Application Note

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Determination of Catecholamines by Agilent Ultivo LC/TQ with an InfinityLab Poroshell 120 Aq-C18 Column

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Abstract

In this application note, a quantification method was established for catecholamines (3-MT, DA, E, NE, MN, and NMN) using the Agilent Ultivo liquid chromatography/triple quadrupole mass spectrometer (LC/TQ) system with a newly developed Agilent InfinityLab Poroshell 120 Aq-C18 column. The Poroshell 120 Aq-C18 column shows good performance with baseline separation of these six catecholamine compounds. The validation test results give a wide linearity range of 5 to 500 pg/mL in bovine serum albumin (BSA) matrices with excellent accuracy.

Introduction

Catecholamines (CA) include norepinephrine (NA or NE), epinephrine (AD or E), and dopamine (DA), the metabolic products of which, in vivo, are methoxynorepinephrine (NMN), methoxyepinephrine (MN), and 3-methoxytyramine (3-MT), respectively. Catecholamines are a kind of neurotransmitter that plays an important regulatory role in the nervous system, cardiovascular system, endocrine system, and other tissue systems. Due to their strong molecular polarity, a newly developed Agilent InfinityLab Poroshell 120 Aq-C18 column was used for catecholamines analysis. This column has been developed based on superficially porous particles with optimized C18 ligands and a proprietary endcapping process on the particle surface. A larger pore size of 120 Å coupled with the newly optimized C18 chemistry enables column use with a highly aqueous mobile phase to avoid retention loss. Also, the column has stronger retention for the polar compounds such as the catecholamines separated in this application note.

Experiment

Standards and reagents

Catecholamine compounds were purchased from Alta Scientific Co., Ltd. (Tianjin, China); the BSA matrices were purchased from Sigma-Aldrich. Other reagents and solvents were LC/MS grade, including acetonitrile, ultrapure water, and other chemical reagents.

Instruments and equipment

The Agilent 1260 Infinity II LC coupled with the Agilent Ultivo LC/TQ was configured as follows. The liquid chromatograph was equipped with a binary pump (part number K7112B), a high-throughput autosampler (part number K7167A), and a column compartment (part number K7116A). The Ultivo LC/TQ was equipped with MassHunter acquisition 1.2, qualification, and quantification software. Sample pretreatment equipment included a low-temperature centrifuge (centrifugal force ≥ 12,000 × g) and a vortex mixer.

Solution preparation and sample pretreatment

Preparation of standard working solution: 0.1% formic acid aqueous solution was used to prepare 1,000 ng/mL six catecholamine stock solution (3-MT, DA, E, NE, MN, and NMN). The stock solution was mixed and diluted to the secondary stock solutions with the same solution in sequence, and the final concentration was listed in Table 1.

Table 1. Concentrations of secondary stock solutions.

Compound	Mixed Catecholamines
S1 pg/mL	50
S2 pg/mL	100
S3 pg/mL	200
S4 pg/mL	500
S5 pg/mL	1,000
S6 pg/mL	2,000
S7 pg/mL	5,000

Then, 100 µL of the preceding serial standard solutions were accurately pipetted into 900 µL of 1% BSA matrices (1 g BSA in 100 mL water that contains 0.1% formic acid and 25 mg/mL citric acid). This step was followed by vortexing to mix thoroughly and obtain the final matrix standard working solutions, which had a concentration range listed in Table 2. The working solutions were then stored at 2 to 8 °C for later use.

Table 2. Concentrations of matrix standard working solutions.

Compound	Mixed Catecholamines with 1% BSA
S1 pg/mL	5
S2 pg/mL	10
S3 pg/mL	20
S4 pg/mL	50
S5 pg/mL	100
S6 pg/mL	200
S7 pg/mL	500

The low- and high-concentration spiked matrix solutions (QCs) were prepared as in the previous method, with concentrations of 8 pg/mL, 40 pg/mL, and 400 pg/mL, respectively.

