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Quantitation of plasma metanephrine and normetanephrine by derivatization using an integrated LC-MS/MS analyzer equipped with fully-automated sample preparation device

ASMS 2017 ThP-099

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Introduction

- Although LC-MS/MS analysis manifests high repeatability in measurement, overall reproducibility of an assay is compromised by errors associated with manual sample pretreatment. This also hinders standardization of assay across multiple laboratories.
- Derivatization of is employed in LC-MS/MS analysis to achieve better chromatographic separation or to enhance favorable detection in MS. However, it involves series of reagent addition and vortexing, making the pretreatment prone to errors and hence low reproducibility.
- In this investigation, we evaluated tested whether or not automated sample preparation device can be employed to carry out complex sample pretreatment procedures, such as required for chemical derivatization. For model experiment plasma metanephrine and normetanephrine was derivatized by reductive amination as shown in Fig. 1 to improve reversed phase column retention.

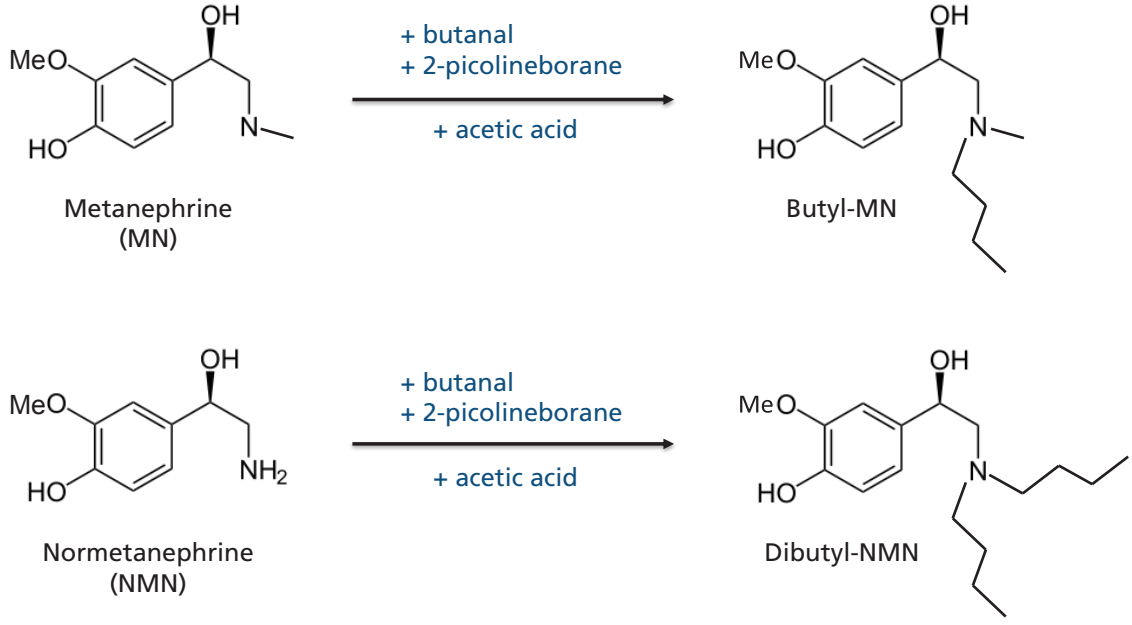

Fig. 1 Target compounds and their derivatization products obtained by reductive amination using butanal as aldehyde and 2-picolineborane as reducing agent.

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Methods

Sample and reagents

Commercially available pooled plasma was used as sample matrix. IS solution (1 ppb of standard compounds and deuterated internal standard in water), Reagent 1 (butanal/acetic acid = 25:75), Reagent 2 (7% 2-picolineborane in EtOH, w/v) and Reagent 3 (5% aq. ammonia) were prepared in 6 mL glass container and placed in CLAM-2000 as reagent reservoir.

Automated sample preparation

Fig. 2 illustrates the general appearance of the instrument setting, with CLAM-2000 connected to LCMS-8060 to comprise an integrated "turnkey" type analyzer system. The pretreatment steps carried out are shown in the flow chart that include 5 steps of reagent addition and 4 steps of vortexing. The procedure completes within 6 minutes, and it can be ran parallelly with LC-MS/MS measurement so resulting in no wait-time between sequential analyses.

