Nota de aplicación

Simultaneous, Sensitive LC-MS Analysis of Plasma Metanephrines

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This Application brief demonstrates the simultaneous and high sensitivity analysis of plasma metanephrines by LC-MS for clinical research.

Benefits

Measurement of several important plasma biogenic amines

Introduction

Development of LC-MS methods for simultaneous measurement of plasma metanephrines has been challenging. Efficient sample pre-treatment strategies have yet to be defined and measurement of these molecules can be complicated by the difficult chromatographic separation of metanephrines such as 3-

methoxytyramine (3-MT), from other metanephrines. Development of a good separation has proven problematic and can lead to overestimation of metanephrine levels. Many laboratories also do not measure 3-MT as the assays they use may not have the analytical sensitivity to measure this low-level analyte. To effectively study these important biogenic amines, a research method to measure metanephrine, normetanephrine and 3-MT simultaneously is needed.

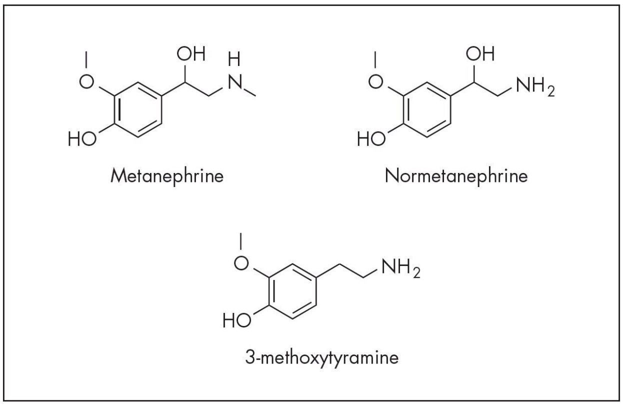


Figure 1: Structures of biogenic amines.

Experimental

Method details

LC System:	ACQUITY UPLC with ACQUITY UPLC Online SPE Manager
Mass Spectrometer:	Xevo TQ-S
Column:	Atlantis HILIC, 2.1 x 50 mm, 3 µm
Sample Preparation:	ACQUITY UPLC Online SPE Manager (OSM)
SPE:	MassTrak WCX OSM Cartridge

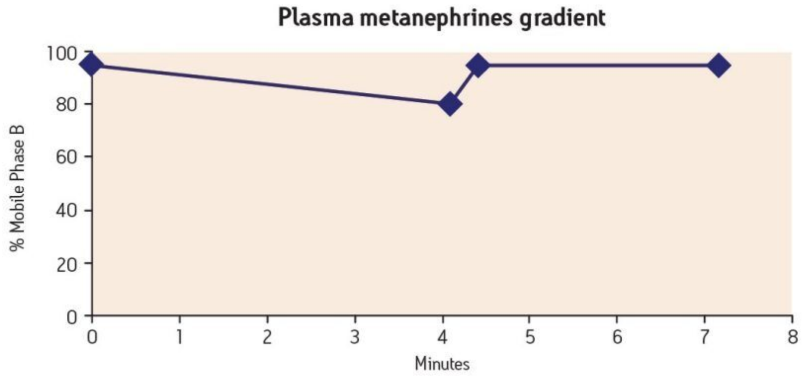
Sample preparation method

Plasma samples were diluted 1:1 with deuterated internal standard and then centrifuged through a 10 k MW cutoff centrifugation spin filter device to remove proteins from the sample.

After filtration, an aliquot of sample was injected into the online SPE equipped LC-MS system. SPE was performed by the online SPE system as follows:

Step	Solvent	Volume (µL)
Cartridge conditioning	0.1% formic acid (v/v) in acetonitrile	200
Cartridge conditioning 2	0.1% formic acid (v/v) in acetonitrile	200
Cartridge conditioning 3	20% 10 mmol/L ammonium formate pH 3.2:80% acetonitrile	250
Cartridge conditioning 4	95% acetonitrile	250
Cartridge equilibration	H_2O	250
Sample load	H_2O	250
Cartridge wash	H_2O	200
Cartridge wash 2	95% acetonitrile	200
Clamp flush	95% acetonitrile	250

After SPE, samples were analyzed by LC-MS using the following gradient conditions:



Mobile phase A:	100 mmol/L ammonium formate pH 3.2
Mobile phase B:	Acetonitrile

Results and Discussion

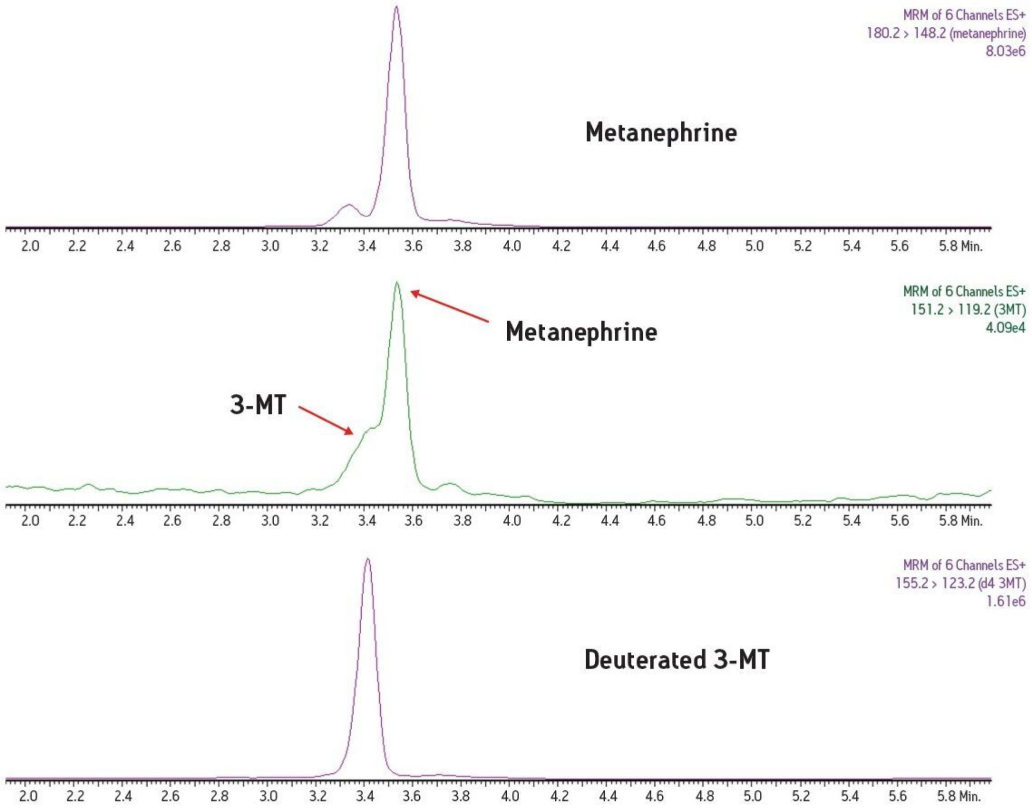


Figure 2: LC-MS Analysis of metanephrine and 3-methoxytyramine. The 3-MT elutes before the metanephrine to help avoid potential interference from ionic crosstalk and isobaric interfaces from metanephrine that could lead to the overestimation of 3-MT in the analysis of these molecules.

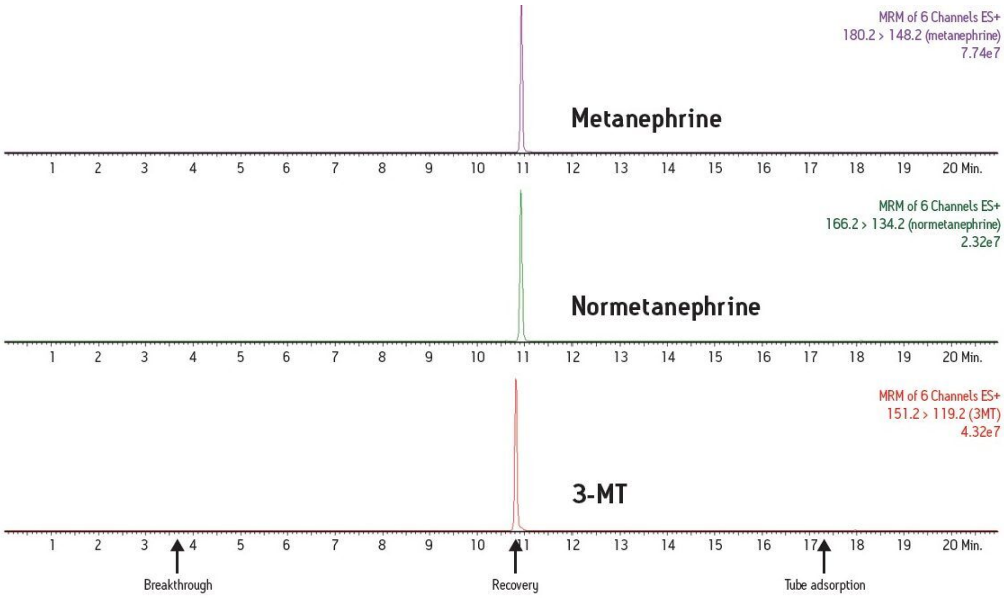


Figure 3: Recovery determination of online SPE method for biogenic amines. The breakthrough, recovery, and and adsorption were determined for each analyte. The online SPE method demonstrated no breakthrough or adsorption and excellent recovery for all three analytes.

