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Simultaneous Quantitation of Plasma Catecholamines and Metanephrines by LC-MS/MS.

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ABSTRACT

The quantitation of the low levels of catecholamines and metanephrines in biological fluids is important for clinical screening of pheochromocytoma/paraganlioma and diagnosis of overtraining syndrome in athletes. We introduce a novel, accurate and sensitive liquid chromatography-tandem mass spectrometry method for simultaneous quantitation of these biogenic amines in human plasma. Simple protein precipitation combined with rapid liquid-liquid extraction with ethyl acetate and 2-aminoethyl diphenylborinate as the complexing reagent allow us to quantify catecholamines and metanephrines over broad concentration ranges. Target analytes and their respective deuterated internal standards were monitored in positive electrospray ionization mode by multiple reaction monitoring. Method performance was validated for linearity, lower limit of quantitation, limit of detection, intra-day and inter-assay precision and carry-over. The assay was linear within analytical range 25–1 000 pg/mL for epinephrine, 30–2 500 pg/mL for norepinephrine, 15–1 000 pg/mL for dopamine, 25–2 000 pg/mL for metanephrine and 50–10 000 pg/mL for normetanephrine, with lower limits of quantification of 15, 20, 10, 15 and 30 pg/mL, respectively The intra- and inter-day precisions for all compounds ranged from 0.6 to 2.4% and from 2.3 to 7.6%, respectively. The efficiency of novel method was confirmed by assaying external quality control samples and perfect results with consistency to the target ranges were obtained.

Keywords: Catecholamines, metanephrines, LC-MS/MS, plasma, overtraining, pheochromocytoma.

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INTRODUCTION

Catecholamines (dopamine, norepinephrine and epinephrine) are the class of chemical neurotransmitters and hormones which took 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 induced by catecholamines metabolism could be 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 importance as allowing more effectively eliminate or confirm the presence of hyperplastic process [6,7,8]. This test demonstrates high sensitivity and selectivity for compounds produced by the tumor. In the 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 work [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 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: 1) at rest – to compare with the normal physiological range; 2) after training specific for given sport discipline – to assess athlete's response to normal training inducements; 3) 24, 48 and 72 hours 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 self-cost, whereas HPLC-FLD-based techniques are negatively characterized by high limit of detection, time-consuming sample preparation and long-lasting analysis 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 [19,20,21,22]. High performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) has the greatest diagnostic accuracy for the determination of catecholamines [23,24,25] and metanephrines [26,27] in human biofluids. Currently there are few methods for the simultaneous determination of catecholamines and their 3-O-methylated metabolites developed only for human urine [28]. Quantitation of these biogenic amines in plasma within single run has a great potential for clinical diagnostics.

The main difficulty of their plasma quantitation is due to low reference values [29]. 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 the 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 [30,31,32], or the use of boric acid elution [33,34]. 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 [35]. The most common cleanup technique for plasma metanephrines is solid phase extraction on weak cation exchange resins (WCX) [36,37,38].

The aim of current work is to demonstrate effective LC–MS/MS approach for simultaneous plasma catecholamines and metanephrines quantitation 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

Epinephrine (E), norepinephrine (NE), dopamine (DA), metanephrine (MN), normetanephrine (NMN) and formic acid (FA) were purchased from Sigma-Aldrich (USA). Respective deuterated internal standards (IS) – E-d3, NE-d6, DA-d4, MN-d3 and NMN-d3 were obtained from Toronto Research Chemicals (Canada). Endocrine plasma normal and pathological range controls were obtained from ChromSystems GmbH (Germany). The LC-MS-grade acetonitrile (ACN) and LC-MS-grade methanol (MeOH) were supplied by Fisher Scientific (UK). HPLC-grade Ethyl acetate, 2-aminoethyl diphenylborinate (2-APB), ethylenediaminetetraacetic acid (EDTA), hydrochloric acid (37%) and ammonium chloride were obtained from Sigma-Aldrich (USA), ammonium hydroxide solution (25%) was purchased from Merck (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, and aliquots were stored at -70°C prior to use. Deionized water with specific electro conductivity 18.2 MOhm*cm^{-1} was prepared employing Millipore Integral 3 (France).

