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ORIGINAL ARTICLE

A Rapid, Precise, and Sensitive LC-MS/MS Method for the Quantitative Determination of Urinary Dopamine Levels *via* a Simple Liquid-liquid Extraction Technique

Basit Sıvı-sıvı Ekstraksiyon Tekniği ile İdrar Dopamin Düzeylerinin Kantitatif Tayini için Hızlı, Kesin ve Hassas Bir LC-MS/MS Yöntemi

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ABSTRACT

Objectives: Dopamine (DA) is a prominent biochemically complex neurotransmitter and immunomodulator. The quantification of DA could contribute to a better understanding of how endocrine system, cardiovascular and renal functions are regulated. The study aims to develop a rapid, precise, and extremely sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for routine clinical quantification of DA in urine.

Materials and Methods: Urine samples were extracted *via* one simple and rapid liquid-liquid extraction technique; then analyzed using a sensitive LC-MS/MS method developed by multiple reaction monitoring mode.

Results: DA and internal standard (IS) retention durations were found to be 2.28 min and 2.24 min, respectively. The mean extraction recovery of DA and DA-IS in urine was above 95.62%. DA calibration curve in urine was linear (r^2≥0.998) ranging from 20 ng/mL to 1000 ng/mL. The maximum intra-day and inter-day precisions were 5.87 and 2.81, respectively and coefficients of variation were 10.55% and 7.57%, respectively.

Conclusion: A rapid, precise, sensitive and quantitative LC-MS/MS detection of DA without the use of derivatization, evaporation, reconstitution and ion-pairing reagents has been developed with a simple and non-invasive sample technique for clinical laboratory applications, basic neuroscience research and drug development studies.

Key words: Dopamine, LC-MS/MS, urine, method validation

ÖZ

Amaç: Dopamin (DA), biyokimyasal olarak kompleks önemli bir nörotransmitter ve immünomodülatördür. DA'nın kantitatif tespiti, endokrin sistem, kardiyovasküler ve renal fonksiyonların düzenlenmesinin daha iyi anlaşılmasını sağlar. Bu çalışmanın amacı, idrarda DA'nın rutin klinik kantitasyonu için hızlı, kesin ve son derece hassas olan sıvı kromatografisi-tandem kütle spektrometresi (LC-MS/MS) yöntemi geliştirmektir.

Gereç ve Yöntemler: İdrar numuneleri, basit ve hızlı bir sıvı-sıvı ekstraksiyon tekniği ve ardından çoklu reaksiyon izleme modu kullanılarak geliştirilen hassas bir LC-MS/MS yöntemi ile hazırlanmıştır.

Bulgular: DA ve internal standardın alıkonma zamanları sırasıyla 2,28 ve 2,24 dakika olarak bulunmuştur. İdrarda DA ve DA-IS'nin ortalama ekstraksiyon geri kazanımı %95,62'nin üzerinde belirlenmiştir. İdrardaki DA kalibrasyon eğrisi, 20 ila 1000 ng/mL arasında doğrusaldır (r^2≥0,998). Maksimum gün içi ve günler arası kesinlik sırasıyla 5,87 ve 2,81 ve varyasyon katsayıları sırasıyla %10,55 ve %7,57'dir.

Sonuç: Türevlendirme, buharlaştırma, yeniden yapılandırma veya iyon eşleştirme reaktifleri kullanılmadan, DA'nın hızlı, hassas ve kantitatif LC-MS/MS yöntemi ile tespiti, invazif olmayan numune ve temel nörobilim ve ilaç geliştirme çalışmaları gibi klinik laboratuvar uygulamaları için kolay hazırlama tekniği ile geliştirilmiştir.

