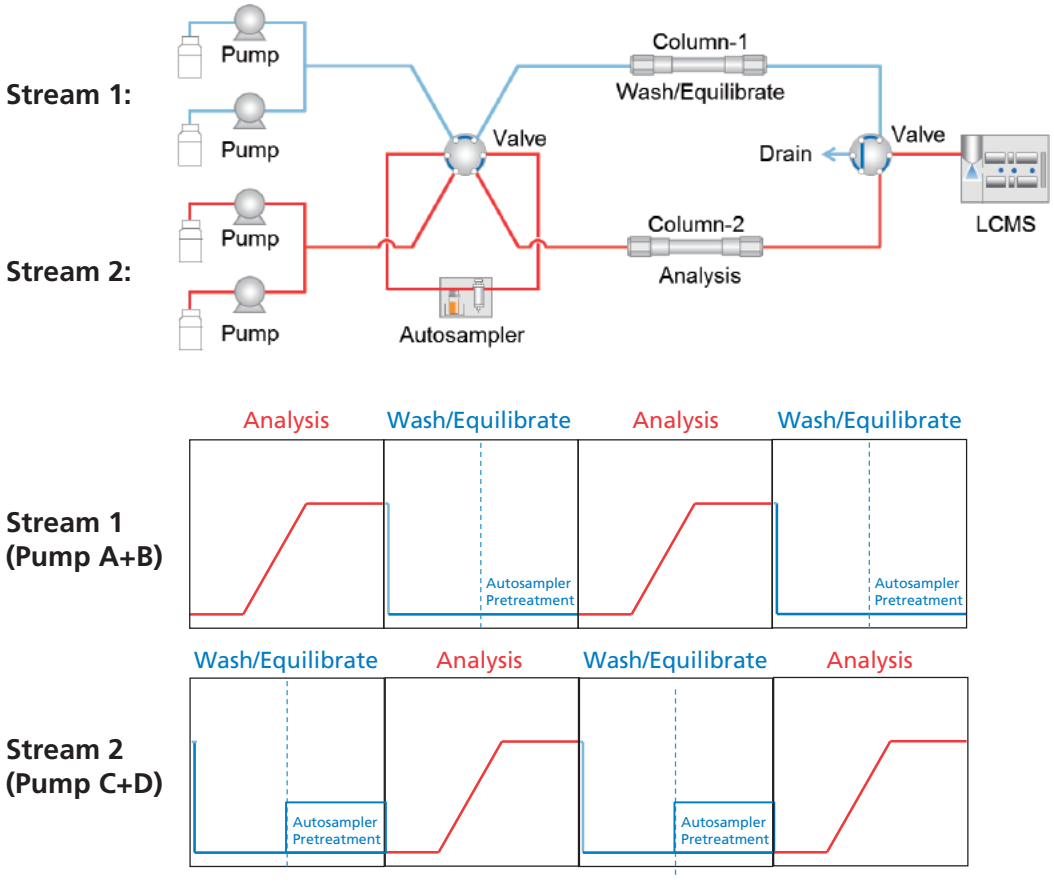

Figure 2. Nexera MX with Dual Stream Technology (DST)

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Figure 3. Nexera MX with LCMS-8060

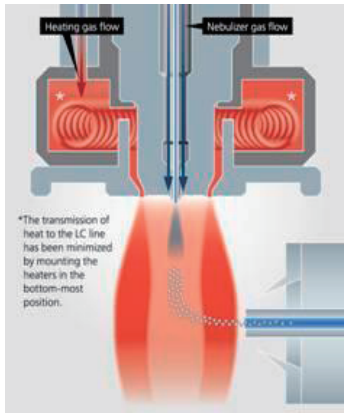

Figure 4. Heated ESI probe

LCMS-8060 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 3), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.
In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 4) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.
The details of analytical conditions are given in Table 1.