Preparation of Calibrators and Controls

Catecholamines, metanephrines and internal standards stock solutions at 1 mg/mL as well as calibrator solutions were prepared in 0.1M HCl. 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 25–1 000 pg/mL for E; 30–2 500 pg/mL for NE; 15–1 000 pg/mL for DA; 25–2 000 pg/mL for MN and 50–10 000 pg/mL for NMN. QC samples were prepared at two levels (low – QCL and high – QCH) – 75 and 850 pg/mL for E; 50 and 2 250 pg/mL for NE; 45 and 750 pg/mL for DA, 75 and 1 500 pg/mL for MN; 150 and 8 500 pg/mL for NMN. An IS working solution including E-d3 (1 ng/mL), NE-d6 (5 ng/mL), DA-d4 (10 ng/mL), MN-d3 (5 ng/mL) and NMN-d3 (10 ng/mL) was prepared in 0.1M HCl. All the solutions were stored at -20°C until analysis.

ChromSystems 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 and supernatant was transferred into clean tube. 30 µL of 5% ammonium hydroxide solution, 0.4 mL of 2-aminoethyl-diphenylborinate solution [39] and 1.5 mL of ethyl acetate were added and analytes were extracted by vigorously mechanically shaking for 10 minutes. The tube was then centrifuged (5 min at 3000 g) and 1.0 mL of organic layer was separated following evaporation to dryness under a flow of nitrogen at 35°C. Dry residue was reconstituted with 150 µL of mobile phase A and transferred into vial.

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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 0.7 ml/min. Injection volume was 20 µL.

Detection was performed on Shimadzu 8060 triple quadrupole mass spectrometer (Shimadzu, Japan) using positive electrospray ionization mode. Quantitative data were obtained by multiple reaction monitoring (MRM) scanning mode of the protonated precursor ion at the form $[M+H]^+$ or at the form $[M+H-H_2O]^+$. Two specific transitions were chosen for each analyte, one for confirmation (the "qual") and one for quantification (the "quan") 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 4 kV, CID gas was maintained at 17 kPa. Nebulizer gas was set at 3 L/min, drying and heating gas flow were kept 10 L/min. Temperature of interface, desolvation line and heat block were 300°C, 250°C and 400°C, respectively. The MRM acquisition settings summarized in Table 1.

Table 1. Optimized MRM parameters of catecholamines and metanephrines (CE – collision energy).

Compound	Precursor ion, *m/z*	Product ion, *m/z*	Q1 Pre Bias, V	CE, V	Q3 Pre Bias, V	Dwell time, ms
NE	151.9	77.1*	14.0	34.0	16.0	30
		107.1	16.0	18.9	19.0	
NE-d_6	158.0	111.0	16.0	18.9	19.0	30
E	183.9	166.2*	16.0	13.1	15.0	30
		107.0	16.0	23.7	10.0	
E-d_3	187.0	107.0	16.0	23.7	10.0	30
DA	153.9	137.2*	14.0	14.7	12.0	30
		91.1	15.0	26.0	17.0	
DA-d_4	157.0	94.0	15.0	26.0	17.0	30
NMN	165.9	134.2*	15.0	12.8	12.0	30
		121.2	23.0	17.9	23.0	
NMN-d_3	169.0	137.0	15.0	12.8	12.0	30
MN	179.9	148.2*	19.0	20.5	14.0	30
		165.2	17.0	19.2	10.0	
MN-d_3	183.0	168.0	17.0	19.0	10.0	30

* Quantification transition

Assay Validation

The method performance was evaluated by means of linearity, lower limit of quantitation (LLOQ), limit of detection (LOD), intra- and inter-assay precision and carry-over. 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 sequence: 1) upper calibration level extract in six replicates; 2) blank sample extract; 3) lower calibration level extract. Carry-over expressed as accuracy should be within ± 20% of expected concentration in the lower calibration level sample.

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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-labelled 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 standards with molecular masses of more than 3 a.m.u. than those for analyzed compounds to eliminate the possible effect of natural isotopic ions of the target analytes on the intensity of the precursor-ions of the internal standards, with a subsequent underestimation of the true values.

Chromatographic separation of catecholamines and metanephrines was achieved on Zorbax Eclipse XDB-C18 column. The separation of E and NMN is especially critical since these compounds share common precursor-ions. Without proper chromatographic separation, fragmentation of these compounds can cause interferences with one another and lead to inaccurate quantitation. We have achieved effective separation of compounds within 6 min. Retention times and MRM chromatograms 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 [40]. Previously it has been reported [39] 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 [39] except for adding plasma protein precipitation stage and using lower biomaterial volume.

