http://dx.doi.org/10.21577/0103-5053.20200033

J. Braz. Chem. Soc., Vol. 31, No. 7, 1467-1474, 2020
Printed in Brazil - ©2020 Sociedade Brasileira de Química

Article

Simultaneous Quantification of Plasma Catecholamines and Metanephrines by LC-MS/MS

Marina Dikunets,[a] *Grigory Dudko,*[*,a] *Pavel Glagovsky*[b] *and Ilgar Mamedov*[b]

[a]*Federal State Budgetary Institution "Federal Science Center for Physical Culture and Sport" (FSBI FSC VNIIFK), 105005, Moscow, Russia*

[b]*OOO ChromsystemsLab, 119049, Moscow, Russia*

The quantification of the low levels of catecholamines and metanephrines in biological fluids is important for clinical screening of pheochromocytoma/paraganglioma and diagnosis of overtraining syndrome in athletes. We introduce a novel, accurate and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous quantification of these biogenic amines in human plasma. Simple protein precipitation combined with rapid 2-aminoethyl diphenylborinate-assisted liquid-liquid extraction allow us to quantify catecholamines and metanephrines over broad concentration ranges. Target analytes were monitored in positive electrospray ionization mode by multiple reaction monitoring. Method performance was validated for linearity, lower limit of quantification, limit of detection, intra-day and inter-assay precision, carry-over, recovery, and matrix effect. The assay was linear within analytical range 25-1000 pg mL^{-1} for epinephrine, 30-2500 pg mL^{-1} for norepinephrine, 15-1000 pg mL^{-1} for dopamine, 25-2000 pg mL^{-1} for metanephrine and 50-10000 pg mL^{-1} for normetanephrine, with lower limits of quantification of 15, 20, 10, 15 and 30 pg mL^{-1}, respectively. The intra- and inter-day precisions for all compounds ranged from 0.4 to 6.9% and from 0.9 to 6.6%, respectively. The efficiency of novel method was confirmed by assaying external quality control samples which demonstrated consistent and accurate results.

Keywords: catecholamines, metanephrines, LC-MS/MS, plasma, overtraining, pheochromocytoma

Introduction

Catecholamines (dopamine, norepinephrine and epinephrine) are the class of chemical neurotransmitters and hormones which take key role in the regulation of physiological mechanisms and the expansion of neurological, psychiatric, endocrine and cardiovascular diseases. Current understanding in catecholamines metabolism in terms of ongoing physiological processes and clinical significance has been reported.[1] The main catecholamines metabolism pathway is intraneuronal deamination whilst secondary way of their biotransformation to metanephrines (metanephrines, normetanephrine) is extraneuronal 3-*O*-methylation caused by catechol-*O*-methyltransferase.[2] The only origin of metanephrines in healthy subjects is the adrenal medulla. Alternatively, significant levels of metanephrines may result from catecholamines metabolism yielded by neuroendocrine tumor cells (pheochromocytoma, paraganglioma).[3,4]

The occurrence of pheochromocytoma/paraganglioma is described by hypertension associated with elevated concentrations of catecholamines. Historically, tumor screening via detecting urinary catecholamines and their metabolites in subjects with paroxysmal hypertension and genetic predisposition to the tumor has led to false negative results.[5] Simultaneous analysis of plasma catecholamines and metanephrines has a crucial diagnostic value as it allows to more effectively eliminate or confirm the presence of hyperplastic process.[6-8] This test demonstrates high sensitivity and selectivity for compounds produced by the tumor. In most patients with pheochromocytoma/paraganglioma, plasma normetanephrine and metanephrine levels are 2-3 times higher in comparison with the upper reference intervals established for healthy individuals.[9]

Catecholamines modulate metabolic and cardiocirculatory reactions as well as adaptation to physical and psychological activity,[10] hence they are proposed as biochemical markers for the early diagnosis of overtraining syndrome (OT),[11,12] and the adrenaline/noradrenaline concentration ratio as a factor of sympathetic nervous

*e-mail: dudko@vniifk.ru

system adrenomedural response.[13] Plasma catecholamines levels more accurately reflect stress-related sympathetic response than their urine concentrations.[14] Theoretically, to insure accurate diagnosis of OT it is essential to conduct following tests: (*i*) at rest, to compare with the normal physiological range; (*ii*) after training specific for given sport discipline, to assess athlete's response to normal training inducements; (*iii*) 24, 48 and 72 h after exercise, to size the possibility of athlete's body to recover and its adaptation to the training load.[15] It is important to emphasize that shifts of biochemical markers which occur during physical exercise are individual for each athlete, for that matter interpretation of the results obtained during the study should be individualized and consider the circadian rhythm and seasonal variations,[16] since catecholamines secretion is not only obeyed by the daily rhythm, but also seasonal changes related to the influence of the ambient temperature on sympathetic nervous system activity.[17]