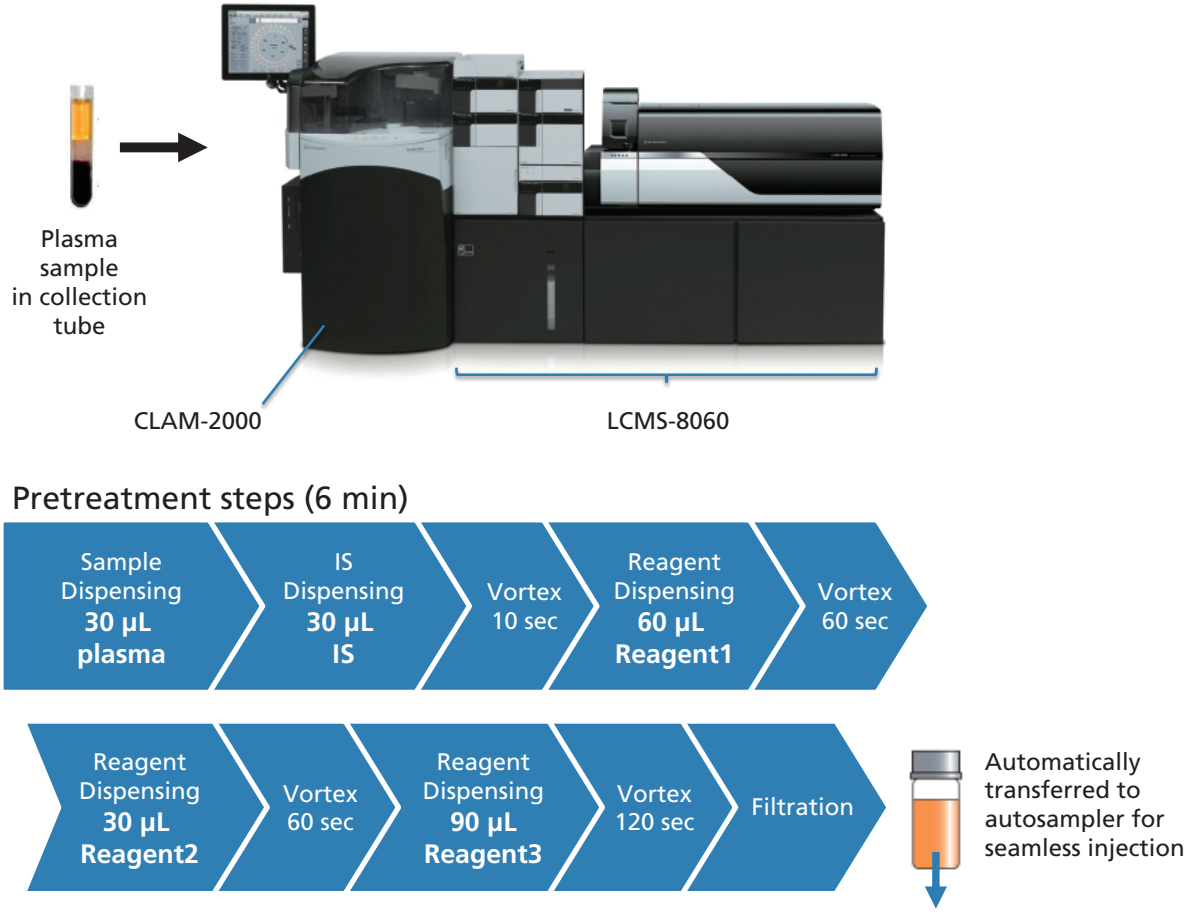

Fig. 2 Appearance of CLAM-LCMS system, and the pretreatement steps carried out for derivatization.

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Analytical conditions

Shown below are the list of target compounds, their MRM transitions and the HPLC condition for LC/MS/MS analysis.

Compound	MW	Precursor *m/z* (derivatized)	Product *m/z*	CE (V)
Metanephrine (MN)	197.23	254.15	236.15	-14
MN-d3	200.23	257.15	154.10	-21
Normetanephrine (NMN)	183.20	296.20	278.20	-17
NMN-d3	186.20	299.20	154.10	-21

Column	: Shimpack GISS C18 (100 mm x 2.0 mm, 3 µm)
Mobile phase A	: 0.1% formic acid in water
Mobile phase B	: Methanol
Flow rate	: 0.4 mL/min
Column temp.	: 40 °C
Injection volume	: 1 µL