Good linearity was achieved using a $1/x^2$ weighted quadratic regression for all compounds. All calibration curves had r^2 values of 0.99 or greater. The assay was linear within analytical range 25–1 000 pg/mL for E, 30–2 500 pg/mL for NE, 15–1 000 pg/mL for DA, 25–2 000 pg/mL for MN and 50–10 000 pg/mL for NMN. LLOQs for E, NE, DA, NM and NMN were determined to be 15, 20, 10, 15 and 30 pg/mL, respectively, which were sufficient for accurate measurement of all analytes in original 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 for E, NE and MN, 5 pg/mL for DA and 20 pg/mL for NMN. The LOD, LLOQ and the linearity parameters are presented in Table 2. Table 3 summarized the intra- and inter-day precisions by analyzing two levels of QC samples. Carry-over was found insignificant as accuracy (RE) for E, NE, DA, MN and NMN was 89.6, 107.2, 86.5, 83.4 and 112.6%, respectively.

Table 2. Parameters for linearity range, LOD and LLOQ for catecholamines and metanephrines.

Compound	R^2	Equation of the curve	Linear range, pg/mL	LLOQ, pg/mL	LOD, pg/mL
E	0.9980	$Y = (2.221e^{-8})X^2 + (1.074e^{-4})X$	25–1 000	15	10
NE	0.9994	$Y = (6.771e^{-9})X^2 + (2.378e^{-4})X$	30–2 500	20	10
DA	0.9969	$Y = (2.037e^{-7})X^2 + (6.385e^{-4})X$	15–1 000	10	5
MN	0.9975	$Y = (-1.220e^{-8})X^2 + (3.927e^{-4})X$	25–2 000	15	10
NMN	0.9982	$Y = (-4.286e^{-9})X^2 + (4.923e^{-4})X$	50–10 000	30	20

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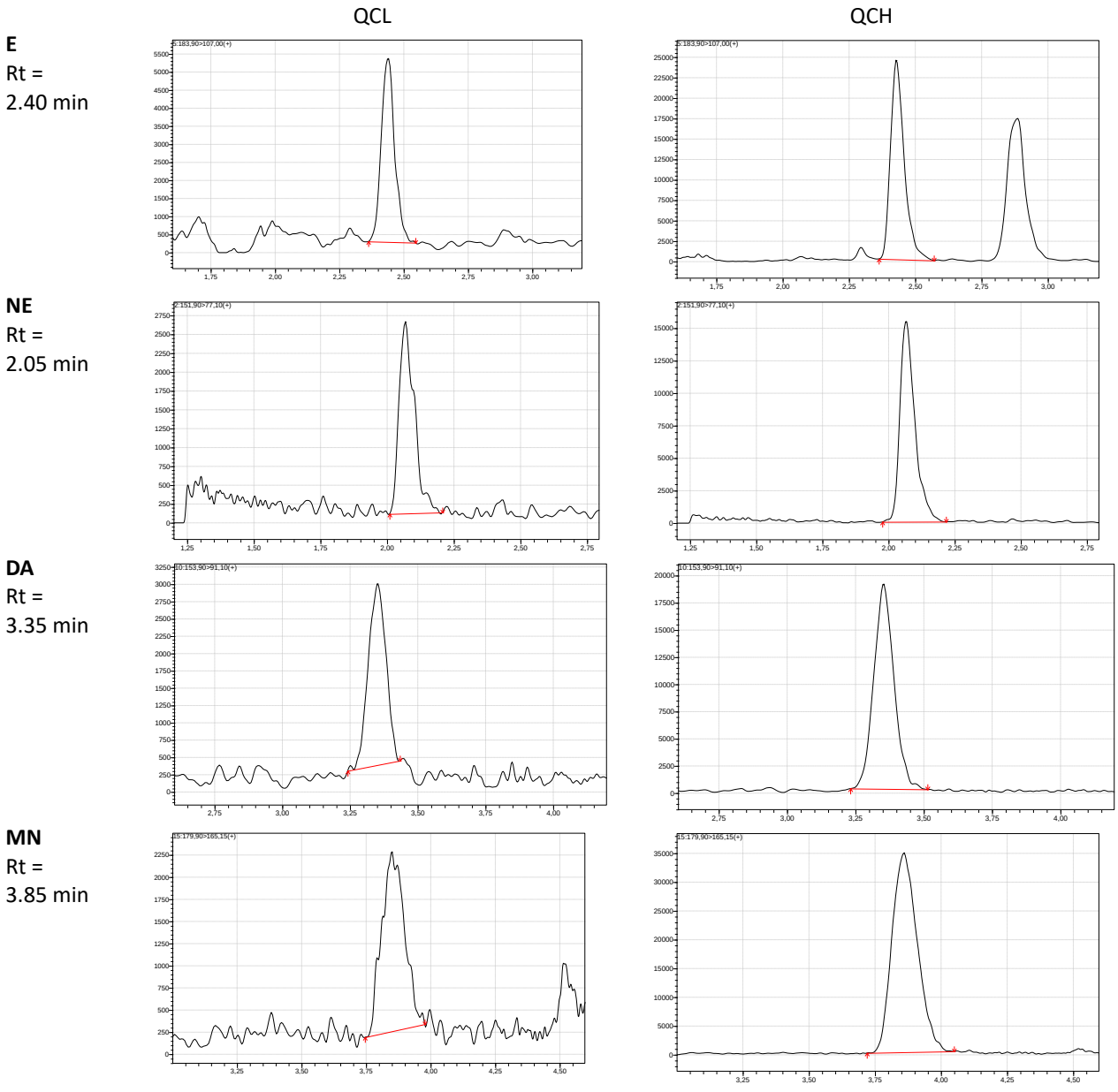
Table 3. Summary of intra- and inter-day precisions.