In general, high-performance liquid chromatography methods combined with electrochemical (HPLC-ECD) or fluorescence detection (HPLC-FLD) are most commonly used to determine plasma catecholamines. The main disadvantages of HPLC-ECD approaches are high background signal, low sensitivity, poor reproducibility of the results, interfering effects of matrix co-eluting components and the high cost, whereas HPLC-FLD-based techniques are negatively characterized by high limit of detection, time-consuming sample preparation and lengthy run time.[18]

The application of chromatography-mass-spectrometry methods in the practice of clinical diagnostic laboratories allows not only to reduce analysis time, but also to ensure highly sensitive and selective determination of compounds of interest since the identification of analytes is based on their unique physicochemical properties: retention time, precursor-ion and ion-products.[19-22] High performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS) has the greatest diagnostic accuracy for the determination of catecholamines[23-25] and metanephrines[26,27] in human biofluids.

The main difficulty of their plasma quantification is due to low reference values.[28] With the arrival of more sensitive LC-MS/MS instruments along with appropriate sample preparation certain analytes can now be accurately measured in body fluids at low concentrations. LC-MS/MS in combination with deuterated internal standards has a huge potential to provide high specificity, accuracy, and sensitivity of measurement. An effective sample cleanup for the complex plasma matrix prior to analysis is essential. Generally, pre-treatment of plasma samples for catecholamines analysis involved extraction onto acid washed alumina at basic pH media,[29-31] or the use of boric acid elution.[32,33] The main analytical goal is to achieve the satisfactory sensitivity for the low levels of plasma catecholamines while restricting the co-elution of many endogenous and exogenous compounds that remain following such a non-selective process as alumina extraction.[34] The most common cleanup technique for plasma metanephrines is solid phase extraction on weak cation exchange resins (WCX).[26,35,36]

Currently, there are few methods for the simultaneous determination of catecholamines and their 3-*O*-methylated metabolites developed only for human urine.[37] Quantification of these biogenic amines in plasma within single run has a great potential for clinical diagnostics.

The aim of current work is to demonstrate novel, rapid and cost-effective LC-MS/MS approach for simultaneous plasma catecholamines and metanephrines quantification for clinical and sport medicine purposes, establish method performance through systematic validation study, which could lay a solid foundation for its further application in diagnostic laboratories.

Experimental

Materials and reagents

Catecholamines and metanephrines of high purity (≥ 98.0%): epinephrine (E), norepinephrine (NE), dopamine (DA), metanephrine (MN), normetanephrine (NMN) and formic acid (FA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Respective deuterated internal standards (IS) of high chemical and isotopic purity (≥ 92.0%): E-d_3, NE-d_6, DA-d_4, MN-d_3 and NMN-d_3 were obtained from Toronto Research Chemicals (Toronto, Canada). Endocrine plasma normal and pathological range controls were obtained from ChromSystems GmbH (Munich, Germany). The LC-MS-grade acetonitrile (ACN) and LC-MS-grade methanol (MeOH) were supplied by Fisher Scientific (Loughborough, UK). HPLC-grade ethyl acetate, 2-aminoethyl diphenylborinate (2-APB), ethylenediaminetetraacetic acid (EDTA), hydrochloric acid (37%) and ammonium chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA); ammonium hydroxide solution (25%) was purchased from Merck (Darmstadt, Germany). All of the chemicals and solvents were of the highest purity available from commercial sources and used without further purification. Dialyzed plasma was acquired from internal Reagent Laboratory (Moscow, Russia), and aliquots were stored at −70 °C prior to use. Deionized water with specific electro conductivity 18.2 MΩ cm was prepared employing Millipore Integral 3 (France). 2-APB

solution (2 g L^{-1}) was prepared in 2 mol L^{-1} ammonium chloride (pH 4.5) with presence of EDTA and pH was adjusted to 8.5 by adding 25% ammonium hydroxide.

Preparation of calibrators and controls

Catecholamines, metanephrines and internal standards stock solutions at 1 mg mL^{-1} as well as calibrator solutions were prepared in 0.1 mol L^{-1} HCl. Quality control (QC) and calibration samples were prepared from dialyzed plasma. Calibration curves for catecholamines and metanephrines were established by using six calibration standards. Linearity ranges were 25-1000 pg mL^{-1} for E; 30-2500 pg mL^{-1} for NE; 15-1000 pg mL^{-1} for DA; 25-2000 pg mL^{-1} for MN and 50-10000 pg mL^{-1} for NMN. QC samples were prepared at two levels (low: QCL, and high: QCH): 75 and 850 pg mL^{-1} for E; 50 and 2250 pg mL^{-1} for NE; 45 and 750 pg mL^{-1} for DA; 75 and 1500 pg mL^{-1} for MN; 150 and 8500 pg mL^{-1} for NMN. An IS working solution including E-d_3 (1 ng mL^{-1}), NE-d_6 (5 ng mL^{-1}), DA-d_4 (10 ng mL^{-1}), MN-d_3 (5 ng mL^{-1}) and NMN-d_3 (10 ng mL^{-1}) was prepared in 0.1 mol L^{-1} HCl. All the solutions were stored at −20 °C until analysis.

