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Application News

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Liquid Chromatography Mass Spectrometry

High-Sensitivity Determination of Catecholamines in Plasma Using the LCMS-8060 Triple Quadrupole LC/MS/MS

Catecholamines are a family of signaling molecules found in brain, adrenal medulla and other nervous systems. Catecholamines in plasma, namely norepinephrine (NE), epinephrine (EP) and dopamine (DA), are commonly measured in clinical research. Analysis of catecholamines in plasma requires both high sensitivity and high throughput.

Presented here is a platform designed to demonstrate the capability to detect catecholamines in plasma, comprising multiplexed plasma sample preparation by Biotage EVOLUTE WCX solid phase extraction followed by high-sensitivity quantitation by LCMS-8060.

MRM Analysis of Deuterated Standards to Estimate LLOQ

Three catecholamine compounds (NE, EP and DA) were separated by Shimpack MAqC-ODS Ⅰ, a reversed-phase column that contains metal ions on the particle surface. The result shown in Fig. 1 demonstrates that the cation-exchange property of MAqC-ODS retained NE well, which is very poorly retained by conventional C18 columns.

Since plasma samples contain endogenous catecholamines, it is difficult to evaluate the LLOQ in plasma matrix. Here we used deuterated catecholamine compounds as standards to estimate the LLOQ in plasma matrix, rather than as internal standards for quantitation. A neat standard curve was prepared by serial dilution in HPLC solvent, whereas a matrix-matched standard curve was prepared by dilution with pooled plasma sample treated with WCX-SPE as described below. Table 1 summarizes the quantitation results, which convincing demonstrate the capability of LCMS-8060 to detect catecholamines at ultra-high sensitivity without matrix interference.

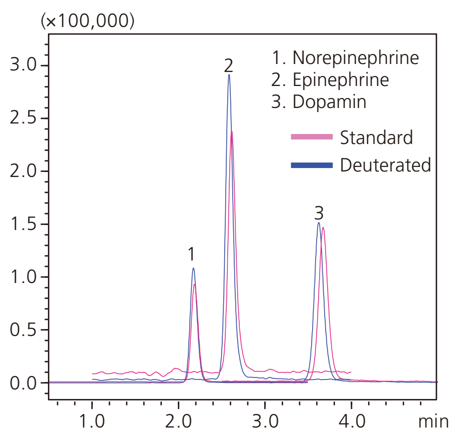

Fig.1 Representative MRM Chromatograms of 3 Catecholamines

Table 1 Quantitative Range of Neat and Matrix-Matched Calibration Curves

Compound name	Neat standard curve		Matrix-matched	
	Range (pg/mL)	Linearity (r^2)	Range (pg/mL)	Linearity (r^2)
Noepinephrine-d6 (158.1 > 111.1)	2.5 – 2000	0.9999	2.5 – 2000	0.9997
Epinephrine-d6 (190.1 > 172.1)	10 – 2000	0.9999	10 – 2000	0.9994
Dopamine-d4 (158.1 > 95.1)	5 – 2000	0.9999	10 – 2000	0.9995

In the actual quantitation assay, deuterated catecholamines are spiked as internal standard at 500 pg/mL in plasma, which is then treated with WCX-SPE and analyzed by LCMS-8060.

Fig. 2 shows the MRM chromatograms of spiked and endogenous catecholamines in plasma.

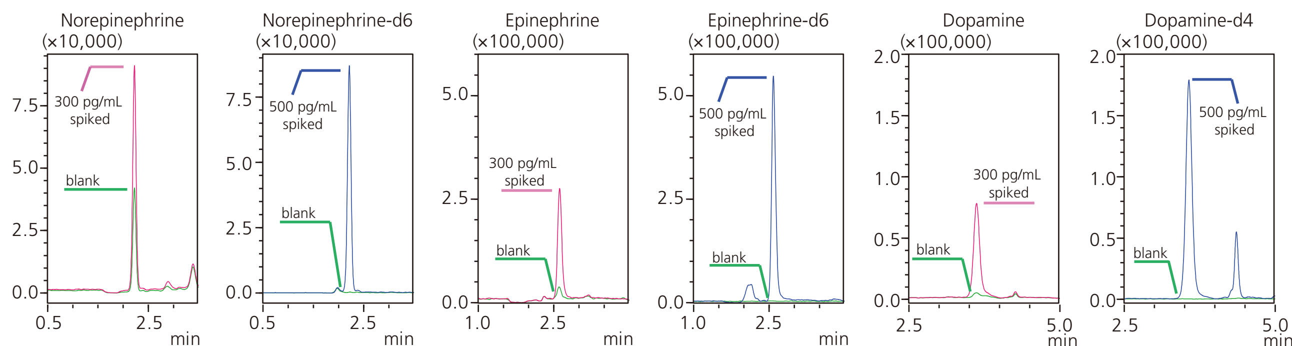

Fig. 2 Detection of NE, EP and DA and Their Deuterated Internal Standards in Plasma

Analytical Performance of QC Samples

We evaluated the analysis performance of catecholamine determination by a series of QC samples that were prepared by spiking to control plasma a fixed amount of deuterated compounds as internal standard and varying amounts of normal catecholamine compounds.

