

Simultaneous, Sensitive LC/MS Analysis of Plasma Metanephrines

Joanne Adaway and Brian Keevil,
University Hospital of South Manchester, UK

GOAL

To demonstrate the simultaneous and high sensitivity analysis of plasma metanephrines by LC/MS for clinical research.

BACKGROUND

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.

Measurement of several important plasma biogenic amines.

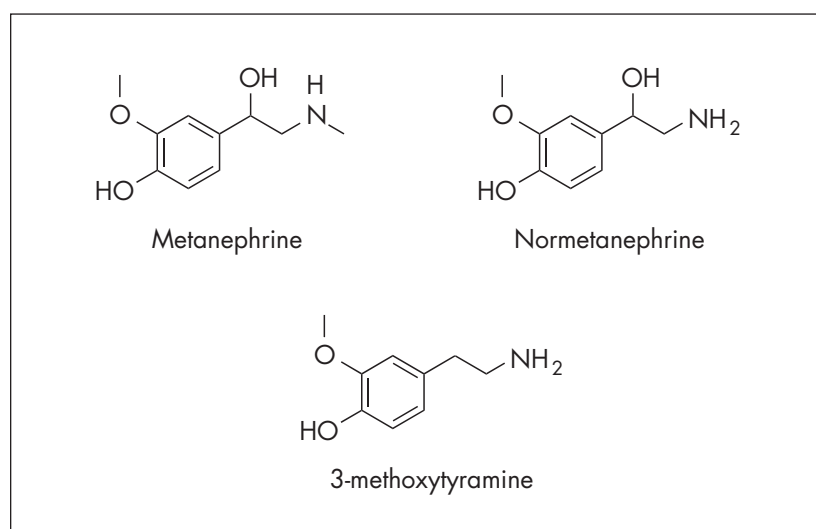


Figure 1: Structures of biogenic amines.

THE SOLUTION

In this study, a method for simultaneously measuring three biogenic amines (metanephrine, normetanephrine, 3-methoxytyramine) from plasma has been developed. This method takes advantage of the capabilities of an online SPE system (ACQUITY UPLC® Online SPE Manager) to enable the accurate measurement of these molecules.

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

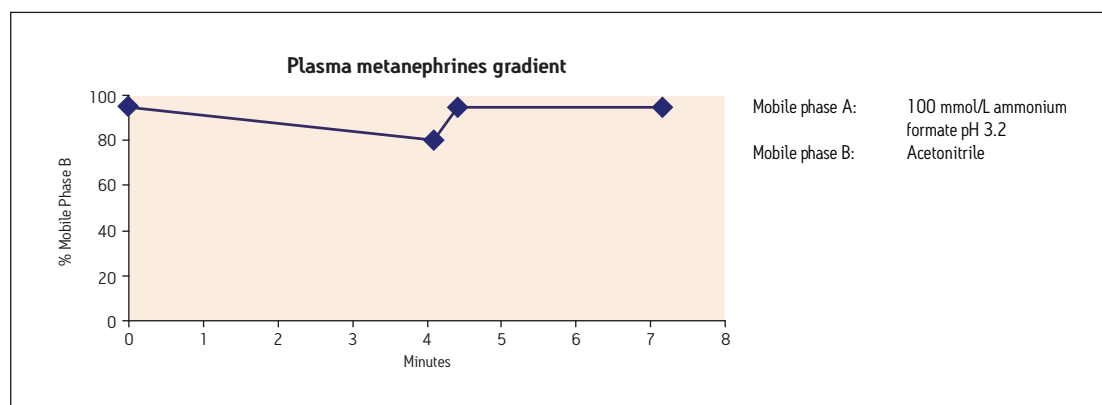
Sample Preparation Method

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 ₂ O	250
Sample load	H ₂ O	250
Cartridge wash	H ₂ O	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:



RESULTS

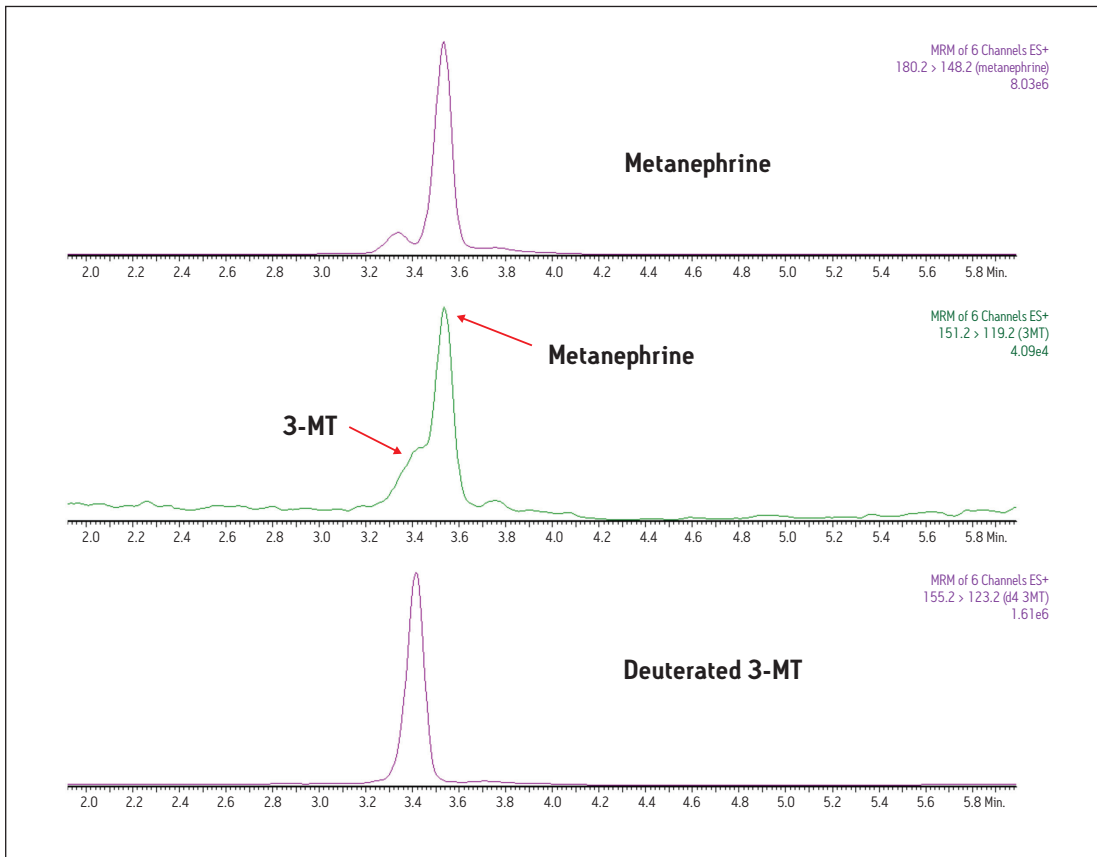


Figure 2: LC/MS Analysis of metanephrine and 3-methoxytyramine. The 3-MT elutes before the metanephrine to help avoid potential interference from ionic crosstalk and isobaric interferences from metanephrine that could lead to the overestimation of 3-MT in the analysis of these molecules.