Anahtar kelimeler: Dopamin, LC-MS/MS, idrar, yöntem validasyonu

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INTRODUCTION

Dopamine (DA) is a basic chemical neurotransmitter with many neurological functions, particularly in the brain (Figure 1). The reward response is intricately linked to DA pathways, otherwise known as dopaminergic pathways.[1] Dopaminergic pathways are commonly activated in response to or in anticipation of reward. DA is assumed to significantly influence motivation and reward-associated satisfaction.[2] The relationship between dopaminergic signaling and reward response has considerable crosstalk, if not a direct influence, on dopaminergic signaling in a vast range of psychotropic pharmaceuticals and illicit drug substances. Therefore, dopaminergic system abnormalities may cause a wide variety of neurological disorders. The neurological and non-neurological syntheses of DA are considered to be largely independent of each other because it is a poor penetrator of the blood-brain barrier.[3] The majority of blood DA is considered to be synthesized in the mesentery or obtained from food digestion, with about 95% of it circulating as the biologically inactive DA sulfate.[4] Thus, unconjugated "(free)" DA is primarily responsible for the biological activity of blood DA. Such activities include vasodilation and noradrenaline release inhibition. There are indications, however not well illuminated, that DA may be released into the bloodstream in response to hypoxic conditions.[5] The immune system is also responsive to DA, with lymphocytes being the most affected. Some of these cells may synthesize and release DA themselves. Although the function of DA in these cells is unclear, it is considered to be an integral component of immunogenetics through lymphocyte activation modulation.[6,7] The possibility for interactions between the nervous and immune systems through DA has been touted as a potential route of interaction between the two systems, with malfunctions of this interaction linked to autoimmune disorders.[7,8]

In the detection and continued monitoring of relatively recent xenobiotic exposure, urine retains its position as the primary matrix of choice owing to several advantages, including its relatively wide temporal envelope of detection, which can last for several days, an increased xenobiotic concentration due to its nature as a concentrating waste carrier, and the ease and non-invasiveness of sample procurement; the patient urinating into a sterile container. Using the aforementioned non-invasive techniques, urine can be used to detect DA in the diagnosis and continued surveillance of several diseases, including stress-induced diseases and sympathoadrenal system dysfunction. Urine is chemically simpler than comparable detection media, obviating the need for tedious, effort-intensive, and complicated preparatory steps. Thus, the sample pretreatment was as simple as dilution in a micellar solution, followed by filtration, and then direct injection.

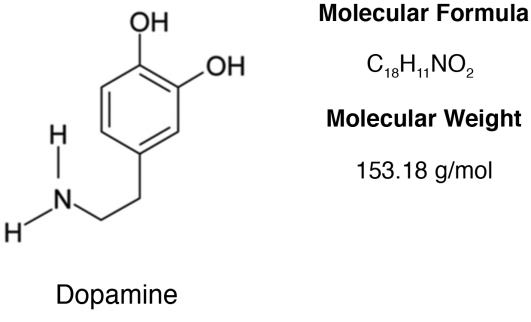

Figure 1. Chemical structure of dopamine

There are many published studies on the analysis of DA and associated species in biological fluids. This creates a landscape containing a variety of methods, including spectrophotometry,[9,10] liquid chromatography (LC)-fluorometry,[11,12] enzyme immunoassays, and LC-electrochemical detection for detecting and quantifying DA.[13-16] LC- tandem mass spectrometry (MS)-based methods are considered the frontrunner because tandem LC-MS methodologies can provide increased selectivity while relying on chromatographic separation only minimally.[17-26] While these methods provide a good combination of selectivity, sensitivity, and ease of use, they are ill-equipped to provide the same quality of output for multitarget analysis, which forms the basis of the present study, thus necessitating the development of novel methods. The simultaneous generation of optimal results for all relevant analytes necessitates substantial method modification and optimization. As a result of these modifications, the method discussed herein has achieved optimal quality in terms of sensitivity, selectivity, and robustness as it applies to DA detection.[27]

The simplicity, robustness, sensitivity, and specificity aspects were the major consideration criteria for this study, with the main objective being to develop an LC-MS/MS method that incorporates and meets all of these conditions. A cost-effective solution was also developed, with a simplified and highly effective liquid-liquid extraction step using a small sample volume, which will be invaluable for routine clinical testing.

Despite its superiority in both selectivity and specificity over other alternatives, an LC-MS/MS platform has not been extensively developed in the past to the degree discussed herein, to the best of the authors' knowledge.[13,14] Thus, this study also serves as a testbed for demonstrating and establishing the method discussed herein in terms of applicability and reproducibility to a larger sample population, as well as the scarcely-discussed clinical applications.[28]

MATERIALS AND METHODS

Chemicals and materials

Analytical grade chemicals were used in all steps of the process. Artificial urine, DA, and creatinine were procured from Sigma-Aldrich (St. Louis, MO, USA). $^{13}C_{12}$-DA (99%) and creatinine-d3 standards (both isotopically labeled) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). High performance liquid chromatography (HPLC)-grade methanol, formic acid, and hydrochloric acid fuming 37% were obtained from Merck (KGaA, Darmstadt, Germany). All aqueous solutions were made with deionized water (18.2 MΩ) treated with a Millipore (Simplicity, 185) Milli-Q water purification system (Elga Labwater Veolia, Anthony, France).

