

A Simple and Sensitive LC-MS/MS Method for Simultaneous Measurement of Metanephrine, Normetanephrine, and 3-Methoxytyramine

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Background

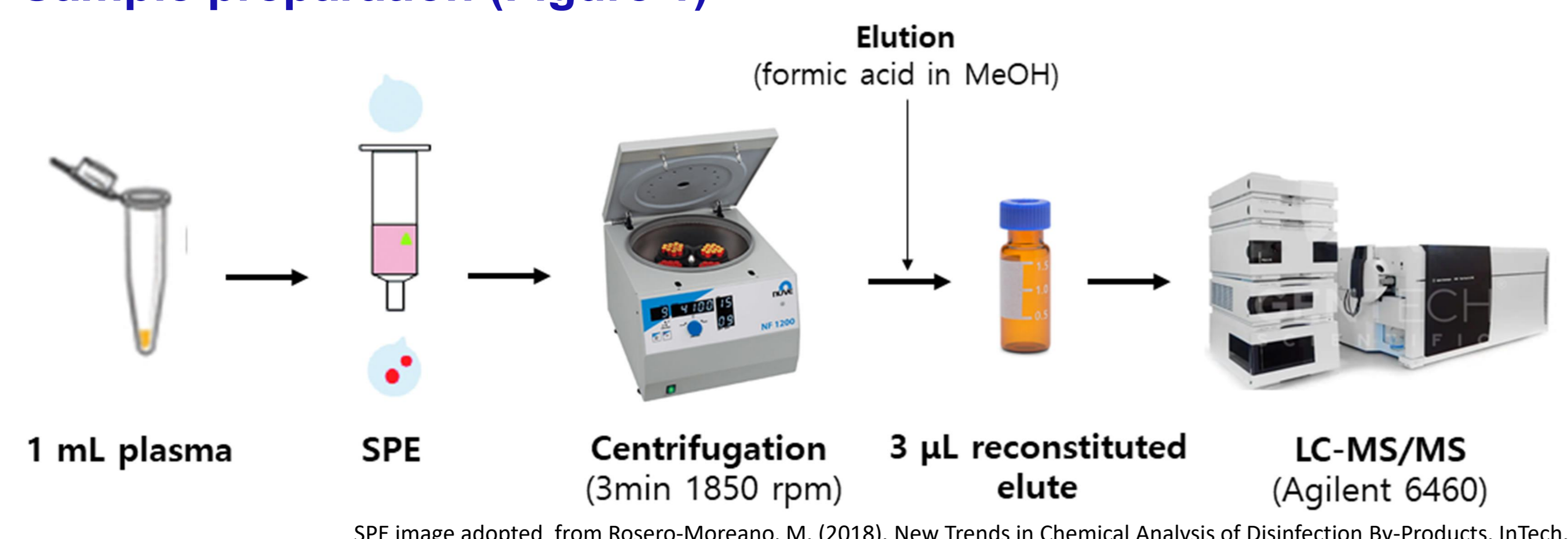
Plasma 3-methoxytyramine (3-MT), the O-methylated **dopamine metabolite**, is useful for detecting **dopamine-producing pheochromocytomas and paragangliomas (PPGLs)**. Due to the difficulty in measuring the **very low concentrations in plasma**, its measurements have not been widely offered as part of routine workup for PPGLs.

Objectives

We developed a highly sensitive and specific mass spectrometric method for **simultaneous measurement of plasma free metanephrine (MN), normetanephrine (NMN), and 3-MT** by incorporating 3-MT measurement into previously established MN and NMN assay, and applied it to clinical practice.

Methods & Materials

Sample preparation (Figure 1)



- Specimen type: plasma
- Extraction using solid-phase extraction (SPE) method
 - Strata CW-X extraction cartridges (Phenomenex, CA, USA)
 - Pretreatment with 1 mL methanol, 1 mL deionized water, and 1 mL of 0.5M ammonium acetate.
- 3 µL of reconstituted eluate was injected into LC-MS/MS system

LC-MS/MS method

• HPLC

Agilent 1260 HPLC system (Agilent Technologies)
Column: Unison UK C18 column, 2.0×100 mm, 3.0 µm (Imtakt)
Mobile A: 0.1% Formic acid in deionized water
Mobile B: 0.1% Formic Acid in acetonitrile