Preparation of isotope internal standard solution (10 ng/mL): 0.1% formic water solution was used to prepare six catecholamines internal standard stock solution with a final mixed solution concentration of 10 ng/mL, which was stored at 2 to 8 °C for later use.

Sample pretreatment

Each matrix standard was precisely pipetted to 200 µL, then 5 µL of 10 ppb mixed internal standard solution was added. The solution was vortexed to mix well, followed by the addition of 80 µL of 10% TCA aqueous solution to precipitate the protein. After centrifugation, 100 µL of supernatant was aspirated for sample analysis.

LC conditions

Table 3. LC conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-742)
Flow Rate	0.5 mL/min
Column Temperature	40 °C
Mobile Phase	A: 10 mmol NH_4Ac + 0.25 mmol NH_4F in water; B: Acetonitrile
Injection Volume	25 µL
Gradient Program	Time (min) %A %B 0 98 2 1.5 98 2 4.0 85 15 7.0 5 95 9.0 5 95 Flow (mL/min): 0.5 Maximum pressure limit (bar): 600

MS conditions

Table 4. MS conditions.

Parameter	Value
Ion Source	Jet Stream Technology ion source, positive mode (AJS+)
Capillary Voltage	2,000 V
Nebulizer Gas Pressure	45.0 psi
Drying Gas Temperature	180 °C
Drying Gas Flow Rate	5 L/min
Sheath Gas Temperature	370 °C
Sheath Gas Flow Rate	11 L/min
Nozzle Voltage	0 V

Table 5. MRM parameters.

Compound Name	Precursor (*m/z*)	Product (*m/z*)	Fragmentor (V)	CE (V)
3-MT*	168.1	91	80	26
3-MT	168.1	65	80	42
3-MT-D4	172.1	95.1	80	26
DA*	154.1	91	75	26
DA	154.1	65	75	38
DA-D4	158.1	141.1	75	6
E*	184.1	166	70	6
E	184.1	107	70	22
E-D3	187.1	169.1	70	6
MN*	198.1	180.1	70	6
MN	198.1	165.1	70	18
MN-D3	201.1	183.1	70	6
NE*	170.1	152.1	70	2
NE	170.1	107	70	18
NE-D6	176.1	158	70	2
NMN	184.1	78.9	80	30
NMN*	166.1	134	110	14
NMN-D3	187.1	169.1	80	2

* Quantitative transition

Results and discussion

The chromatogram in Figure 1 showed a good peak shape of six catecholamines with proper retention time. All had a linear range of 5 to 500 pg/mL, except epinephrine with a range of 10 to 500 pg/mL. The recovery of the spiked sample was between 80 and 120%.