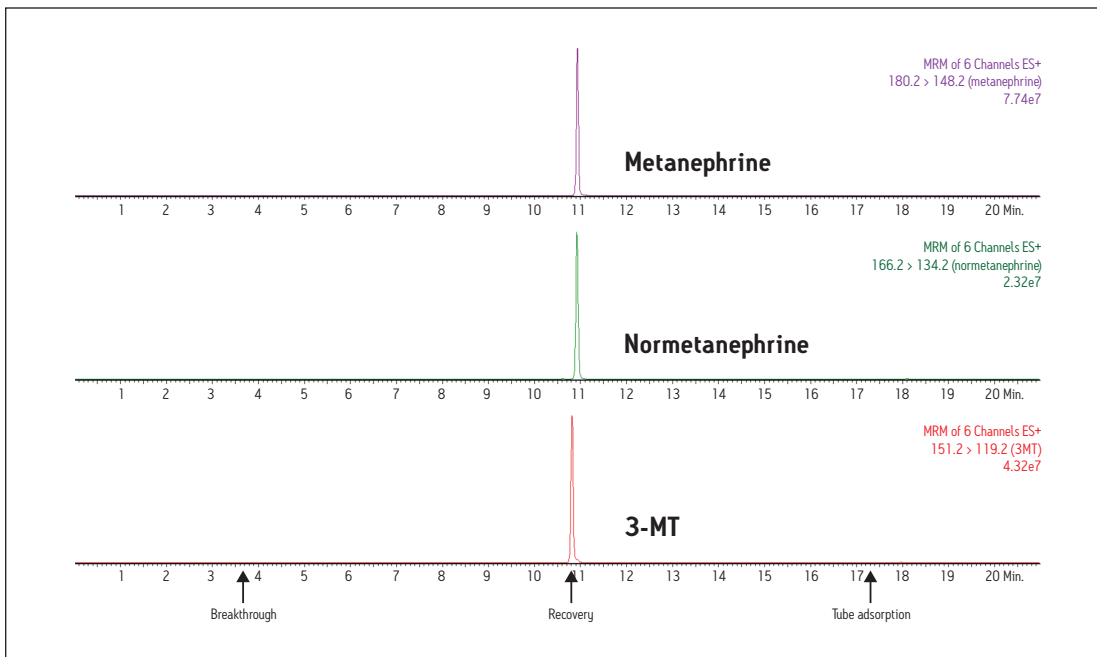


Figure 3: Recovery determination of online SPE method for biogenic amines. The breakthrough, recovery, and adsorption were determined for each analyte. The online SPE method demonstrated no breakthrough or adsorption and excellent recovery for all three analytes.

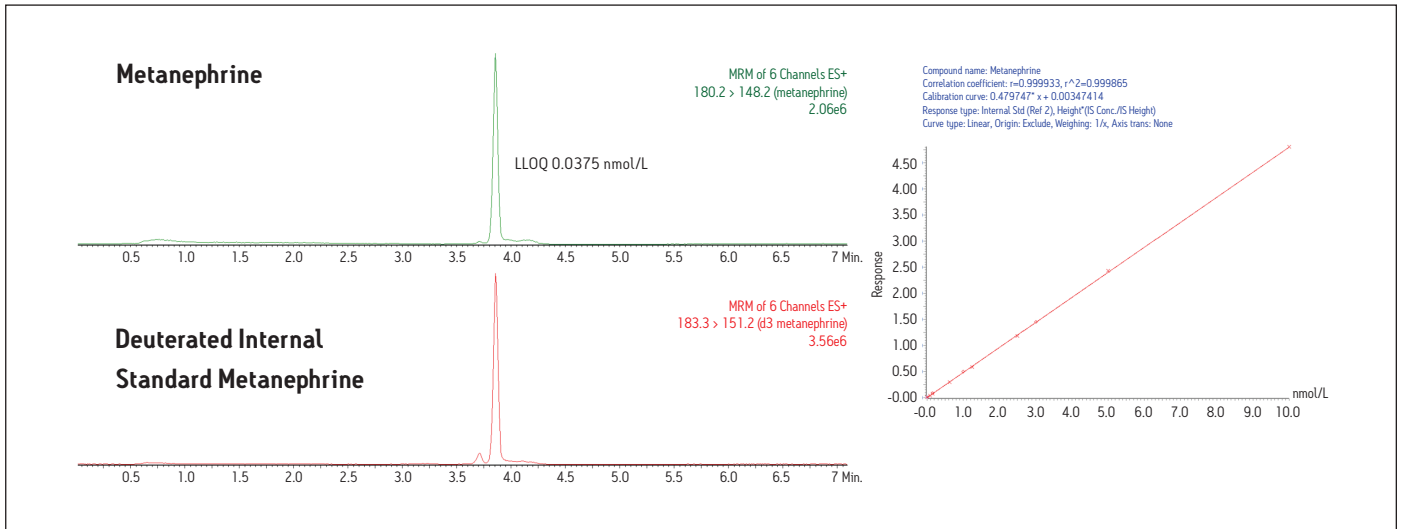


Figure 4: Plasma metanephrine analysis using LC/MS with online SPE.