Table 1. LC/MS/MS conditions for levosalbutamol

Column	: Grace Alltima HP HILIC (50 mm L x 2.1 mm I.D.; 3 µm)
Mobile phase	: A: 2 mM ammonium acetate in water B: acetonitrile
Gradient program (B %)	: 0.0-2.5 min → 90 (%); 2.5-3.0 min → 90-20 (%); 3.0-4.0 min → 20(%); 4.0-4.2 min → 20-90 (%); 4.2-6.0 min → 90 (%)
Flow rate	: 0.5 mL/min
Oven temperature	: 40 °C
Injection volume	: 10 µL
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 5 L/min
Zero air flow	: Heating gas 15 L/min
MS temperature	: Desolvation line 200 °C; Heating block 400 °C Interface 380 °C

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Results

Nexera MX analysis results of levosalbutamol

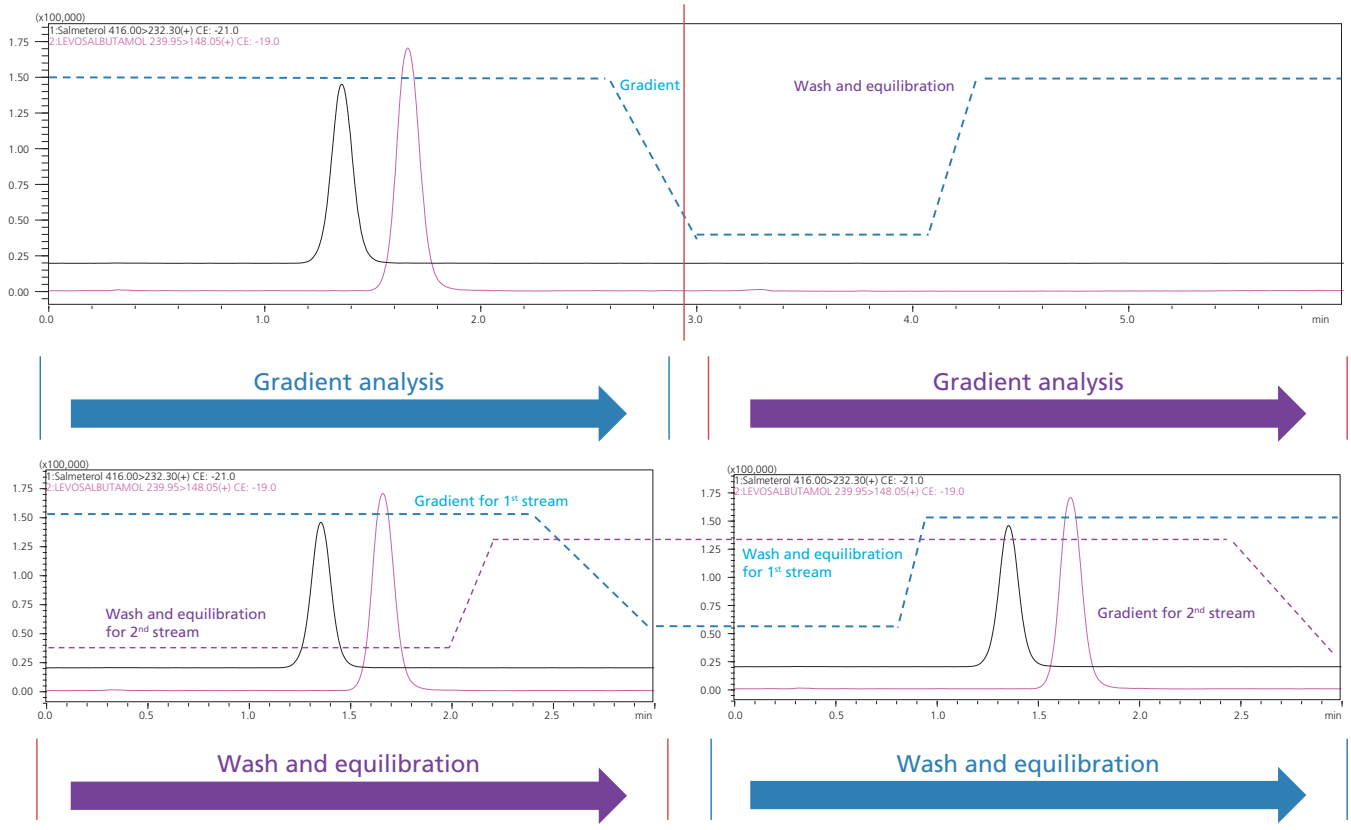

Figure 5. Comparison between conventional analysis and MX analysis showing maximum LC/MS/MS data acquisition by alternately using two streams to inject samples into LC/MS/MS system

Nexera MX enabled parallel HPLC systems to synchronize with a single mass spectrometer. In Nexera MX while one stream is analyzing, the other washes and equilibrates the column. This parallel processing reduces the cycle time for sample analysis. This helps to acquire data simultaneously without keeping MS idle during washing and equilibration step. In this way, non productive time was utilized for acquiring data for next sample, thus increasing the throughput.