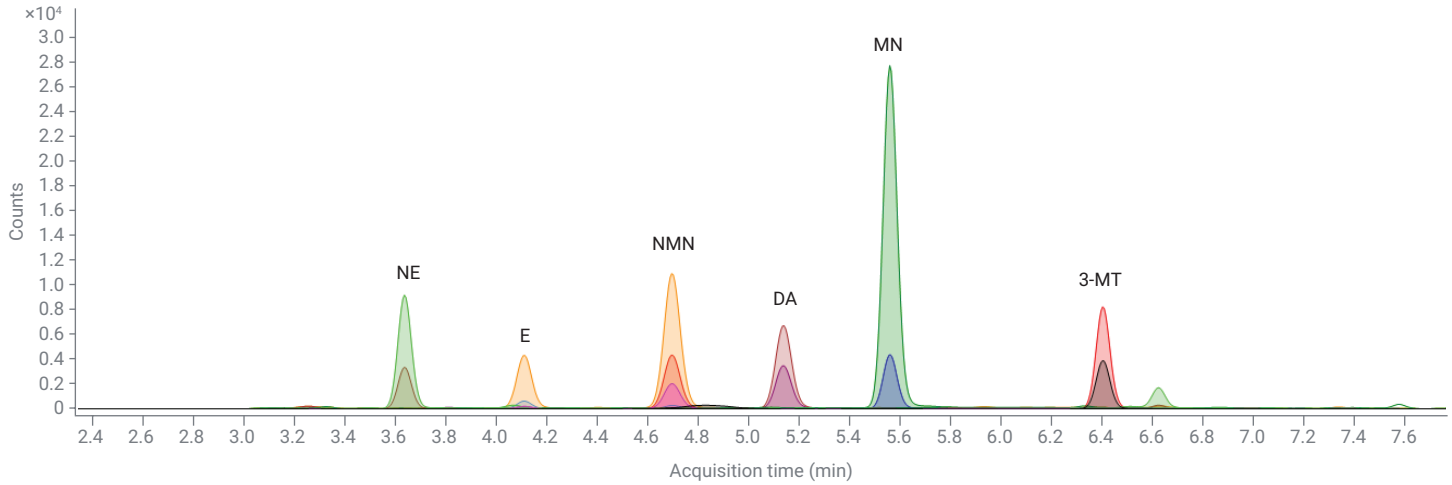
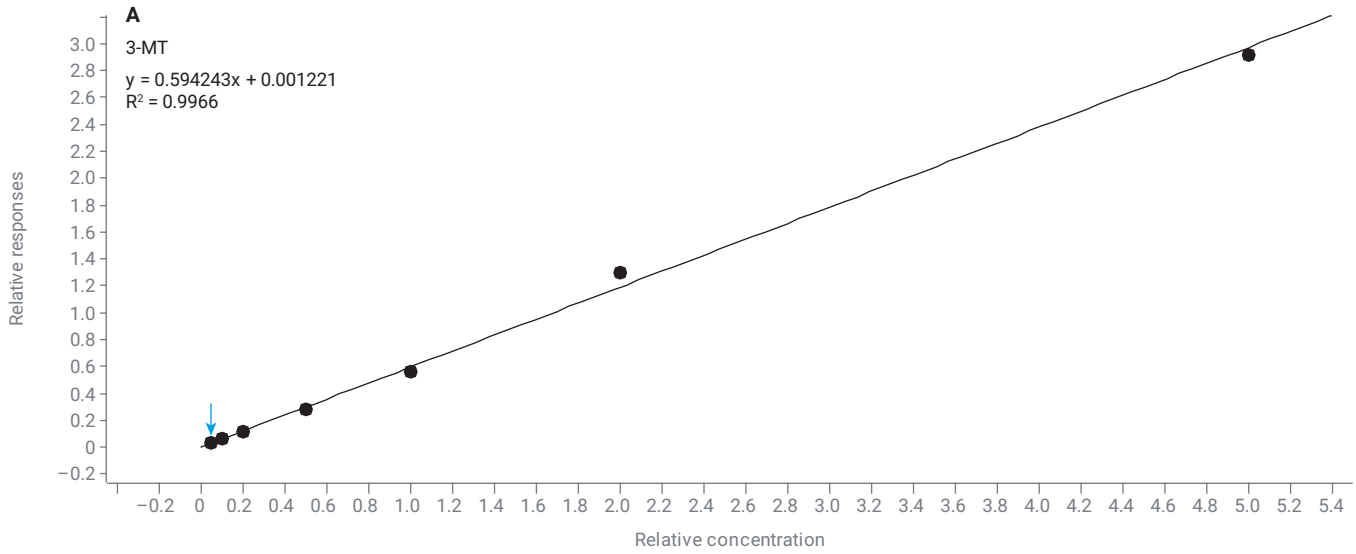
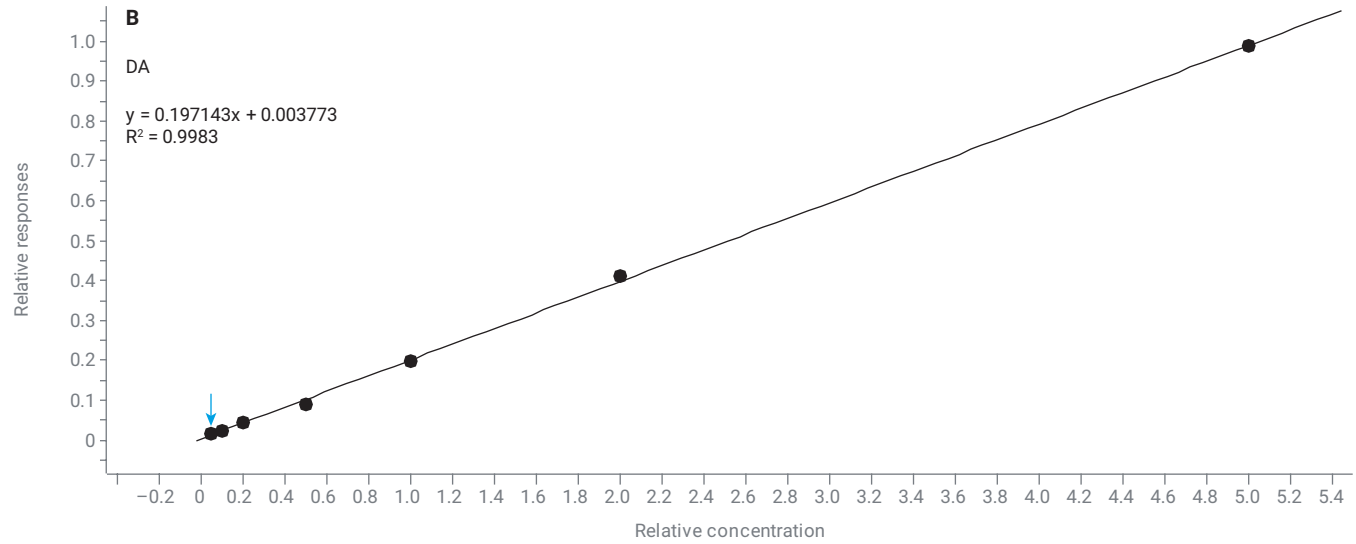
