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A simple analysis of catecholamines in cell medium by LC/MS/MS using an ion-pairing reagent added to final extracts

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1. Overview

- A new LC-MS/MS method for the quantification of neurotransmitters catecholamines, including dopamine, L-DOPA, L-tyrosine and norepinephrine in cell medium
- A new analytical method using 1-hepane sulfuric acid (HSA) as an ion-pairing reagent added to the final extract which can improve the retention of the highly polar compounds for LC-MS/MS analysis

2. Introduction

The catecholamines L-tyrosine, L-DOPA, dopamine and norepinephrine are well established neurotransmitters and have been widely employed in various types of cell lines for the study of the mechanism (Fig. 1), pharmacology and biochemistry of neurosecretion [1]. But there are always challenges for the determination of catecholamines in biological samples as they are small very polar molecules which always poorly retained on reversed-phase C18 column. The major disadvantages of existing analytical methods include short retention time and difficulty in separating from each other and potential interference from endogenous compounds present in the matrix. It would be ideal to develop a simple and rapid LC-MS/MS method for the determination of the catecholamines for quantitative monitoring of these compounds in cell cultures.

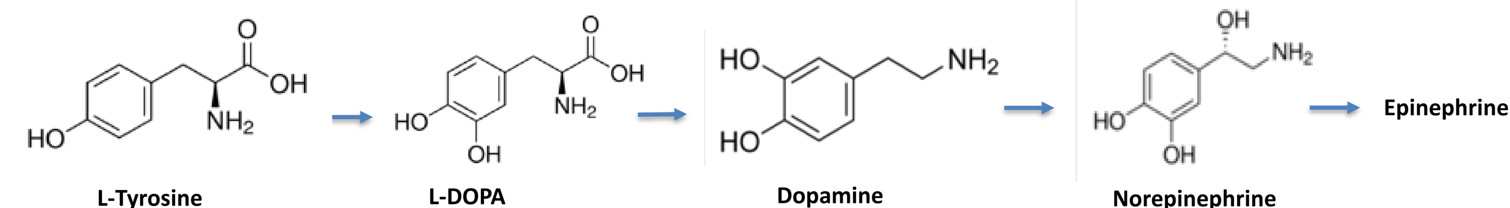

Figure 1. The metabolism pathway of catecholamines in this study in cells

3. Experimental

L-tyrosine, L-DOPA, dopamine and norepinephrine, formic acid (analytical grade), 1-hepane sulfuric acid (HSA, reagent grade) were purchased from Sigma-Aldrich. They were dissolved in the Milli-Q water (stock solution, 1000 μg/mL) and diluted to different working solutions to create the calibration curves and quality control standards, ranging from 2.5 to 2500 μg/mL.

L-tyrosine, L-DOPA, dopamine and norepinephrine standards were further diluted with DMEM cell media or MQ water containing HSA at 30 mM as the final concentration for the preparation of working solutions. Each sample was transferred into HPLC vial and 20 μL was injected to Shimadzu LCMS-8060 for analysis after centrifugation at 15,000 × *g* for 10 min. The analytical conditions are shown in Table 1.

Table 1. Analytical conditions of catecholamines on LCMS-8060

Column	Shim-pack Velox Biphenyl (100 mm. x 2.1mm I.D., 1.9μm)
Flow Rate	0.3 mL/min
Mobile Phase	A : 0.05% formic acid (FA) in milli-Q water B : 0.05% Formic acid in methanol (MeOH)
Elution Mode	Gradient elution, LC program 7 minutes 5%B (0.01 min to 0.50 min)→8%B (3.00 min to 4.00 min) →15% B (5.50 min to 6.00 min) →5%B (6.50min)
Oven Temp.	40 ºC
Injection Vol.	20μL

Interface & Temp.	ESI, 250 ºC
MS Mode	MRM, Positive
Heat Block temp.	400ºC
DL temp.	250ºC
Nebulizing gas flow	Nitrogen, 3.0 L/min
Drying gas flow	Nitrogen, 10.0 L/min
Heating gas flow	Zero air, 10 L/min
CID gas	270 kPa (Ar)