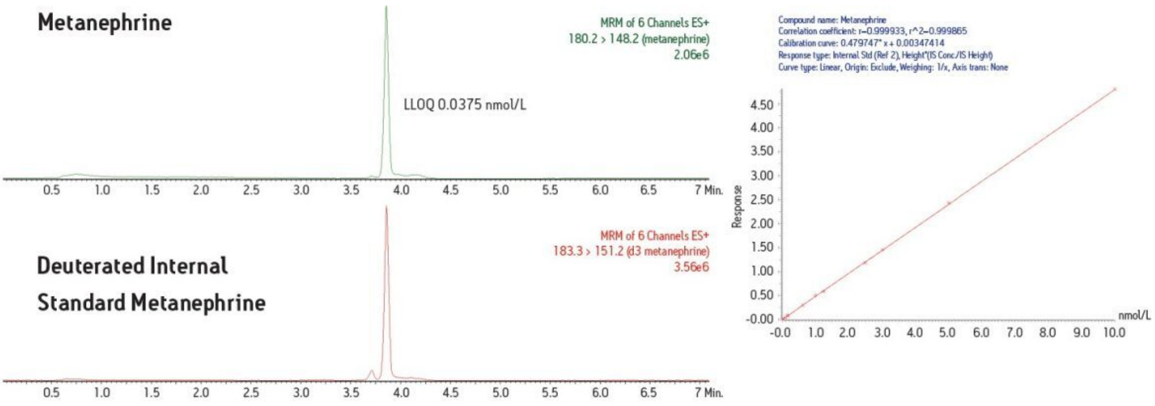


Figure 4: Plasma metanephrine analysis using LC-MS with online SPE.

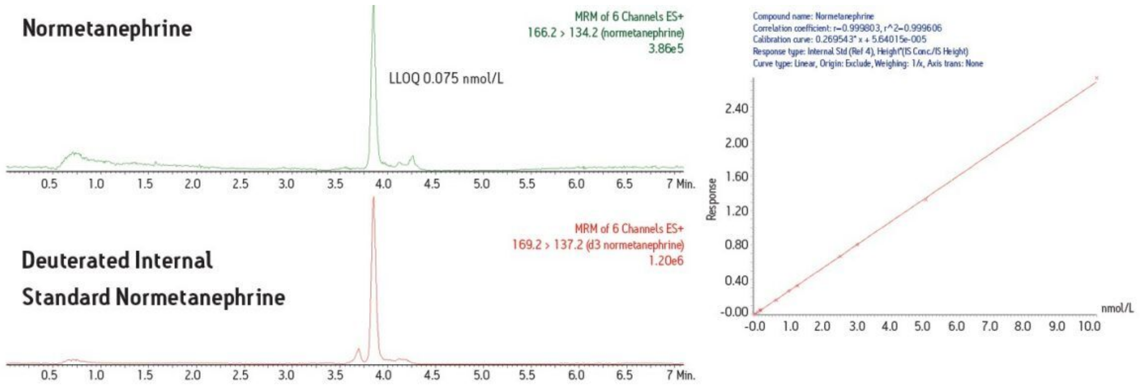


Figure 5: Plasma normetanephrine analysis using LC-MS with online SPE.

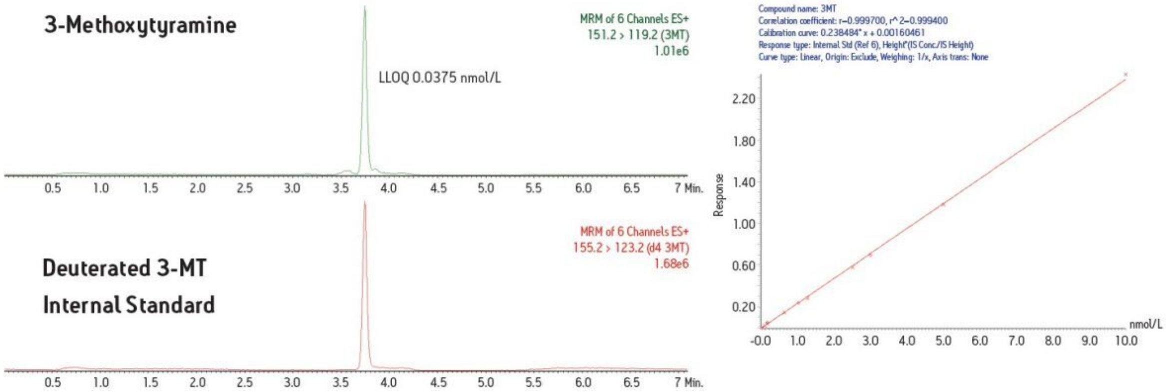


Figure 6: Plasma 3-methoxytyramine analysis using LC-MS with online SPE.

Conclusion

In this study, a clinical research method has been developed for the simultaneous measurement of three plasma biogenic amines. The method utilizes LC-MS and an online SPE system. This combination of SPE sample preparation coupled with the analytical power of LC-MS is able to deliver an efficient assay for these important plasma metanephrines.

The method developed here provides:

- Simultaneous analysis of metanephrine, normetanephrine, and 3-methoxytyramine from plasma

- Effective separation of 3-MT from metanephrines to avoid interferences and overestimation
- Highly efficient SPE sample preparation integrated with LC-MS
- Very good analytical sensitivity (LLOQs for all analytes in the low picomolar ranges)

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