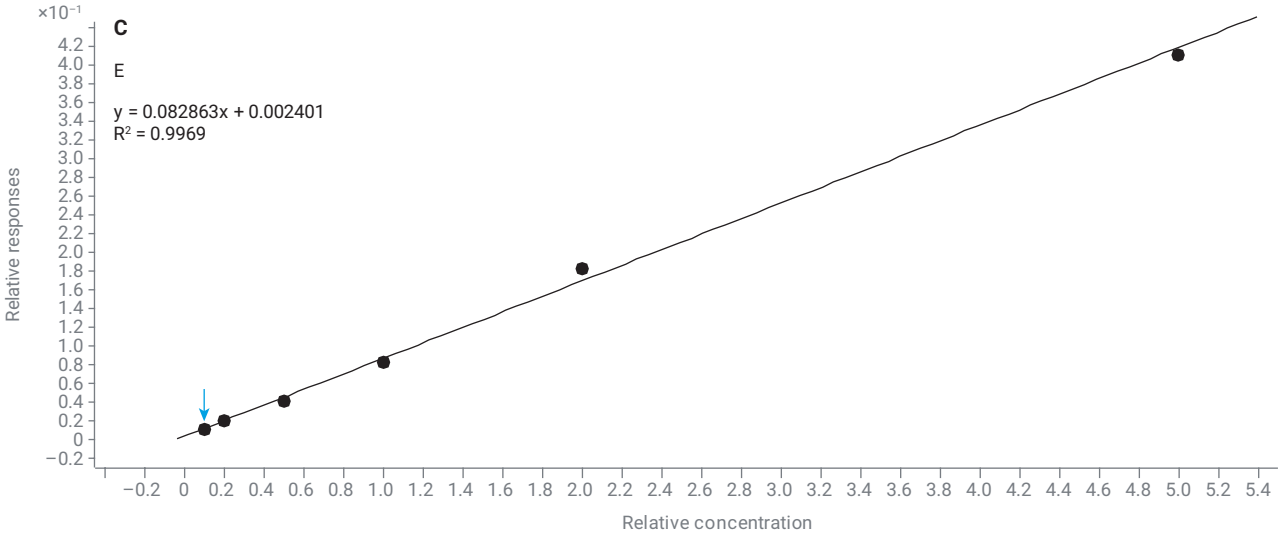
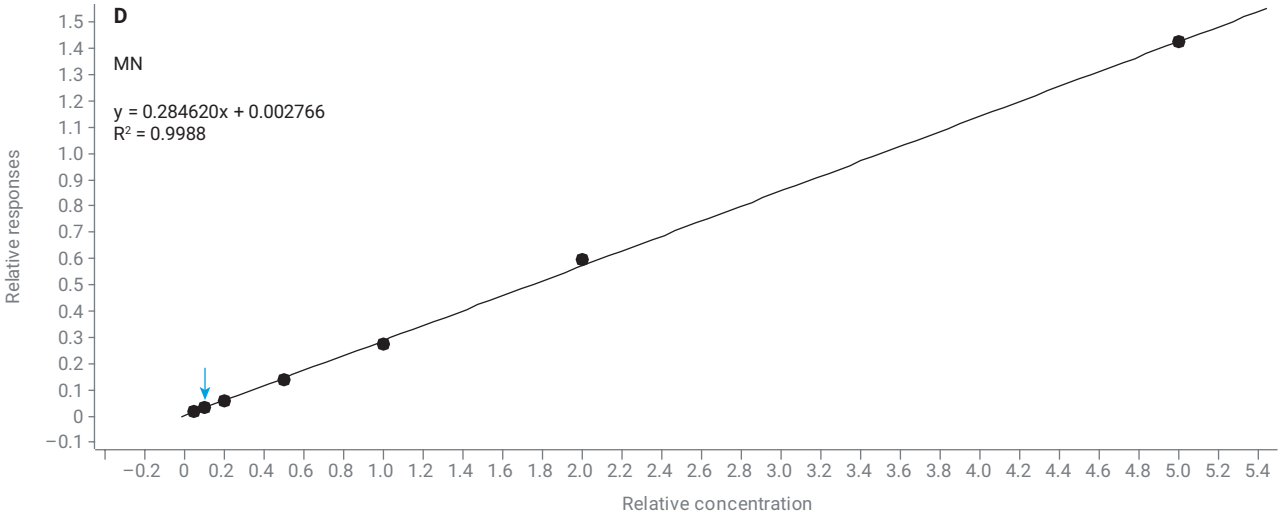