Normal and pathological range controls were prepared according to manufacturer's protocol. Multiple vials of reconstituted controls were pooled, aliquoted, and stored at −20 °C until use (up to two months).

Sample preparation

500 µL of sample specimens, calibrators and controls were mixed with 10 µL of IS working solution and 500 µL of ACN. After vigorous stirring for 60 s using a vortex apparatus, mixture was centrifuged (5 min at 2000 rpm) and 850 µL of supernatant was transferred into clean tube. 30 µL of 5% ammonium hydroxide solution, 0.4 mL of 2-aminoethyl-diphenylborinate solution and 1.5 mL of ethyl acetate were added and analytes were extracted by vigorous mechanical shaking for 10 min. The tube was then centrifuged (5 min at 3000 rpm) and 1.0 mL of organic layer was separated followed by evaporation to dryness under a flow of nitrogen at 35 °C. Dry residue was reconstituted with 150 µL of mobile phase A (aqueous 0.1% FA) and transferred into vial.

HPLC-MS/MS

Chromatography was performed on Nexera X2 UPLC system (Shimadzu, Japan) equipped with Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm, Agilent, USA) coupled with the guard column Zorbax Eclipse XDB-C18 (12.5 × 4.6 mm, 5 µm, Agilent, USA) at 60 °C. Mobile phases were aqueous 0.1% FA (mobile phase A) and 0.1% FA in MeOH (mobile phase B). Gradient elution was as follows: 0.0 min, 2% (B); 2.0 min, 5% (B); 2.7-3.2 min, 95% (B); 3.3-6.0 min, 2% (B) at flow rate of 0.7 mL min^{-1}. Injection volume was 20 µL.

Detection was performed on 8060 triple quadrupole mass spectrometer (Shimadzu, Japan) using positive electrospray ionization (ESI) mode. Quantitative data were obtained in multiple reaction monitoring (MRM) mode of the protonated precursor ion $[M + H]^+$ or following the in-source loss of water $[M + H - H_2O]^+$. Two specific transitions were chosen for each analyte, one for confirmation (the "qualitation transition") and one for quantification (the "quantification transition") as displayed in Table 1. LabSolutions software (Shimadzu, Japan) version 5.86 was used for instrument control, data acquisition and processing. Interface voltage was set at 4 kV, collision-induced dissociation (CID) gas was maintained at 17 kPa. Nebulizer gas was set at 3 L min^{-1}, drying and heating gas flow were kept at 10 L min^{-1}. Temperature of interface, desolvation line and heat block were 300, 250 and 400 °C, respectively. The MRM acquisition settings are summarized in Table 1.

Assay validation

The method performance was evaluated by means of linearity, lower limit of quantification (LLOQ), limit of detection (LOD), intra- and inter-assay precision, carry-over, analytes recovery, ion suppression. Analytes stability in matrix, stock solutions and samples when stored under different temperature conditions was also assessed. Linearity was assessed by analyzing calibrators at six levels which were prepared by spiking 50 µL of respective calibrator solution to dialyzed plasma. Each of the six concentration levels were analyzed at three replicates. The acceptance criterion for linearity was correlation factor (r^2) ≥ 0.99. LLOQ was determined as the lowest measured concentration with accuracy within 80-120% of expected value and precision (relative standard deviation, RSD) < 20%. LOD was estimated as the lowest measured concentration with signal-to-noise ratio 3:1. Intra-assay precision was determined by measuring each level of QC samples in six replicates (n = 6) within single batch. Inter-assay precision was assessed by measuring each level of QC samples in six replicates over three consecutive days (n = 18). The criteria for intra- and inter-assays acceptance was precision (RSD) within ± 10% and accuracy within 90-110% of nominal concentration. Carry-over was measured by injecting the following