Compound	Nominal concentration, pg/mL	Intra-day precision (n=6)		Inter-day precision (n=18)	
		RE, %	RSD, %	RE, %	RSD, %
E	50	98.5	2.4	98.1	3.2
	850	92.9	1.3	98.6	4.5
NE	50	90.0	2.2	92.2	5.5
	2500	97.5	0.6	99.2	3.1
DA	45	107.5	0.7	105.8	3.4
	750	94.1	0.6	99.2	4.1
MN	75	98.4	6.6	99.0	7.6
	1500	96.8	2.1	97.8	2.5
NMN	150	98.0	0.9	97.4	3.2
	8500	99.3	1.9	100.0	2.3



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NMN
Rt =
2.90 min

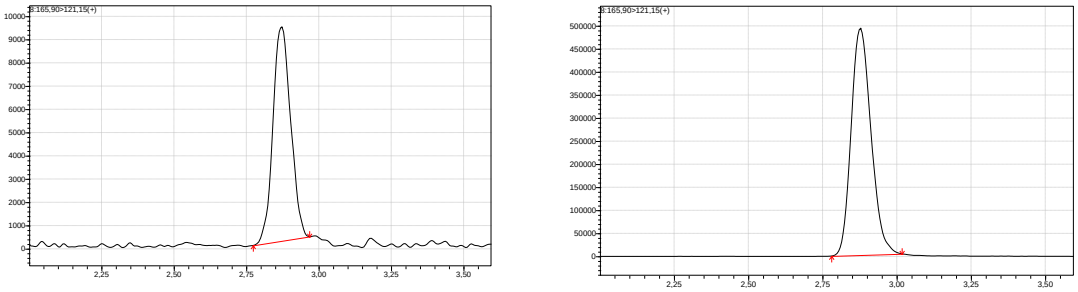

Figure 1. Typical MRM chromatograms and retention times of target analytes in QC samples extracts (concentrations are presented in Table 3).

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) ranged 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.

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

Compound	Endocrine plasma controls					
	Normal range (n=15)			Pathological range (n=15)		
	Measured, pg/mL	Target / Range (HPLC-ED)	RSD, %	Measured, pg/mL	Target / Range (HPLC-ED)	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

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

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REFERENCES

[1] Eisenhofer G, Kopin IJ., Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacological Reviews. 2004;56(3):331–349.

[2] Kopin IJ. Catecholamine metabolism: basic aspects and clinical significance. Pharmacological Reviews. 1985;37(4):333–364.

[3] Eisenhofer G, Keiser H, Friberg P, Mezey E, Huynh TT, Hiremagalur B, Ellingson T, Duddempudi S, Eijsbouts A, Lenders JW. Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors. The Journal of Clinical Endocrinology and Metabolism. 1998;83(6):2175–2185.