All QC samples were pretreated with EVOLUTE WCX and analyzed by LCMS-8060. A matrix calibration curve was plotted with blank-subtracted area ratio to determine catecholamine concentration in plasma sample, and its performance was evaluated as summarized in Table 2.

Table 2 Catecholamine Determination in QC Samples

Compound name	Spiked Conc. (pg/mL)	Measured IS Area Ratio	Linearity (r^2)	Theoretical Conc. (pg/mL)	Determined Conc. (pg/mL)	Accuracy	Repeatability (n=2)
Norepinephrine (152.1 > 107.1)	0 18.1 72.5 300 600 1200	0.4969 0.4862 0.6023 1.0176 1.5652 2.6390	0.9994	292.2 310.3 364.7 592.2 892.2 1492.2	- 282.0 346.6 577.3 881.5 1478.1	- 92.3 % 95.8 % 97.2 % 97.9 % 97.6 %	0.6 % 4.5 % 3.4 % 0.5 % 1.4 % 0.1 %
Epinephrine (184.1 > 166.1)	0 18.1 72.5 300 600 1200	0.1590 0.2056 0.3947 1.1140 2.0267 4.2128	0.9986	52.2 70.3 124.7 352.2 652.2 1252.2	- 66.3 123.7 341.8 618.5 1281.3	- 94.4 % 99.2 % 97.0 % 94.8 % 102.3 %	1.2 % 2.4 % 3.7 % 0.7 % 3.9 % 2.7 %
Dopamine (154.1 > 91.1)	0 18.1 72.5 300 600 1200	0.0223 0.0511 0.1260 0.4281 0.8436 1.6754	0.9999	13.5 31.6 86.0 313.5 613.5 1213.5	- 34.6 89.4 310.5 614.5 1223.2	- 109.4 % 104.0 % 99.0 % 100.2 % 100.8 %	6.0 % 4.3 % 4.7 % 1.7 % 0.2 % 0.1 %

High-throughput Plasma Preparation

In order to detect plasma catecholamines with high sensitivity and accuracy, and minimize instrument maintenance intervals, plasma samples need preparation to remove interfering molecules such as proteins. Such routine work is best multiplexed and automated to maximize efficiency. Fig. 3 describes a preparation protocol using EVOLUTE WCX extraction plate.

Fig. 3 Plasma Sample Preparation by Biotage EVOLUTE WCX Plate

HPLC Conditions

Analytical column	: Shimpack MAqC-ODSⅠ(150 mm × 2.0 mm, 5 µm)
Mobile phase A	: 0.1 % Formic acid in water
Mobile phase B	: 0.05 % Formic acid in methanol
Time program	: 1 %B. (0 - 0.5 min) → 50 %B. (3 min) → 99 %B. (3.1 - 7 min) → 1 %B. (7.1 - 12 min)
Flowrate	: 0.2 mL/min.
Injection volume	: 5 µL
Post-column addition	: Mobile phase B at 0.2 mL/min

MS Conditions (LCMS-8060, ESI positive)

Interface voltage	: +0.6 kV
Nebulizer gas flow	: 2.2 L/min
Drying gas flow	: 3 L/min
Heating gas flow	: 17 L/min
DL temp.	: 250 °C
Interface temp.	: 250 °C
Heat block temp.	: 400 °C

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Table 3 Sample Preparation Protocol

Plasma prep	Commercially available human plasma was aliquoted, spiked with deuterated IS compounds (500 pg/mL) and normal standard compounds. Mix 300 µL of plasma with equal volume of 50 mM ammonium acetate (pH 7.0).
Step 1	To each well of EVOLUTE WCX, add 900 µL of methanol and wash.
Step 2	Add 900 µL of ammonium acetate (pH 7.0) and equilibrate the well.
Step 3	Add diluted plasma samples (600 µL) to wells. Discard flow-through
Step 4	Add 300 µL of 10 % methanol and discard flow-through
Step 5	Add 300 µL isopropanol to wells and discard flow-through
Step 6	Add 1000 µL of 5 % formic acid 95 % methanol to elute target compounds. Collect eluate.
Step 7	Evaporate the eluate by N_2 purge at 40 °C
Step 8	Reconstitute with 150 µL of 0.1 % formic acid, ready for LCMS analysis.

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