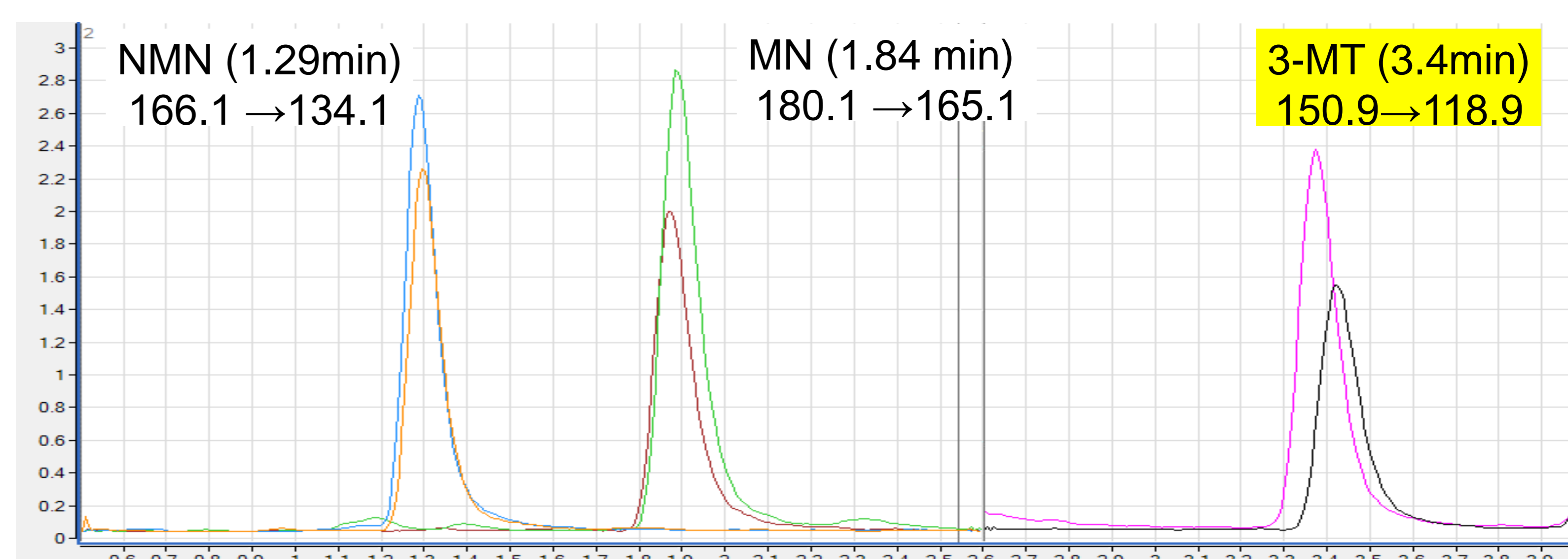
• MS/MS

Agilent 6490 tandem mass spectrometer (Agilent Technologies)
Ionization mode: Electrospray ionization
Acquisition mode: Positive mode MRM

Total analytical runtime : 6 min

Total sample preparation time: 1.5 hr/batch

Chromatogram (Figure 2)



Method validation

Performed according to the CLSI guideline C62-A

linearity, precision, accuracy, lower limits of quantification (LLOQ) and detection (LOD), extraction recovery, matrix effect, and carry-over

Table 1. Analytical performance of LC-MS/MS method

Parameter		Result
Linearity (AMR)		R2 0.99 (0.05-58 nM)
Precision	Intra-day (n=5)	%CV 2.7-6.0%
	Inter-day (n=5)	%CV 3.3-7.8%
Accuracy (QC sample & blank plasma spiked with pure STD)		91.5-107.7%
LLOQ		0.03 nM
Extraction recovery		96.3-107.2%
Matrix effect		92.8-98.8%
Carryover		Not significant

Clinical specimen analyses

- 3-MT was analyzed in a total of 2,947 clinical specimens requested for MN and NMN measurement between August 2022 and August 2023.
- **1.4% (42/2,947)** showed elevated plasma 3-MT (cutoff < 0.1 nmol/L).
- 3-MT can be elevated by dietary dopamine and other amines, common to many foods, therefore, results should be interpreted in the context of other clinical data.

Conclusions

- A **highly sensitive and specific LC-MS/MS method for 3-MT quantitation in plasma** that could be incorporated in an **established LC-MS/MS assay for metanephrines**, was developed and successfully applied to clinical practice.
- By allowing simultaneous measurement of three catecholamine metabolites, this method would thus contribute to **increasing accessibility to 3-MT measurements in clinical laboratories**, and provide valuable information to clinicians when diagnosing and monitoring patients with PPGL.