Table 1. Optimized MRM parameters of catecholamines and metanephrines

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 / V	CE / V	Q3 / V	Dwell time / ms
NE	152.0	77.2	−6.0	−33.0	−30.0	20
		107.1[a]	−6.0	−20.0	−24.0	
NE-d_6	158.2	112.2	−16.0	−19.0	−19.0	20
E	184.1	166.0	−12.0	−10.0	−12.0	20
		107.2[a]	−12.0	−21.0	−24.0	
E-d_3	187.2	107.1	−14.0	−21.0	−24.0	20
DA	154.1	137.1[a]	−30.0	−14.0	−28.0	20
		91.1	−11.0	−24.0	−17.0	
DA-d_4	158.2	141.1	−6.0	−15.0	−10.0	20
NMN	166.1	134.1	−12.0	−10.0	−30.0	20
		106.2[a]	−8.0	−20.0	−8.0	
NMN-d_3	169.1	109.2	−12.0	−20.0	−12.0	20
MN	180.2	148.1[a]	−12.0	−20.0	−16.0	20
		165.0	−12.0	−19.0	−12.0	
MN-d_3	183.2	151.1	−12.0	−19.0	−30.0	20

[a]Quantification transition. CE: collision energy; NE: norepinephrine; E: epinephrine; DA: dopamine; NMN: normetanephrine; MN: metanephrine.

sequence: (*i*) upper calibration level extract in six replicates; (*ii*) blank sample extract; (*iii*) lower calibration level extract. Carry-over expressed as accuracy should be within ± 20% of expected concentration in the lower calibration level sample. Assessment of analytes recovery was carried out on two concentration levels of analytes corresponding to those in QC samples in triplicate. Plasma samples were spiked in pooled plasma and along with unspiked aliquots were subjected to current approach. Recovery in percentage was calculated as [(concentration in spiked sample – concentration in unspiked sample) / known spiked concentration]. Ion suppression (in percentage) was evaluated following postextraction addition protocol by comparing peak areas of all analytes added postextraction in dialyzed plasma (at 100 pg mL^{-1}) to those of a pure solution with equivalent amount prepared in mobile phase A. Analytes stability in matrix was estimated using two internal QC samples and ChromSystems endocrine plasma controls. Samples were analyzed and then placed in refrigerator (2-8 °C) for 24 and 72 h. Beyond these time points stored samples were re-analyzed using calibration curve from freshly prepared solutions and the obtained concentrations of analytes were compared to nominal values. Analytes stability in stock solutions at 1 mg mL^{-1} expressed as percentage of peak areas variation was calculated by comparing peak areas of stored in freezer (−18 to −20 °C) solutions to those freshly prepared. For this reason, model solutions were prepared by diluting fresh and stored stock solutions to 10 µg mL^{-1} with aqueous 0.1% FA and analyzed in six replicates. The acceptance criteria for analytes stability in stock solutions was peak areas variety within ± 15%. Analytes stability in samples was assessed by quantification catecholamines and metanephrines in plasma aliquots from volunteers (n = 8) which after initial analysis were stored for: (*i*) 3 h at room temperature; (*ii*) 3 h at 2-8 °C; (*iii*) 24 h at 2-8 °C; (*iv*) 7 days at 2-8 °C; (*v*) 7 days at −20 °C with single freeze-thawing cycle. Concentration alteration in plasma samples during storage not exceeding ± 15% were considered acceptable.

The study protocol (No. 07/19) was approved by the Ethics Committee for biomedical ethics of Federal State Budgetary Institution Federal Science Centre for Physical Culture and Sport. All volunteers participated in the current study gave written permission to use their biomaterial in scientific purposes.

Results and Discussion

Generally, $[M + H]^+$ precursor ion is preferred for generating the product ions spectrum. However, protonated molecular ions of NE, NM and NMN are unstable and undergo loss of water in the ESI source, yielding the more stable $[M + H - H_2O]^+$ ions, which were selected as the precursor ions. Conversely protonated ions of E and DA were monitored in the more stable form of $[M + H]^+$. Automatic optimization to obtain fragment ions was performed using LabSolutions software and the most intense product ions of both target analytes and respective ISs were chosen.

The use of stable isotope-labeled internal standards with equal physicochemical properties of target analytes allows to offset matrix effects which affect the ionization efficiency and take account of extraction losses. We have chosen deuterium labelled internal standards with molecular masses of more than 3 a.m.u. towards analyzed compounds to eliminate the possible interference of isotopic precursor-ions of internal standards on those for target analytes.

One of the most important part of reliable quantification of substances with similar chemical structures is to select optimal chromatographic conditions. Within the scope of our aim this task is complicated inasmuch as E and NMN share common precursor-ions. Without proper chromatographic separation, fragmentation of these compounds can cause interferences with one another and lead to inaccurate quantification. The choice of optimal conditions for chromatographic separation of target substances consisted in selection of mobile phases, chromatographic columns as well as gradient elution program and flow rate. We tested 10 mmol L^{-1} aqueous ammonium formate and 0.1% FA in water as mobile phase A, and 10 mmol L^{-1} ammonium formate in methanol and 0.1% FA in methanol as mobile phase B. During optimization chromatographic separation was performed on Zorbax Eclipse XDB-C18 and Ascentis C18. The most effective separation of analytes and obtaining narrow chromatographic peaks with shape close to that of Gaussian distribution was achieved on Zorbax Eclipse XDB-C18 column and with FA as modifier. Using current gradient elution program and flow rate 0.7 mL min^{-1} we managed to reduce the run time and effectively separate target compounds within just 6 min together with 2 min for column washing and re-equilibration. Retention times and MRM chromatograms of QC samples and real plasma sample are shown in Figure 1.