MRM method of catecholamines

The multiple reaction monitoring (MRM) transitions for L-tyrosine, L-DOPA, dopamine and norepinephrine were optimized and can be seen in Table 2

Table2. The MRM method parameters for catecholamines on LCMS-8060

Compound	MRM		Pause time (msec)	Dwell time (msec)	Q1 Pre Bias (V)	C.E. (V)	Q3 Pre Bias (V)
	Precursor	Product					
L-DOPA	198.15	181.15	3	20	-19	-12	-22
		152.15	3	20	-20	-15	-20
		107.10	3	20	-20	-29	-20
Dopamine	154.15	137.15	3	20	-15	-14	-14
		91.10	3	20	-15	-23	-18
L-Tyrosine	182.20	165.15	3	20	-11	-12	-17
		136.15	3	20	-23	-15	-14
		91.10	3	20	-11	-29	-18
Norepinephrine	170.20	107.10	3	20	-16	-21	-22
	152.15		3	20	-17	-21	-20

Chromatographic behaviour

Catecholamines are small and very polar molecules, which are poorly retained on reversed-phase C18 columns. The short retention times of these catecholamines cause the difficulty to separate them from a large number of endogenous compounds in biological samples and lead to poor ionization in the mass spectrometer.
Several analytical approaches [2-3] have been used to overcome these difficulties, such as HILIC. The HILIC method achieved low sensitivity for all catecholamines which make it unsuitable for the analysis of cell culture samples. Recently, a new approach for ion-pairing chromatography has been successfully applied for different types of highly polar compounds. In current study, a simple and rapid LC-MS/MS method for the quantitation of catecholamines in cell culture samples using this new ion-pairing chromatography approach with 1-hepane sulfuric acid (HSA). The effect of HAS on the retention of catecholamines can be seen in Figure 2.