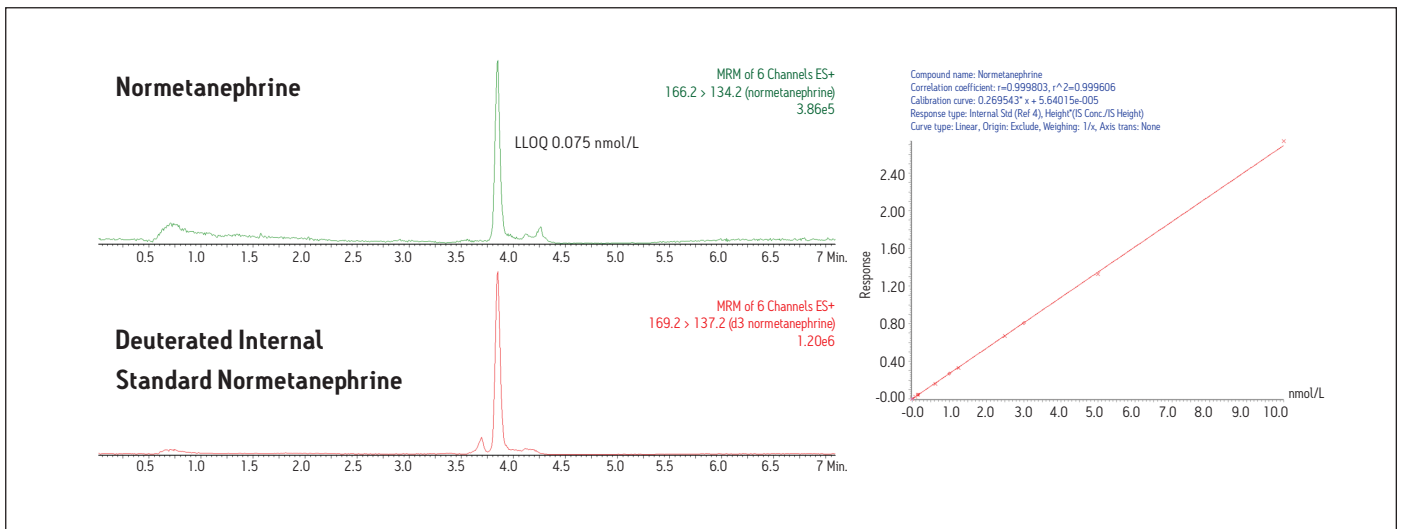


Figure 5: Plasma normetanephrine analysis using LC/MS with online SPE.

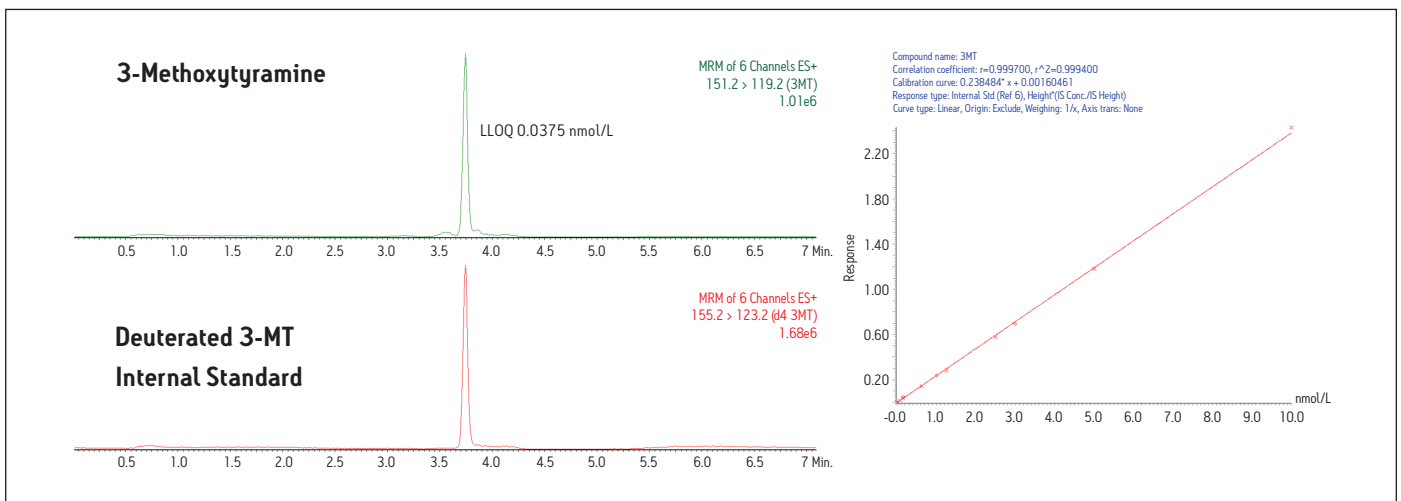


Figure 6: Plasma 3-methoxytyramine analysis using LC/MS with online SPE.

SUMMARY

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)

For research use only. Not intended for use in diagnostic procedures.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters, ACQUITY UPLC, Xevo, Atlantis and The Science of What's Possible are registered trademarks of Waters Corporation. MassTrak is a trademark of Waters Corporation. All other trademarks are the property of their respective owners.

©2013 Waters Corporation. Produced in the U.S.A.
September 2013 720004613EN IH-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com