The sample preparation was accomplished according to the protocol described above. Cleanup was carried out using a simple liquid-liquid extraction (LLE) technique with ethyl acetate and 2-APB as the complexing reagent at pH 9.5. The diphenyl boronate forms a stable, negatively charged complex with *cis*-hydroxyl groups of catecholamines, which has strong affinity for the apolar solvent, when operating in alkali media.[38] It has been reported[39] previously that LLE using ethyl acetate in pH 9.5 and 2-APB showed better results for simultaneous extraction of catecholamines and metanephrines from urine samples. The use of 2-APB has several advantages over extraction methods utilizing the alumina, cation-exchange or boronate sorbents that are commonly used for catecholamines isolation. Thus, our sample preparation scheme was based on earlier established method[38] except for adding plasma protein precipitation stage and using lower biomaterial volume.

Good linearity was achieved using a $1/x^2$ weighted linear regression for all compounds. All calibration curves had r^2 values of 0.99 or greater. The assay was linear within analytical range 25-1000 pg mL^{-1} for E, 30-2500 pg mL^{-1} for NE, 15-1000 pg mL^{-1} for DA, 25-2000 pg mL^{-1} for MN and 50-10000 pg mL^{-1} for NMN. LLOQs for E, NE, DA, NM and NMN were determined to be 15, 20, 10, 15 and 30 pg mL^{-1}, respectively, which were sufficient for accurate measurement of all analytes in patient samples. By measuring a series of sequentially diluted calibrators, the LODs, defined as the concentration that produces a signal 3-fold higher than noise, were 10 pg mL^{-1} for E, NE and MN, 5 pg mL^{-1} for DA and 20 pg mL^{-1} for NMN. The LOD, LLOQ and the linearity parameters are presented in Table 3. Table 2 summarized the intra- and inter-day precisions by analyzing two levels of QC samples. Carry-over was found insignificant as accuracy (relative error (RE)) for E, NE, DA, MN and NMN was 89.6, 107.2, 86.5, 83.4 and 112.6%, respectively. Average (n = 6) recoveries for E, NE, DA, MN and NMN were found 83.2, 86.0, 81.7, 103.2 and 106.5%, respectively. Postextraction addition of target analytes solution has not educed significant alterations in ionization efficiency as ion suppression for all compounds were accounted for no more than 7.8%. Analytes stability in matrix was demonstrated for 72 h when stored at 2-8 °C, in stock solutions (1 mg mL^{-1}) for 4 months at −18 to −20 °C, in samples extracts for at least 7 days at −18 to −20 °C.

ChromSystems endocrine plasma normal and pathological range controls were analyzed using established approach in triplicate within 5 consecutive days. The results shown in Table 4 demonstrate that the measured concentrations for all substances were within acceptable ranges with precision (RSD) ranging from 3.8 to 6.8% for normal control and from 1.2 to 4.9% for pathological control. These findings suggested that the validated method is suitable for the analysis of plasma catecholamines and metanephrines at clinically significant levels.

Conclusions

A robust, selective and reliable LC-MS/MS method was designed to quantify epinephrine, norepinephrine, dopamine, metanephrine and normetanephrine in human plasma. Simple and rapid LLE technique with 2-APB was implemented without need to perform cost intensive and time consuming solid-phase extraction. The minimum amount of organic solvent used as well as the short-time extraction and analysis run time make developed approach rapid and less expensive for routine clinical analysis. LC-MS/MS assay was characterized by excellent linearity, accuracy and precision for catecholamines