[4] Lenders JW, Keiser HR, Goldstein DS, Willemsen JJ, Friberg P, Jacobs MC, Kloppenborg PW, Thien T, Eisenhofer G. Plasma metanephrines in the diagnosis of pheochromocytoma. Annals of Internal Medicine. 1995;123(2):101–109.

[5] Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G. Biochemical diagnosis of pheochromocytoma: which test is best? JAMA. 2002;287(11):1427–1434.

[6] Václavík J, Stejskal D, Lacnák B, Lazárová M, Jedelský L, Kadalová L, Janosová M, Frysák Z, Vlcek P. Free plasma metanephrines as a screening test for pheochromocytoma in low-risk patients. Journal of Hypertension. 2007;25(7):1427–1431.

[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 HJLM., Sinnott R, Beuschlein F, Fassnacht M, Januszewicz A, Lenders JWM. Biochemical diagnosis of chromaffin cell tumors in patients at high and low risk of disease: plasma versus urinary free or deconjugated O-methylated catecholamine metabolites. Clinical Chemistry. 2018;64(11):1646–1656.

[8] Hickman PE, Leong M, Chang J, Wilson SR, McWhinney B. Plasma free metanephrines are superior to urine and plasma catecholamines and urine catecholamine metabolites for the investigation of phaeochromocytoma. Pathology. 2009;41(2):173–177.

[9] Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Phaeochromocytoma. The Lancet. 2005;366(9486):665–675.

[10] Carter JR, Ray CA. Sympathetic neural adaptations to exercise training in humans. Autonomic Neuroscience. 2015;188:36–43.

[11] Hooper SL, Mackinnon LT, Gordon RD, Bachmann AW. Hormonal responses of elite swimmers to overtraining. Medicine and Science in Sports and Exercise. 1993;25(6):741–747.

[12] Lehmann M, Baumgartl P, Wiesenack C, Seidel A, Baumann H, Fischer S, Spori U, Gendrisch G, Kaminski R, Keul J. Training-overtraining: influence of a defined increase in training volume vs training intensity on performance, catecholamines and some metabolic parameters in experienced middle- and long-distance runners. European Journal of Applied Physiology and Occupational Physiology. 1992;64(2):169–177.

[13] Schöfl C, Becker C, Prank K, von zur Mühlen A, Brabant G. Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men. European Journal of Endocrinology. 1997;137(6):675–683.

[14] Lehmann M, Lormes W, Optiz-Gress A, Steinacker JM, Netzer N, Foster C, Gastmann U. Training and overtraining: an overview and experimental results in endurance sports. The Journal of Sports Medicine and Physical Fitness. 1997;37(1):7–17.

[15] Petibois C, Cazorla G, Poortmans JR, Déléris G. Biochemical aspects of overtraining in endurance sports: a review. Sports Medicine. 2002;32(13):867–878.

[16] Hansen AM, Garde AH, Skovgaard LT, Christensen JM. Seasonal and biological variation of urinary epinephrine, norepinephrine, and cortisol in healthy women. Clinica Chimica Acta. 2001;309(1):25–35.

[17] Kruse HJ, Wieczorek I, Hecker H, Creutzig A, Schellong SM. Seasonal variation of endothelin-1, angiotensin II, and plasma catecholamines and their relation to outside temperature. The Journal of Laboratory and Clinical Medicine. 2002;140(4):236–241.

[18] Tsunoda M. Recent advances in methods for the analysis of catecholamines and their metabolites. Analytical and Bioanalytical Chemistry. 2006;386(3):506–514.

[19] Ketha SS, Singh RJ, Ketha H. Role of mass spectrometry in clinical endocrinology. Endocrinology and Metabolism Clinics of North America. 2017;46(3):593–613.

[20] Keevil BG. LC-MS/MS analysis of steroids in the clinical laboratory. Clinical Biochemistry. 2016;49(13–14):989–997.