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LC/MS/MS analysis results of levosalbutamol

LC/MS/MS method was developed for trace level quantitation of levosalbutamol using salmeterol as an internal standard. Analysis was performed using matrix matched standards and quality control samples. MRM transitions used for levosalbutamol and salmeterol are 239.95 > 148.05 and 416.00 > 232.30 respectively. LOQ of 0.1 pg/mL was achieved for levosalbutamol.

Overlay of MRM chromatograms of blank with 0.1 pg/mL level for levosalbutamol and blank with salmeterol matrix matched standard are shown in Figures 6A and 6B respectively. No interfering peaks were observed in blank plasma at the retention time of these compounds, confirming the absence of any interference.

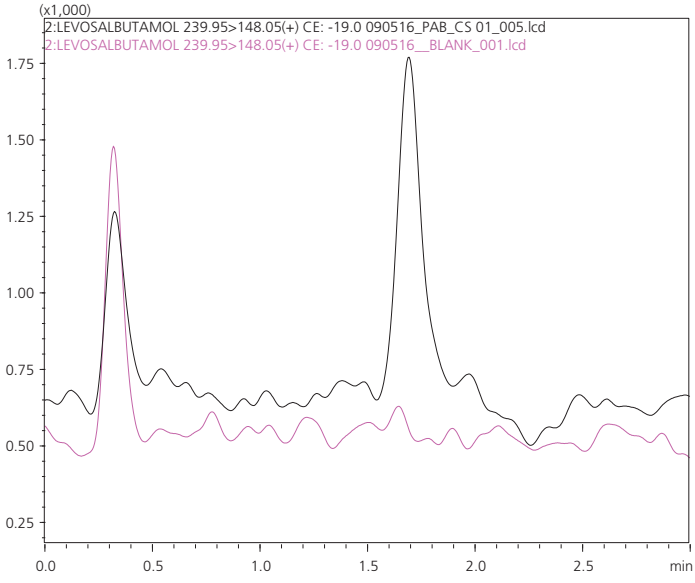

Figure 6A. Overlay of MRM chromatograms of blank and 0.1 pg/mL for levosalbutamol matrix matched standards

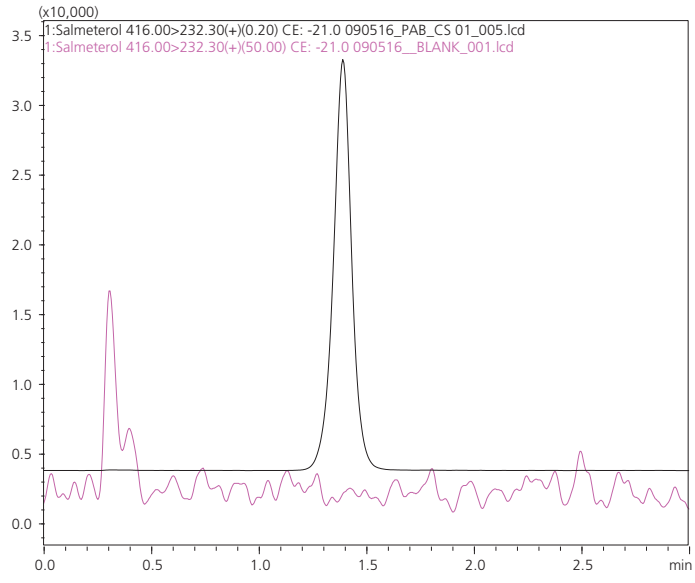

Figure 6B. Overlay of MRM chromatograms of blank and salmeterol as internal standard

Linearity studies were carried out using internal standard calibration method with correlation coefficient of 0.9972 as shown in Figure 6. Accuracy and repeatability results for Levosalbutamol are given in Table 2.