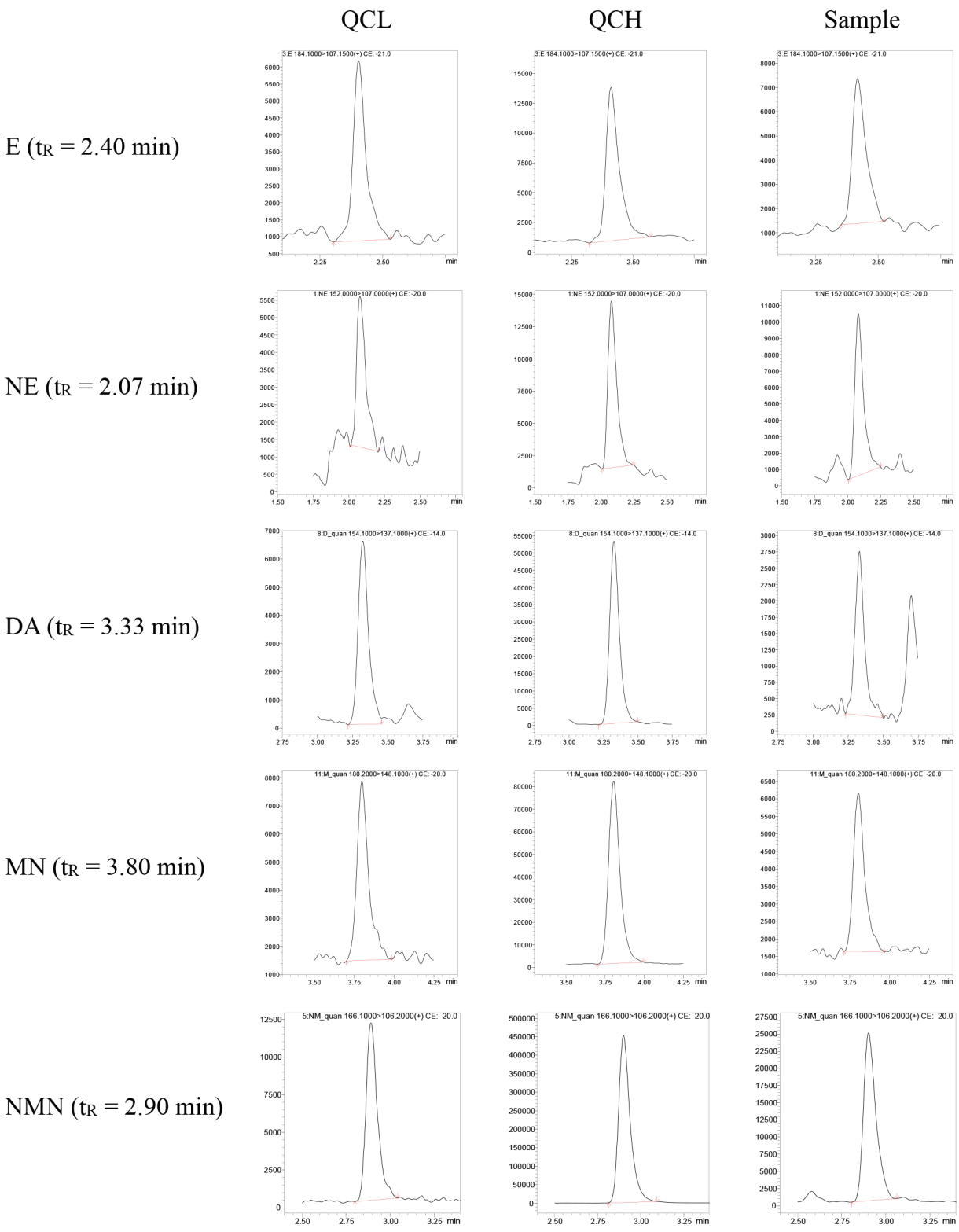

Figure 1. Typical MRM chromatograms and retention times (t_R) of target analytes in QC samples and real plasma extract (concentrations are presented in Table 2).

and metanephrines determination in plasma. Obtained limits of detection and quantification are comparable with other works in literature. Novel method of plasma catecholamines and their 3-*O*-methylated metabolites quantification will contemporaneously allow to study the activity of sympathoadrenal system which plays a key role

Table 2. Summary of intra- and inter-day precisions

Compound	Nominal concentration / (pg mL^{-1})	Intra-day precision (n = 6)		Inter-day precision (n = 18)	
		RE / %	RSD / %	RE / %	RSD / %
E	50	96.9	4.4	98.9	3.5
	850	101.8	1.4	101.2	1.0
NE	50	95.6	5.1	90.9	5.1
	2500	102.7	3.1	97.5	1.0
DA	45	102.5	4.7	107.4	1.9
	750	102.5	0.4	101.1	2.2
MN	75	94.6	6.9	98.4	6.6
	1500	98.4	4.0	96.8	2.1
NMN	150	94.8	3.2	98.0	0.9
	8500	99.3	1.9	99.3	1.9

RE: relative error, accuracy; RSD: relative standard deviation; E: epinephrine; NE: norepinephrine; DA: dopamine; MN: metanephrine; NMN: normetanephrine.