Preparation of calibration standards and quality control (QC) samples

Internal standard (IS) solution

To begin, a dilution solution (DS) was prepared using 50 mL of 1N hydrochloric acid and 1 L of water. After that, a stock solution (500 µg/L) was prepared by diluting 5 mg of DA-*d4* IS with the DS. Next, 0.1 Ml of stock solution was diluted with 10 Ml of the DS for a dilute stock solution (5 ng/mL). Finally, a 200 µL of dilute stock solution (100 ng/mL) diluted with 10 Ml of the DS was employed as an IS solution to be used in the analyses.

Standard solutions

50 mg of DA was diluted with 10 mL DS (4000 ng/mL). To prepare the stock standard solution (100 ng/mL), 200 µL of 5000 ng/mL solution was diluted with 10 Ml of the DS. For the intermediate standard stock solution (2000 µg/L), 100 µL of 100 ng/mL solution was diluted with 10 mL of the DS. Preparation of stock standard solution levels are presented in Figure 2.

Sample pretreatment

The sample was vortex for 3-4 minutes after mixing 200 µL of the IS solution (100 µg/L), 800 µL of the DS, and 100 µL of the artificial urine sample.

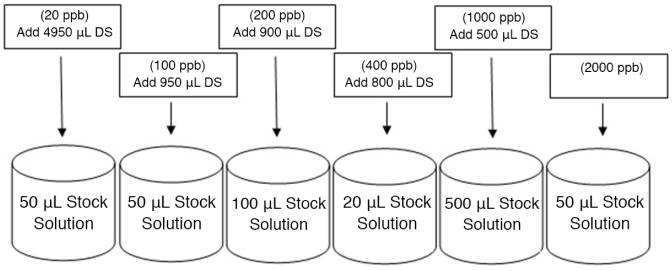

Figure 2. Preparation for stock standard solution levels

LC-MS/MS conditions

Agilent 1200 Series 6460 triple quadrupole mass spectrometry with Jet-Stream atmospheric pressure electrospray ionization source and MassHunter data acquisition/Quantitation software (Santa Clara, USA) were used in the LC system. Chromatographic separation was performed on a Zorbax SB-C18 3.0x50 mm 3.5-micron 600 BAL. The mobile phases comprised 50% formic acid aqueous and methanol (HPLC gradient grade). The flow rate was set to 5 mL/min. The injection volume was set at 40 µL. MS was conducted on an Agilent triple quadrupole mass spectrometer operated in selected reaction monitoring (SRM) mode. The parameters for chromatographic conditions were set up as follows: Capillary voltage: P (1750 V) N (3000V); desolvation gas: 325 L/h; desolvation gas temperature: 375˚C; cone gas: 12 L/min; Nebulizer: 40 psi; Nozzle voltage: 0-0; Chamber current: 0.24 µA; LC stop time: 5.50 min; SRM transitions were monitored at m/z 158.10→141.10 for DA IS (positive) and at m/z 154.0→137.00 for DA (positive). For each analyte, dwell time was set at 150 *ms*.

Method validation

The validation of the LC-MS/MS method was based on the Food and Drug Administration Guidance for Industry on bioanalytical method validation[27] (selectivity, carryover, linearity, lower limit of detection (LLOD) and lower limit of quantification (LLOQ), accuracy, precision, matrix effect, extraction recovery, and stability). Artificial urine was used throughout the validation process owing to a scarcity of genuine blank urine samples without all targeted analytes.