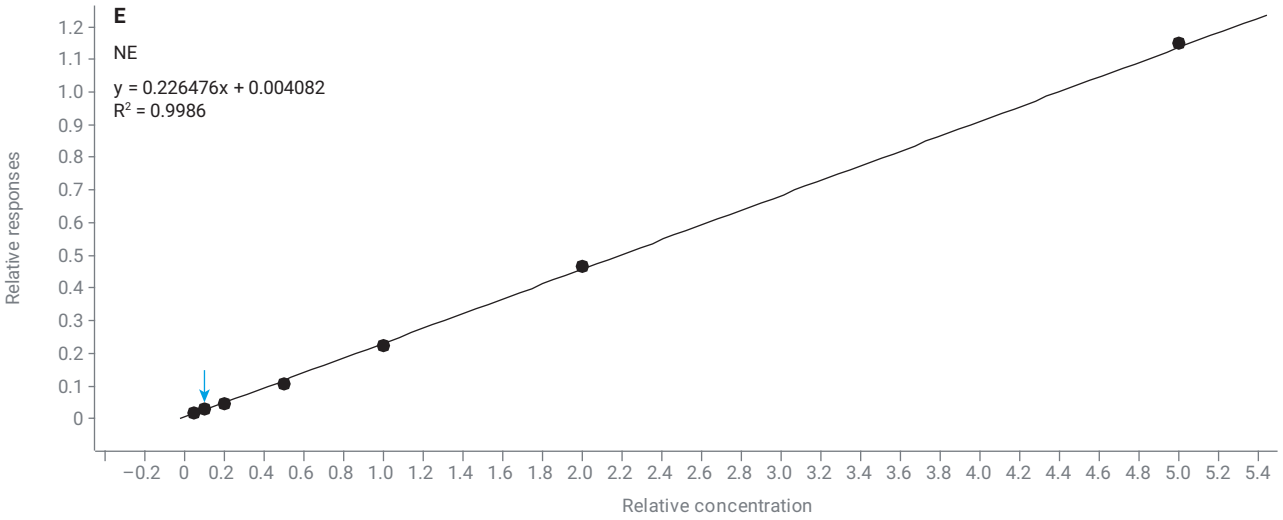
Figure 1. Chromatogram of six catecholamines.