Table 3. Parameters for linearity range, LOD and LLOQ for catecholamines and metanephrines

Compound	r^2	Equation of the curve	Linear range / (pg mL^{-1})	LLOQ / (pg mL^{-1})	LOD / (pg mL^{-1})
E	0.9997	$y = 1.977 \times 10^{-3}x - 1.676 \times 10^{-2}$	25-1000	15	10
NE	0.9943	$y = 1.737 \times 10^{-3}x - 0.199$	30-2500	20	10
DA	0.9999	$y = 5.995 \times 10^{-5}x - 1.142 \times 10^{-4}$	15-1000	10	5
MN	0.9996	$y = 6.270 \times 10^{-4}x + 8.764 \times 10^{-3}$	25-2000	15	10
NMN	0.9999	$y = 6.731 \times 10^{-4}x + 1.307 \times 10^{-2}$	50-10000	30	20

r^2: correlation factor; LLOQ: lower limit of quantification; LOD: limit of detection; E: epinephrine; NE: norepinephrine; DA: dopamine; MN: metanephrine; NMN: normetanephrine.

Table 4. Results of ChromSystems endocrine plasma controls measured by new method (target concentrations and acceptable measurement ranges established by manufacturer using HPLC with electrochemical detection)

Compound	Endocrine plasma control					
	Normal range (n = 15)			Pathological range (n = 15)		
	Measured / (pg mL^{-1})	Target / range (HPLC-ED) / (pg mL^{-1})	RSD / %	Measured / (pg mL^{-1})	Target / range (HPLC-ED) / (pg mL^{-1})	RSD / %
E	116	101 / 70.4-131	6.8	531	533 / 400-666	4.9
NE	326	317 / 222-412	5.2	1988	2122 / 1592-2653	3.1
DA	160	175 / 122-227	3.8	838	854 / 598-1110	2.2
MN	61	60 / 48-72	6.5	1663	1500 / 1200-1800	1.2
NMN	103	100 / 80-120	4.2	7578	7003 / 5602-8403	2.2

HPLC-ED: high performance liquid chromatography with electrochemical detection; RSD: relative standard deviation; E: epinephrine; NE: norepinephrine; DA: dopamine; MN: metanephrine; NMN: normetanephrine.

in the implementation of neurohumoral regulation of vital functions, homeostatic equilibrium under the influence of various factors of external and internal environment, metabolic activity of catecholamines in extraneuronal tissues, along with obtaining appropriate information to diagnose neuroendocrine tumors and to prevent overtraining syndrome in athletes.

Acknowledgments

The authors thank OOO ChromsystemsLab represented by CEO PhD Pavel Glagovsky and Deputy CEO of Innovative Technologies PhD Ilgar Mamedov for funding this research.

