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Quantitative analysis of 6 beta blockers on an LCMS-8045RX

WP 131

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1. Overview

An ultra-trace sensitive LC-MS/MS research method was developed for quantification of beta-adrenergic blocking agents that was able to achieve limits of detection at low concentration levels.

2. Introduction

Beta-adrenergic blocking agents, commonly known as beta blockers, competitively inhibit the binding of catecholamines such as epinephrine and norepinephrine to β-adrenergic receptors, thereby reducing heart rate and blood pressure. These drugs are widely prescribed for the treatment of hypertension, angina and heart failure[1]. However, beta blockers may also be misused by athletes resulting in their inclusion on the World Anti-Doping Agency (WADA) prohibited list[2]. Owing to both their therapeutic importance and potential misuse, sensitive and selective analytical methods are required for reliable detection and quantification. Liquid chromatography tandem mass spectrometry (LC-MS/MS) offers high sensitivity and specificity for such analyses.

3. Method

Nadolol, metoprolol, propranolol, atenolol & carvedilol were purchased from Sigma Aldrich and Thermo Fisher Scientific. Standards were prepared in 1 mg/mL in LCMS grade methanol. Nexera 40-series HPLC system connected to a LCMS-8045RX was used for data acquisition. The mixture of standards was further diluted in LCMS grade methanol (Sigma Aldrich) and Sigmatrix urine diluent to create the standards for the calibration curves. A Shim-pack Velox Biphenyl column was used for the chromatographic separation. All analytes were ionized with electrospray ionization (ESI) in positive ion mode.

Table 1. MRM Transitions used for quantitation

Compound Name	Precursor m/z	Product 1 m/z	Product 2 m/z
Atenolol	267.17	190.10	74.10
Nadolol	310.20	201.10	236.10
Acebutolol	337.21	98.10	56.05
Metoprolol	268.19	74.10	72.05
Propranolol	260.16	183.10	56.10
Carvedilol	470.20	224.10	222.05

Table 2. HPLC and MS acquisition parameters

Nexera X3				LCMS-8045RX	
Analytical Column	Shim-pack Velox Biphenyl column, 2.7 µm, 2.1 × 100 mm			**Interface**	CoreSpray-ESI
Mobile phase	A: 5 mM Ammonium Formate + 0.1% Formic Acid in LCMS grade Water.			**Ionization mode**	Positive
	B: 5 mM Ammonium Formate + 0.1% Formic Acid in 1:1 Methanol : Acetonitrile (LCMS grade).				
Flow rate	0.6 mL/min			**Nebulizing gas flow**	7 L/min
Oven Temp	40 °C			**Heating gas flow**	14 L/min
Injection Volume	1 µL			**Interface Temperature**	400 °C
Gradient	T (min)	A (%)	B (%)	**Desolvation Temperature**	650 °C
	0.00	90	10		
	0.01	90	10	**DL Temperature**	150 °C
	0.50	90	10		
	4.00	5	95	**Heat Block Temperature**	500 °C
	5.00	5	95		
	5.01	90	10	**Drying gas flow**	3 L/min
	7.00	90	10		

4. Results and Discussion

Table 3: Limits of detection and limits of quantification for beta blockers mixture in Sigmatrix urine diluent and methanol. Limits of detection were the same across both matrices evaluated in this study. Calibration ranges were from 0.010 – 100 ng/mL.

Analyte	RT (min)	R^2	Sigmatrix urine diluent and methanol LOD (ng/mL)	Sigmatrix urine diluent LOQ (ng/mL)	Methanol LOQ (ng/mL)
Atenolol	1.04	0.996	0.010	0.010	0.010
Nadolol	1.78	0.997	0.010	0.010	0.025
Acebutolol	2.05	0.996	0.010	0.010	0.010
Metoprolol	2.14	0.996	0.010	0.025	0.025
Propranolol	2.63	0.995	0.010	0.010	0.010
Carvedilol	3.05	0.996	0.010	0.025	0.010

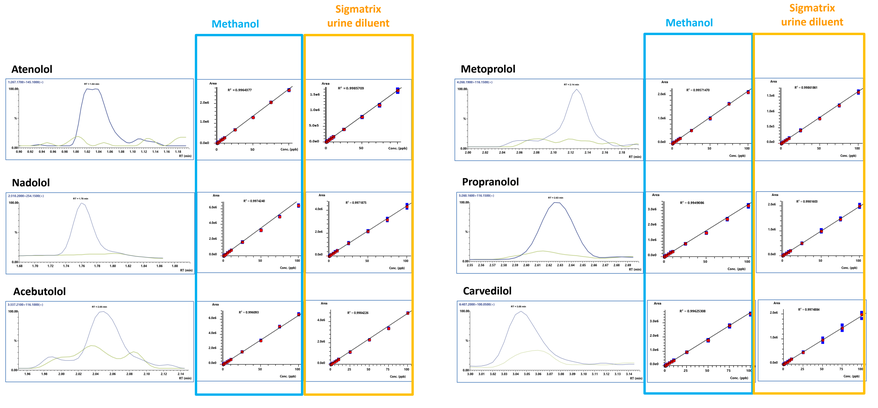

Figure 1. Calibration curves for the 6 analytes in methanol and Sigmatrix urine diluent. Chromatograms are an overlay of blank and LOQ (at accuracy ±20% and S/N > 10) in methanol.

5. Conclusion

- *Superior sensitivity in complex matrices:* Limit of Detection (at accuracy ±20% and S/N > 3) of 0.010 ng/mL for all the analytes evaluated in this study.
- *Excellent linearity and accuracy:* Linear responses (with R^2 > 0.99) observed across the evaluated concentration range, and consistent accuracy within ±20% for all 17 calibrants included in this study.
- *Optimized throughput:* Automated interface and MRM optimization via LabSolutions Connect MRM ensured maximum signal-to-noise determination for this study, while the high-organic flush at the end of each run, maintained column integrity.

6. Reference

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