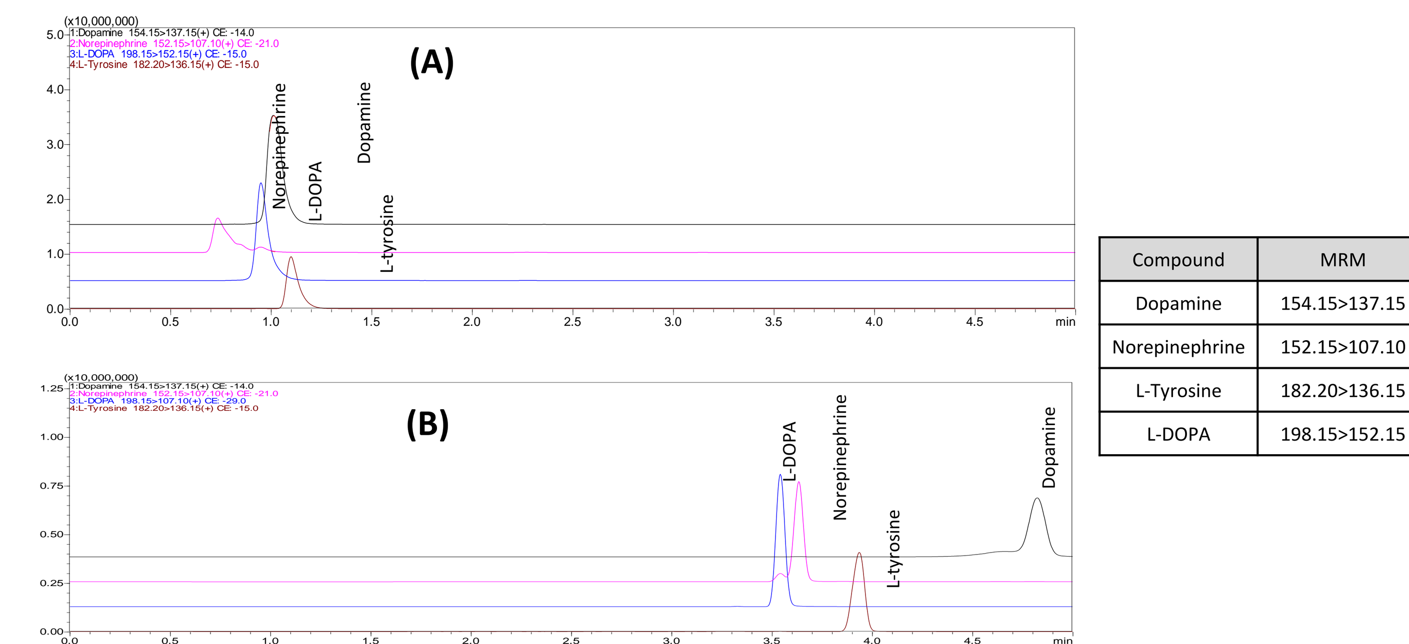

Figure 2. Typical MRM chromatograph for L-tyrosine, L-DOPA, dopamine and norepinephrine (A) without HSA and (B) with HSA at final concentration of 30mM in standard solutions on Biphenyl column using MRM method

4. Results and Discussion

4.1 Chromatography

Recently, a few publications [2-3] have applied a new analytical strategy using ion-pairing chromatography method which demonstrated that the ion-pairing reagent can be added to the injected extract rather than the traditional approach to add the reagent to the mobile phase.

In this study it is shown that these catecholamines elute close to the solvent front with little separation without HSA as ion-pairing reagent (Fig. 2A). By using HSA as ion-pairing reagent added to the final extracted cell medium samples at the final concentration of 30 mM achieved good retention for L-tyrosine, L-DOPA, dopamine and norepinephrine (Fig. 2B). .

In such new technique of addition of the ion-pairing reagent into the final extracts just before the injection for LC-MS/MS analysis can decrease the time consuming for cleaning procedures of mass spectrometer due to the contamination.

4.2 Extraction from the cell culture medium samples

100 μL of cell culture medium sample was treated with 400 μL pf cold methanol by vortex-mixed for 1 min and stand for 10 min. 500 μL of MQ water was added for dilution of the samples. Precipitated protein were removed after centrifugation at 15, 000 x *g* for 10 min. The supernatant was transferred into HPLC vial for LC-MS/MS analysis.
This sample treatment method for biological samples is simple and cost-saved comparing to SPE method.

4.3 Linearity and sensitivity studies

Linearity was evaluated by analysing cell medium samples containing L-tyrosine, L-DOPA, dopamine and norepinephrine at concentrations of 0.25, 0.5, 2.5, 5, 25, 50, 250, 500 ng/mL. The representative calibration curves for all catecholamines are shown in Figure 3.

The LOD and LOQ for catecholamines ranged from 0.003125 to 0.5 ng/mL and 0.025 to 1 ng/mL, respectively as shown in Table 3.