References

1. Eisenhofer, G.; Kopin, I. J.; Goldstein, D. S.; *Pharmacol. Rev.* **2004**, *56*, 331.
2. Kopin, I. J.; *Pharmacol. Rev.* **1985**, *37*, 333.
3. Eisenhofer, G.; Keiser, H.; Friberg, P.; Mezey, E.; Huynh, T. T.; Hiremagalur, B.; Ellingson, T.; Duddempudi, S.; Eijsbouts, A.; Lenders, J. W.; *J. Clin. Endocrinol. Metab.* **1998**, *83*, 2175.
4. Lenders, J. W.; Keiser, H. R.; Goldstein, D. S.; Willemsen, J. J.; Friberg, P.; Jacobs, M. C.; Kloppenborg, P. W.; Thien, T.; Eisenhofer, G.; *Ann. Intern. Med.* **1995**, *123*, 101.
5. Lenders, J. W.; Pacak, K.; Walther, M. M.; Linehan, W. M.; Mannelli, M.; Friberg, P.; Keiser, H. R.; Goldstein, D. S.; Eisenhofer, G.; *JAMA, J. Am. Med. Assoc.* **2002**, *287*, 1427.
6. Václavík, J.; Stejskal, D.; Lacnák, B.; Lazárová, M.; Jedelský, L.; Kadalová, L.; Janosová, M.; Frysák, Z.; Vlcek, P.; *J. Hypertens.* **2007**, *25*, 1427.
7. Eisenhofer, G.; Prejbisz, A.; Peitzsch, M.; Pamporaki, C.; Masjkur, J.; Rogowski-Lehmann, N.; Langton, K.; Tsourdi, E.; Pęczkowska, M.; Fliedner, S.; Deutschbein, T.; Megerle, F.; Timmers, H. J. L. M.; Sinnott, R.; Beuschlein, F.; Fassnacht, M.; Januszewicz, A.; Lenders, J. W. M.; *Clin. Chem.* **2018**, *64*, 1646.
8. Hickman, P. E.; Leong, M.; Chang, J.; Wilson, S. R.; McWhinney, B.; *Pathology* **2009**, *41*, 173.
9. Lenders, J. W.; Eisenhofer, G.; Mannelli, M.; Pacak, K.; *Lancet* **2005**, *366*, 665.
10. Carter, J. R.; Ray, C. A.; *Auton. Neurosci.* **2015**, *188*, 36.
11. Hooper, S. L.; Mackinnon, L. T.; Gordon, R. D.; Bachmann, A. W.; *Med. Sci. Sports Exercise* **1993**, *25*, 741.
12. Lehmann, M.; Baumgartl, P.; Wiesenack, C.; Seidel, A.; Baumann, H.; Fischer, S.; Spori, U.; Gendrisch, G.; Kaminski, R.; Keul, J.; *Eur. J. Appl. Physiol. Occup. Physiol.* **1992**, *64*, 169.
13. Schöfl, C.; Becker, C.; Prank, K.; von zur Mühlen, A.; Brabant, G.; *Eur. J. Endocrinol.* **1997**, *137*, 675.
14. Lehmann, M.; Lormes, W.; Optiz-Gress, A.; Steinacker, J. M.; Netzer, N.; Foster, C.; Gastmann, U.; *J. Sports Med. Phys. Fitness* **1997**, *37*, 7.
15. Petibois, C.; Cazorla, G.; Poortmans, J. R.; Déléris, G.; *Sports Med.* **2002**, *32*, 867.
16. Hansen, A. M.; Garde, A. H.; Skovgaard, L. T.; Christensen, J. M.; *Clin. Chim. Acta* **2001**, *309*, 25.
17. Kruse, H. J.; Wieczorek, I.; Hecker, H.; Creutzig, A.; Schellong, S. M.; *J. Lab. Clin. Med.* **2002**, *140*, 236.
18. Tsunoda, M.; *Anal. Bioanal. Chem.* **2006**, *386*, 506.
19. Ketha, S. S.; Singh, R. J.; Ketha, H.; *Endocrinol. Metab.* **2017**, *46*, 593.
20. Keevil, B. G.; *Clin. Biochem.* **2016**, *49*, 989.
21. van den Ouweland, J. M. W.; Kema, I. P.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2012**, *883-884*, 18.
22. Jannetto, P. J.; Fitzgerald, R. L.; *Clin. Chem.* **2016**, *62*, 92.
23. Ji, C.; Walton, J.; Su, Y.; Tella, M.; *Anal. Chim. Acta* **2010**, *670*, 84.
24. Kushnir, M. M.; Urry, F. M.; Frank, E. L.; Roberts, W. L.; Shushan, B.; *Clin. Chem.* **2002**, *48*, 323.
25. Shen, Y.; Cheng, L.; Guan, Q.; Li, H.; Lu, J.; Wang, X.; *Biomed. Chromatogr.* **2017**, *31*, e4003.
26. Petteys, B. J.; Graham, K. S.; Parnás, M. L.; Holt, C.; Frank, E. L.; *Clin. Chim. Acta* **2012**, *413*, 1459.
27. Clark, Z. D.; Frank, E. L.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 3673.
28. Burtis, C. A.; *Tietz Textbook of Clinical Chemistry*, 6th ed.; Burtis, C. A.; Ashwood, E. R.; Bruns, D. E., eds.; WB Saunders: Philadelphia, USA, 2008.
29. Anton, A. H.; Sayre, D. F.; *J. Pharmacol. Exp. Ther.* **1962**, *138*, 360.
30. Hjemdahl, P.; *Acta Physiol. Scand.* **1984**, *527*, 43.
31. Holly, J. M. P.; Makin, H. J. L.; *Anal. Biochem.* **1983**, *128*, 257.
32. Hansen, C.; Agrup, G.; Rorsman, H.; Rosengren, A. M.; Rosengren, E.; *J. Chromatogr. A* **1978**, *161*, 352.
33. Smedes, F.; Kraak, J. C.; Poppe, H.; *J. Chromatogr.* **1982**, *231*, 25.
34. Macdonald, I. A.; Lake, D. M.; *J. Neurosci. Methods* **1985**, *13*, 239.
35. Peitzsch, M.; Prejbisz, A.; Kroiss, M.; Beuschlein, F.; Arlt, W.; Januszewicz, A.; Siegert, G.; Eisenhofer, G.; *Ann. Clin. Biochem.* **2013**, *50*, 147.
36. Wright, M. J.; Thomas, R. L.; Stanford, P. E.; Horvath, A. R.; *Clin. Chem.* **2015**, *61*, 505.
37. Peitzsch, M.; Pelzel, D.; Glöckner, S.; Prejbisz, A.; Fassnacht, M.; Eisenhofer, G.; *Clin. Chim. Acta* **2013**, *418*, 50.
38. Talwar, D.; Williamson, C.; McLaughlin, A.; Gill, A.; O'Reilly, D. S.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2002**, *769*, 341.
39. Diniz, M. E. R.; Vilhena, L. S.; Paulo, B. P.; Barbosa, T. C. C.; Mateo, E. C.; *J. Braz. Chem. Soc.* **2015**, *26*, 1684.

Submitted: September 10, 2019
Published online: March 4, 2020

This is an open-access article distributed under the terms of the Creative Commons Attribution License.