C
E
y = 0.082863x + 0.002401
R² = 0.9969
×10⁻¹
Relative responses
Relative concentration


D
MN
y = 0.284620x + 0.002766
R² = 0.9988
Relative responses
Relative concentration


E
NE
y = 0.226476x + 0.004082
R² = 0.9986
Relative responses
Relative concentration

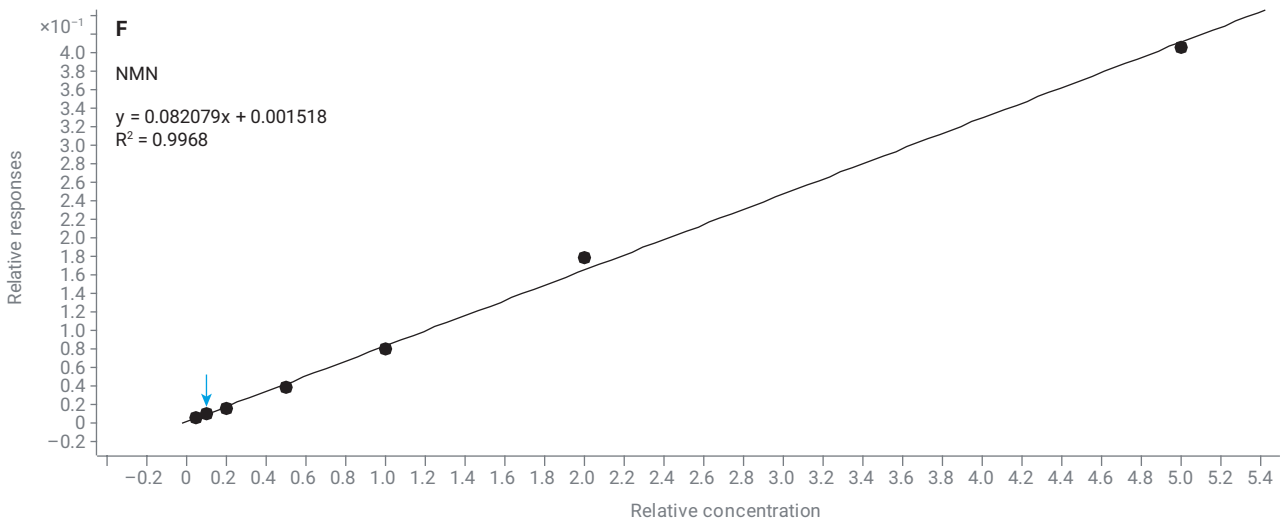


Figure 2. Calibration curves.

Conclusion

The method using Agilent Ultivo LC/TQ with an AJS source and the Agilent InfinityLab Poroshell 120 Aq-C18 column was developed for determination of catecholamines in BSA matrices. The method gives a wide linearity range of 5 to 500 pg/mL in BSA matrices (10 to 500 pg/mL for epinephrine), as well as excellent accuracy.

Statement

The experimental results involved in this document were obtained by Agilent under certain conditions. The document only serves as a technical reference for proving that the Agilent instrument is able to test and analyze target analyte under certain conditions. For any inquiries, please contact the Agilent Customer Center.

Reference

1. Fu, R.-J.; Wei, T.-C. Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance, *Agilent Technologies application note*, publication number 5994-5555EN, **2022**.

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