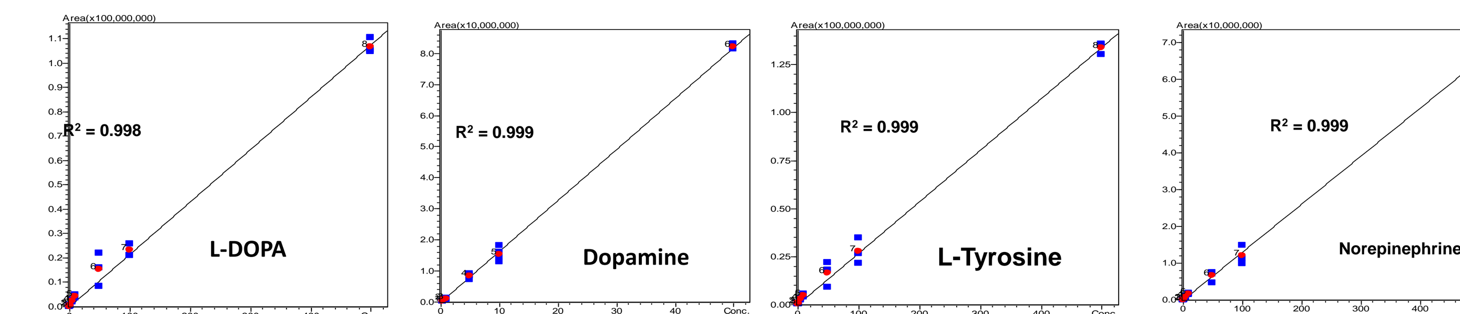

Figure 3. Representative calibration curves of catecholamines

Table 3. Linearity, LOD and LOQ of catecholamines in DMEM cell medium

Compound	Range (ng/mL)	R^2	Estimated LOD (ng/mL)	Estimated LOQ (ng/mL)
L-DOPA	0.25 – 500	0.998	0.0625	0.25
Dopamine	0.25 – 50	0.999	0.0625	0.25
L-Tyrosine	0.25 – 500	0.999	0.003125	0.025
Norepinephrine	0.25 – 500	0.999	0.5	1

4.4 Accuracy of analysis

The accuracy and precision tests were performed by testing QC samples at low, medium and high concentrations within their respective calibration ranges. Quality control samples were accurate and precise, ranging from 87.9-113.9% with RSD less than 15%.

4.5 Matrix effect

Matrix effects were also investigated at low, medium and high concentration level. A little decrease in response (about 9 – 23%) for catecholamines was observed in cell medium spiked samples (data not shown).

4.6 Analysis of cell medium samples

The developed quantitation method was applied for the analysis of catecholamines from *in-vitro* cell medium samples (Fig.4). This simple sample treatment method and the established calibration curve were suitable for the real cell medium samples with variable concentration levels of catecholamines in this study.

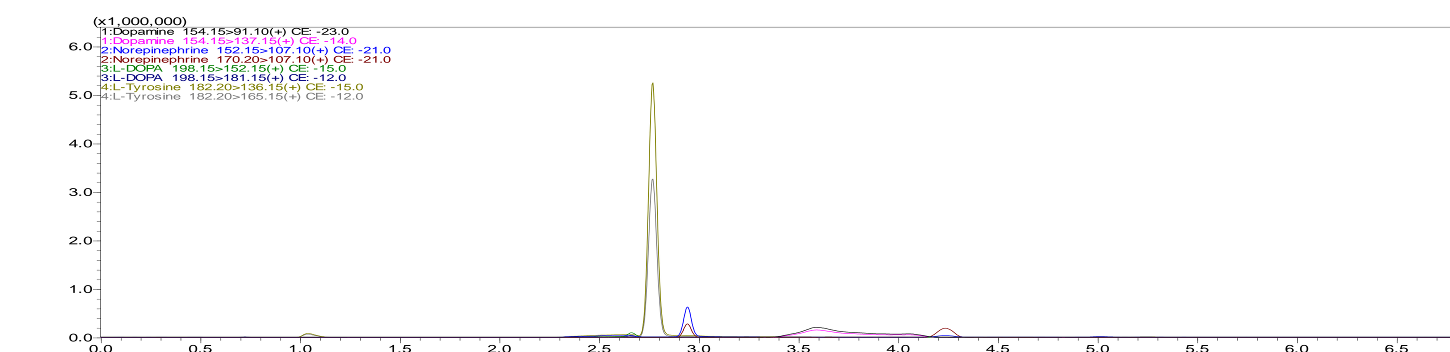

Figure 4. The detected catecholamines from *in-vitro* cell medium sample

5. Conclusions

We described the development of a novel analytical strategy to ion-pairing chromatography using 1-hepane sulfuric acid as an ion-pairing reagent which was added to injected extracts. The addition of 1-hepane sulfuric acid solution at the final concentration of 30 mM to the final extract after a simple sample dilution method permitted the simultaneous analysis of four catecholamines in cell medium samples. This new LC-MS/MS method was applied for the separation and quantification of L-tyrosine, L-DOPA, dopamine and norepinephrine in cell medium samples using this simple LC-MS/MS method.

References

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