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Fast determination of plasma catecholamines and metanephrines by solid-phase extraction and liquid chromatography–tandem mass spectrometry applicated to clinical assay

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1. Introduction

Catecholamines (dopamine, epinephrine, and norepinephrine) are the main neurotransmitters that regulate various functions of the central nervous system, such as motor control, cognition, emotions, memory processing and endocrine regulation. Their determination with their methoxylated derivatives (3-methoxytyramine, metanephrine and normetanephrine), allows the diagnosis of pheochromocytomas, paragangliomes in adults and neuroblastomas in children.

A sensitive method combining Solid Phase Extraction (SPE) and Liquid Chromatography - tandem Mass Spectrometry (LC-MS/MS) has been developed for the determination of catecholamines and their methoxylated derivatives in human plasma. LC-MS/MS is an ideal tool for specific analysis in a complex matrix. We monitored the MRM quantifier and qualifier transitions and used deuterated internal standards for each analyte to ensure accurate and reproducible quantification.

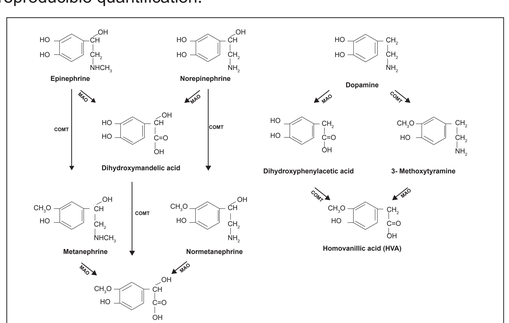

Figure 1 : Metabolic pathway of catecholamines and metanephrines

Source : Gabriela (Paula) Finkielstain , Smita Jha , Deborah Merke, Chapter 9 - Adrenal disorders, 2021, pages 267-296, Biochemical and molecular basis of pediatric disease (filfth edition)

2. Method and Material

Calibrators and quality controls were prepared using a spiked free synthetic plasma and extracted by Evolute Express 96-Well SPE plate, an anion exchange phase. Stable isotope labelled compounds were used as internal standards for extraction and quantification to compensate for matrix effects and to increase the accuracy and precision.

Following SPE purification of catecholamines and their methoxylated derivatives from human plasma samples, the extract was injected onto an PFP column using a triple quadrupole mass spectrometer 8060NX (Shimadzu Corporation, Kyoto, Japan) coupled to a Nexera LC40x3 model UHPLC system.

High Speed Mass Spectrometer
Ultra Fast Polarity Switching
- 5ms
Ultra Fast MRM
- Max. 555 transitions/sec

Figure 2 : LCMS-8060NX triple quadrupole mass spectrometer

UHPLC conditions (Nexera LC40 system)

Column : PFP
Mobile phase A : H2O + additifs
Mobile phase B : MeOH + additifs
Flow rate : 0.25 mL/min
Injection volume : 5 µL
Column temperature : 30°C

MS conditions (LCMS-8060NX)

Compound	Polarity	MRM Quantification
Adrenaline	+	184.1 > 166.2
Adrenaline D3	+	187.05 > 169.2
Noradrenaline	+	152.2 > 107.1
Noradrenaline D6	+	157.9 > 111.05
Dopamine	+	154.1 > 137.1
Dopamine D4	+	157.1 > 139.05
Metanephrine	+	180.1 > 165.1
Metanephrine D3	+	183.1 > 168.3
Normetanephrine	+	166.1 > 134
Normetanephrine D3	+	169.1 > 137.1
3-Methoxythyramine	+	168.1 > 91.05
3-Methoxythyramine D4	+	172.3 > 95.1

Table 1 : MRM transitions

3. Results and discussion

Figure 3 shows MRM chromatograms of 6 targeted biomarkers. It tooks 9 min per one LC-MS/MS analysis. Excellente separation and high sensitive detection were obtained.

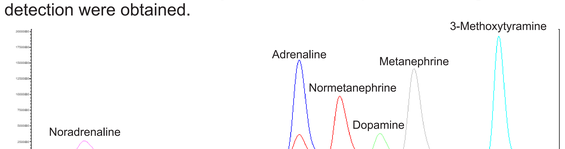

Figure 3 : Mass Chromatograms of Catecholamines and Metanephrines

3-1. Calibration curves

The calibration range was 1-100 nmol/L for all compounds, with r² values of the calibration models above 0,99.

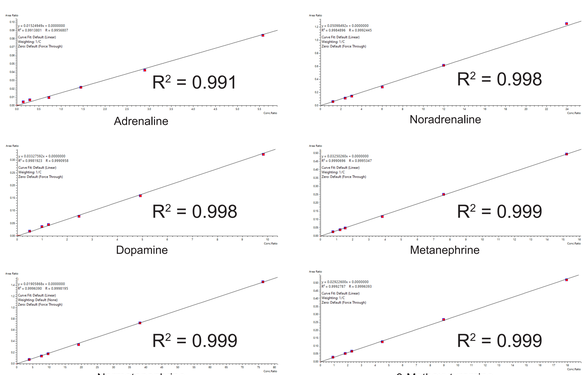

Figure 4 : Calibration curves

4. Performances evaluation

4.1 Intra-days repeatability and Accuracy

Analytical performances of the method were monitored using spiked free synthetic plasma at differente concentrations. Accuracies of controls are between 92 and 111% for all analytes, and RSD values (n=30 intra-day) were below 10%.

Compound	Epinephrine			Norepinephrine			Dopamine		
Level	Low Controls	Medium Controls	High Controls	Low Controls	Medium Controls	High Controls	Low Controls	Medium Controls	High Controls
Average (nmol/L)	3.33	8.28	41.75	3.02	8.80	43.19	3.02	8.28	45.75
CV	2.1%	1.5%	2.0%	5.5%	4.5%	5.4%	5.5%	1.6%	5.1%

Compound	Metanephrine			Normetanephrine			3-Methoxythyramine		
Level	Low Controls	Medium Controls	High Controls	Low Controls	Medium Controls	High Controls	Low Controls	Medium Controls	High Controls
Average (nmol/L)	3.15	7.98	41.34	2.90	8.15	43.22	3.32	8.30	42.97
CV	2.1%	2.8%	2.7%	6.5%	3.4%	2.4%	1.4%	1.1%	1.9%

Table 2 : Repeatability over 30 intra-days injections

Compound	Accuracy		
	Low Controls	Medium Controls	High Controls
Adrenaline	111%	104%	93%
Noradrenaline	101%	110%	96%
Dopamine	101%	104%	102%
Metanephrine	105%	100%	92%
Normetanephrine	97%	102%	96%
3-Methoxythyramine	111%	104%	95%

Table 3 : Controls accuracies

4.2 Limits of quantification

The low limits of quantification (LLOQ) in plasma were determined below 0.5 nmol/L for all analytes, as described in the table.

Molecule	LOQ (nmol/L)
Adrenaline	0.2
Noradrenaline	0.5
Dopamine	0.3
Metanephrine	0.1
Normetanephrine	0.1
3-Methoxythyramine	0.1

Table 4 : Limits of quantification

5. Conclusion

The method allows the determination of catecholamines and their methoxylated derivatives at very low levels (0.1-0.5 nmol/L for catecholamines and 0.1 for all their methoxylated derivatives) according to the need of clinical diagnosis with very good accuracy and precision. Simultaneous analysis strengthens clinical diagnosis by having enlarged vision (metabolites and catabolites).

The method shows a good linearity with a r² >0.991. Intra-days precision was less than 6.5% for all compounds. High accuracy was achieved (between 92 and 111%) using spiked plasma controls. No carryover was measured for all compounds.