[21] van den Ouweland JMW, Kema, IP. The role of liquid chromatography–tandem mass spectrometry in the clinical laboratory. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2012;883–884:18–32.
[22] Jannetto PJ, Fitzgerald RL. Effective use of mass spectrometry in the clinical laboratory. Clinical Chemistry. 2016;62(1):92–98.
[23] Ji CJ, Walton J, Su Y, Tella M. Simultaneous determination of plasma epinephrine and norepinephrine using an integrated strategy of a fully automated protein precipitation technique, reductive ethylation labeling and UPLC–MS/MS. Analytica Chimica Acta. 2010;670(1–2):84–91.
[24] Kushnir MM, Urry FM, Frank EL, Roberts WL, Shushan B. Analysis of catecholamines in urine by positive-ion electrospray tandem mass spectrometry. Clinical Chemistry. 2002;48(2):323–331.
[25] Shen Y, Cheng L, Guan Q, Li H, Lu J, Wang X. Development and validation of a liquid chromatography tandem mass spectrometry method for the measurement of urinary catecholamines in diagnosis of pheochromocytoma. Biomedical Chromatography. 2017;31(11):e4003.
[26] Petteys BJ, Graham KS, Parnás ML, Holt C, Frank EL. Performance characteristics of an LC-MS/MS method for the determination of plasma metanephrines. Clinica Chimica Acta. 2012;413(19–20):1459–1465.
[27] Clark ZD, Frank EL. Urinary metanephrines by liquid chromatography tandem mass spectrometry: using multiple quantification methods to minimize interferences in a high throughput method. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2011;879(31):3673–3680.
[28] [28] Peitzsch M, Pelzel D, Glöckner S, Prejbisz A, Fassnacht M, Eisenhofer G. Simultaneous liquid chromatography tandem mass spectrometric determination of urinary free metanephrines and catecholamines, with comparisons of free and deconjugated metabolites. Clinica Chimica Acta. 2013;418:50–58.
[29] Burtis CA, Ashwood ER, Bruns D.E. eds. Tietz textbook of clinical chemistry, 6th ed, Philadelphia, WB Saunders, 2008, p. 952.
[30] Anton AH, Sayre DF. A study of factors affecting the aluminium oxide-trihydroxyindol procedure for analysis of catecholamines. The Journal of Pharmacology and Experimental Therapeutics. 1962;138(3):360–375.
[31] Hjemdahl P. Interlaboratory comparison of plasma catecholamine determination using several different methods. Acta Physiologica Scandinavica. Supplementum. 1984;527:43–54.
[32] Holly JMP, Makin HJL. The estimation of catecholamines in human plasma. Analytical Biochemistry. 1983;128(2):257–274.
[33] Hansen C, Agrup G, Rorsman H, Rosengren AM, Rosengren E. Chromatographic separation of catecholic amino acids and catecholamines on immobilised phenylboronic acid. Journal of Chromatography. A. 1978;161:352–365.
[34] Smedes F, Kraak JC, Poppe H. Simple and fast solvent extraction system for selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine. Journal of Chromatography. 1982;231(1):25–39.
[35] Macdonald IA, Lake DM. An improved technique for extracting catecholamines from body fluids. Journal of Neuroscience Methods. 1985;13(3–4):239–348.
[36] Petteys BJ, Graham KS, Parnás ML, Holt C, Frank EL. Performance characteristics of an LC–MS/MS method for the determination of plasma metanephrines. Clinica Chimica Acta. 2012;413(19–20):1459–1465.
[37] Peitzsch M, Prejbisz A, Kroiss M, Beuschlein F, Arlt W, Januszewicz A, Siegert G, Eisenhofer G. Analysis of plasma 3-methoxytyramine, normetanephrine and metanephrine by ultraperformance liquid chromatographytandem mass spectrometry: utility for diagnosis of dopamine-producing metastatic phaeochromocytoma. Annals of Clinical Biochemistry. 2013;50(2):147–155.
[38] Wright MJ, Thomas RL, Stanford PE, Horvath AR. Multiple reaction monitoring with multistage fragmentation (MRM^3) detection enhances selectivity for LC-MS/MS analysis of plasma free metanephrines. Clinical Chemistry. 2015;61(3):505–513.
[39] Diniz MER, Vilhena LS, Paulo BP, Barbosa TCC, Mateo EC. Simultaneous determination of catecholamines and metanephrines in urine by liquid chromatography electrospray ionization tandem mass spectrometry: successful clinical application. Journal of the Brazilian Chemical Society. 2015;26(8):1684–1691.
[40] Talwar D, Williamson C, McLaughlin A, Gill A, O'Reilly, DSTJ. Extraction and separation of urinary catecholamines as their diphenyl boronate complexes using C18 solid-phase extraction sorbent and

high-performance liquid chromatography. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2002;769(2):341–349.