

Sensitive and specific LC-MS/MS analysis of plasma free metanephrines using either on-line or off-line sample cleanup

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Introduction

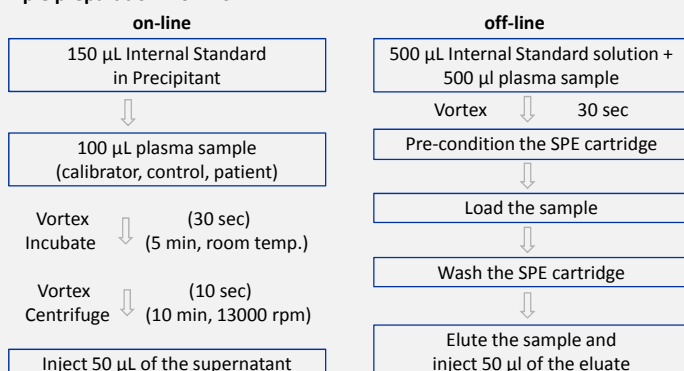
- Quantitation of plasma free metanephrines (PFM) using LC-MS/MS is recommended for sensitive and specific diagnosis of the catecholamine-producing tumors pheochromocytoma and paraganglioma
- PFM comprise the O-methylated forms of catecholamines, namely normetanephrine, metanephrine and 3-methoxytyramine
- Analytical measurement is challenging because of their polar nature, their low molecular weight and their low physiological concentration in human plasma
- Low physiological concentration necessitates enrichment of analytes – either via on-line or off-line sample cleanup
- Up to now, no diagnostic LC-MS/MS complete kit is commercially available for use in clinical routine analysis

Development of RECIPE's ClinMass® LC-MS/MS Complete Kits – Free Metanephrines in Plasma (MS11000, MS11100) for simplified LC-MS/MS analysis and improved standardisation of routine measurement

Materials and Methods

- Analytes / Internal Standards:
 - Normetanephrine / Normetanephrine-d₃
 - Metanephrine / Metanephrine-d₃
 - 3-Methoxytyramine / 3-Methoxytyramine-d₄
- Calibrators and controls:
 - ClinCal® Calibrators: Serum Calibrator Set (lyophilized) (Level 0-4)
 - ClinChek® Controls: Serum Controls (lyophilized) (3 levels)

Sample preparation workflow



LC-system

on-line:	off-line:
Two-dimensional LC-system (injection interval 6 min)	Standard LC-system (injection interval 4 min)
2 binary pumps	1 binary pump
6-port switching valve	autosampler
autosampler	column oven
column oven	

MS/MS system

- Ionisation: ESI⁺
- Mass analysis and detection: Triple quadrupole mass spectrometer

Compound	Parent Ion [m/z]	Product Ion [m/z]
Normetanephrine	166	134
Metanephrine	180	148
3-Methoxytyramine	151	119

Table 1. MRM parameters

Results (on-line method)

Chromatograms

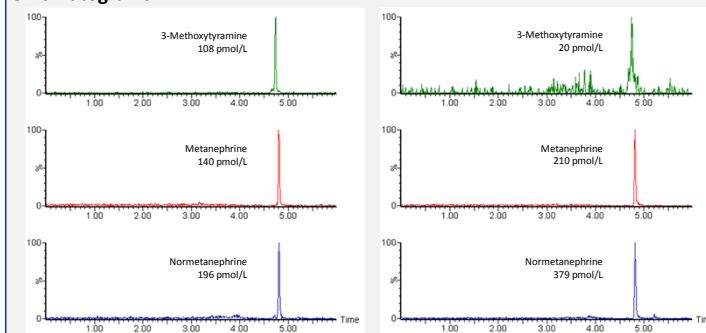


Fig. 1. Chromatogram of ClinCal® Serum Calibrator, level 1

Fig. 2. Chromatogram of human plasma sample

Method evaluation

- Validation parameters:

Performance criteria	Normetanephrine	Metanephrine	3-Methoxytyramine
Linearity [nmol/L]	0.06 - 54	0.03 - 27	0.02 - 61
LLOQ [nmol/L]	0.06	0.03	0.02
Intra-day precision ¹ (CV) [%]			
Low (0.3 nmol/L)	6.1	3.7	4.6
Medium (1.5 nmol/L)	3.1	3.1	2.5
High (4.5 nmol/L)	4.4	2.6	3.0
Inter-day precision ² (CV) [%]			
Low (0.3 nmol/L)	6.1	3.6	4.4
Medium (1.5 nmol/L)	3.6	3.1	3.6
High (4.5 nmol/L)	4.5	2.9	4.3

Table 2. Validation parameters acquired on Waters Acquity / Xevo TQ-S system; ¹n = 8 determinations, ²n = 8 series x 2 determinations

- RCPAQAP proficiency testing scheme results:

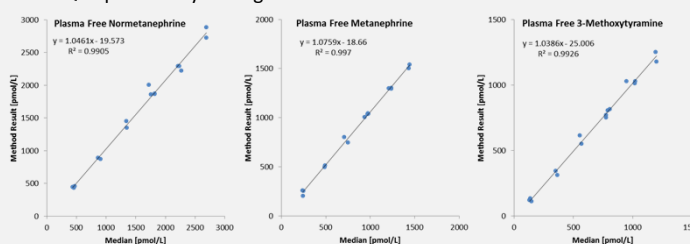


Fig. 3. Comparison of PFM concentrations of RCPAQAP samples found by the LC-MS/MS Complete Kit – Free Metanephrines in Plasma together with reported results from RCPAQAP survey (January 2015 – August 2015, 16 samples)

Summary and Conclusion

RECIPE ClinMass® LC-MS/MS Complete Kits – Free Metanephrines in Plasma (MS11000, MS11100):

- First commercially available CE-IVD labelled LC-MS/MS complete kits
- Highly sensitive and selective LC-MS/MS methods for quantification of free normetanephrine, metanephrine and 3-methoxytyramine in plasma
- Simple sample preparation with SPE cartridges or on-line SPE with one-step protein precipitation
- Effective chromatographic separation of the analytes (run time 6 min on-line; 4 min off-line)
- Accurate and precise quantification using multi-level serum calibrator set (5 levels); Quality control using multi level serum controls (3 levels)
- Matrix effects are compensated using isotope labeled internal standards
- Two options for a fast, easy and reliable analysis of PFM