Results

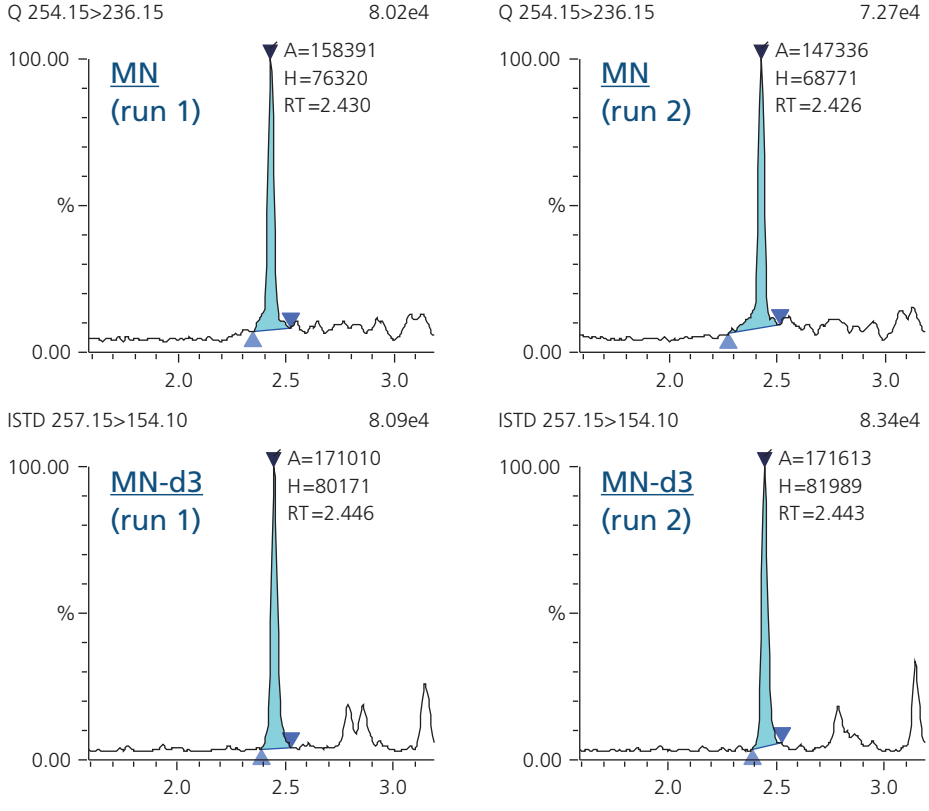

Fig. 3 MRM chromatograms of MN and MN-d3 spiked in control plasma at 1 ng/mL concentration.

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Two consecutive measurement of MN and NMN were made, each starting fresh sample pretreatment. High intensity signal was detected demonstrating that direct one-pot derivatization in plasma was successful, and that there was minimal difference between the two runs. Under the given condition, NMN showed higher signal; derivatization condition was likely more favorable for NMN than for MN.

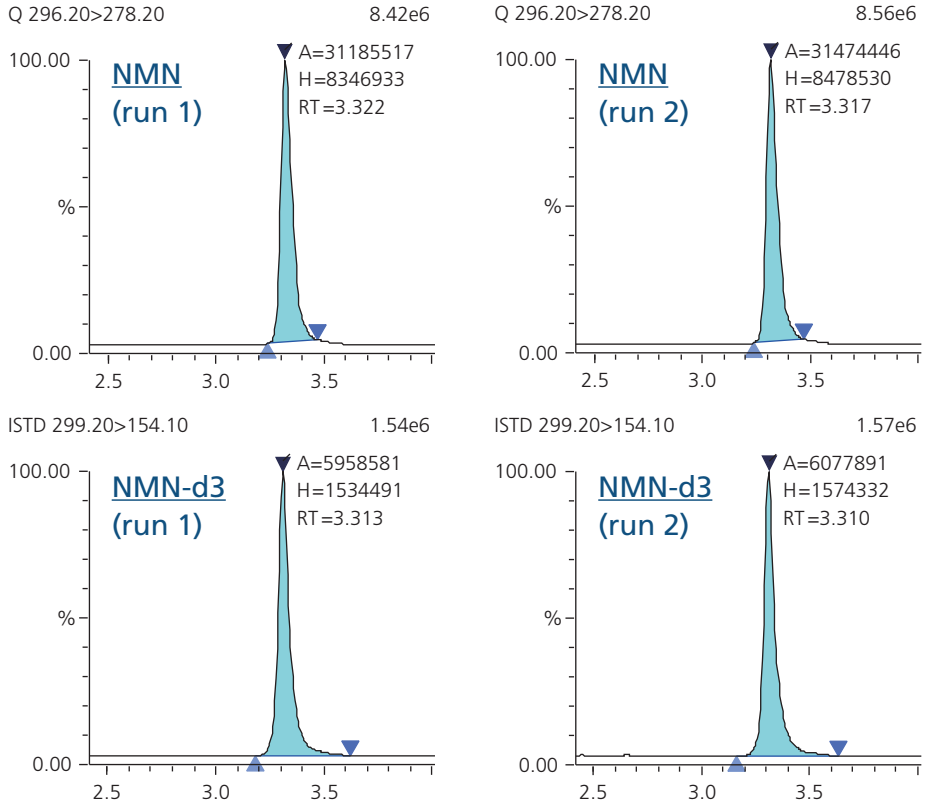

Fig. 4 MRM chromatograms of NMN and NMN-d3 spiked in control plasma at 1 ng/mL concentration.

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Conclusion

- Although still preliminary, the experiment clearly demonstrated that complex sample pretreatment such as derivatization was greatly facilitated by automation by CLAM-2000.
- Due to sequential pretreatment scheme, the incubation times and vortex conditions were kept constant, thus contributing to exceptional inter-run reproducibility for a derivatization method.
- In-depth evaluation and optimization requires the use of synthetic standards for the derivatized compounds – for future work.

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First Edition: June, 2017

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