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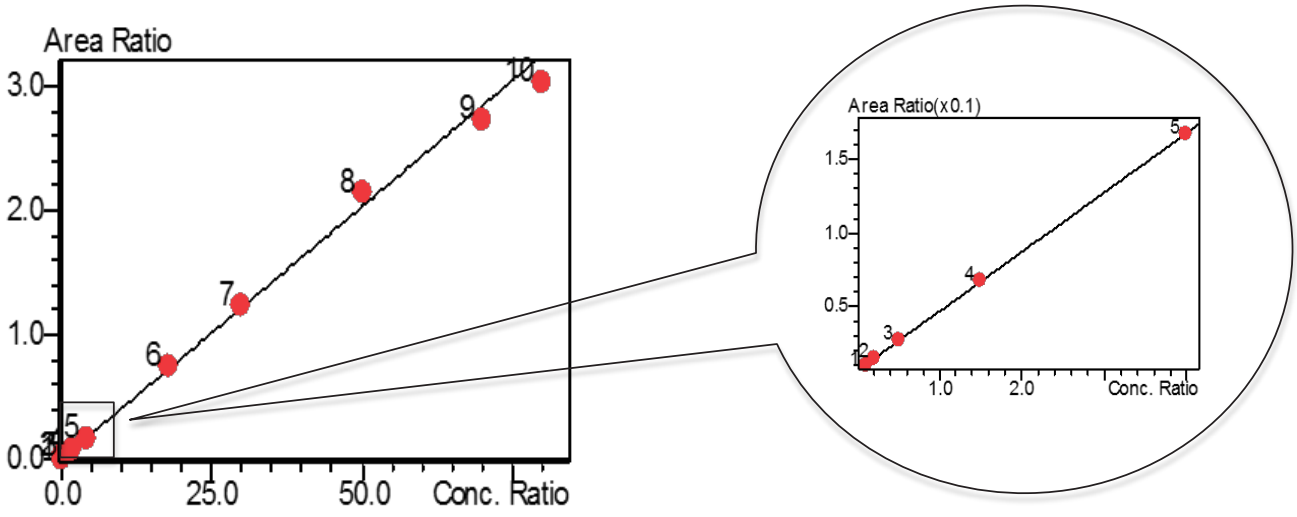

Figure 6. Calibration curve for levosalutamol matrix matched standards

Table 2. Results of accuracy and repeatability for levoslbutamol matrix matched calibration standards and quality control samples

Name of compound	Standard concentration (pg/mL)	Calculated average concentration from calibration graph (pg/mL) (n=6)	Average % accuracy (n=6)	% RSD for area counts (n=6)
Levosalbutamol	0.1	0.09	87.26	5.73
	0.2	0.22	113.88	5.05
	0.5	0.49	101.63	4.47
	1.5	1.50	101.68	1.07
	4.0	3.95	99.59	0.84
	18.0	18.11	101.66	0.83
	30.0	30.89	101.95	0.85
	50.0	53.54	105.72	0.45
	70.0	68.38	95.90	0.36
	80.0	75.75	93.38	0.21
	LQC (0.5)	0.56	112.87	4.87
	M1QC (5.0)	4.99	102.34	0.91
	M2QC (35.0)	36.72	102.38	0.49
	HQC (75.0)	78.11	101.43	0.31

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Conclusion

- Nexera MX enhanced the operation rates of LC/MS/MS and throughput by alternately using two streams to inject samples into the LC/MS/MS system.
- Nexera MX with DST allowed removal of matrix components without increasing cycle time and maintaining consistency of results at trace level quantitation.
- Heated ESI probe of LCMS-8060 helped in achieving LOQ of 0.1 pg/mL for levosalbutamol with considerable reduction in background. Hence, LCMS-8060 system from Shimadzu gives a complete solution for bioanalysis.

References

[1] Sathish Kumar Shetty. A. et al., International Journal of Biological & Pharmaceutical Research, (2012), 320-326.
[2] Snehal Gomes et al., International Journal of Pharmacy and Biological Sciences, Volume 3 Issue 2, (2013), 247-257.

Disclaimer : LCMS-8060 is intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2016

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