Preparation of calibration curve

The linearity of the DA method was quantified with a calibration curve constructed in the range of 20-2000 ng/mL (Figure 3), which included the LLOQ. The acceptance criterium for recalculated standard concentrations was not more than 15% of the nominal values and 20% in the LLOQ. Each validation run

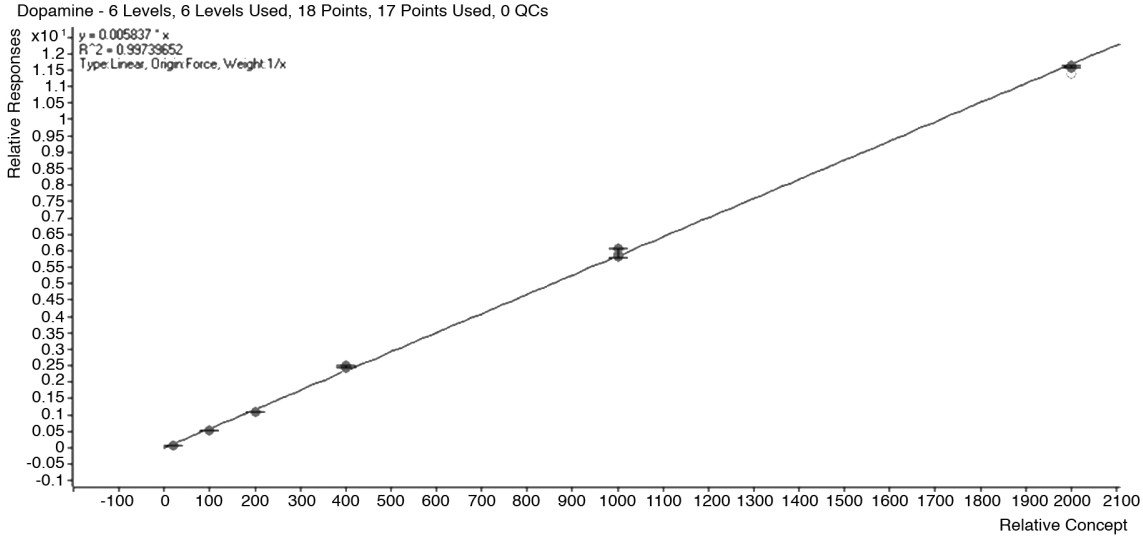

Figure 3. Calibration curve of dopamine

comprised QC samples at three concentrations (n=6, at each concentration). Such validation studies should be completed in three consecutive days.

Accuracy and precision

The accuracy and precision studies should be evaluated and reported as intra-day/within-run accuracy and between-run accuracy with a single injection. Intra-day and inter-day accuracy and precision should be determined using quality control samples at each level of at least five samples and at least four different concentration levels of DA at low-quality control (LQC: 200 ng/mL), medium-quality control (MQC: 400 ng/mL), and high-quality control (HQC: 100 ng/mL) samples, including LLOQ (100 ng/mL). The mean concentration value did not exceed 15%, except at LLOQ of the nominal concentration (20%), and the coefficient of variation (CV %) values was <15% above the calibration range.

Selectivity

The analysis matrix was examined as 6 different lots, the CV % did not exceed 20% of the LLOQ in terms of the substance to be analyzed, and the IS was not affected as it did not exceed 5% of the IS response.

Recovery

By equating the peak area of each analyte, the recovery of DA, six concentration levels, and IS in artificial urine was determined

Stability

The stability of DA was determined using all six replicate samples (LLQC, LQC, MQC, and HQC) stored at +4˚C for one week. The acceptance stability criterion did not exceed 15% of the nominal concentration.

Matrix effect

The matrix effect for DA and its IS is the ratio of the response of the metabolite to be analyzed, added at certain concentrations to six independent blank matrices, to the response of the pure standard solution of the same concentration in the analysis after extraction. The CV % of the matrix factors obtained for 6 different matrices did not exceed 15%.

Statistical analysis

The data were processed using "SPSS v.22". For accuracy, precision, stability, and matrix effect, the results were calculated as mean ± standard deviation (SD), and the relative SD (CV %). The coefficient of regression was also calculated for the linearity parameter.

RESULTS

Selectivity and optimization of chromatographic conditions

To obtain and evaluate the selectivity of the method, six different artificial urine matrices were employed and tested. Subsequently, the interference at the analyte and the IS retention times were also quantified. In the aforementioned chromatographic parameters, DA and IS separation from the artificial urine were both found to be adequate, with retention times of ~2.09 min and ~1.08 min, respectively (Figure 4). The retention time corresponding to DA and the IS showed no significant interference peaks (Figure 5). The results showed that the method developed in this study is highly specific and selective for DA in urine samples.

Linearity, LOQ, and LOD

For DA, the method was validated over the nominal concentration range of 20 ng/mL-2000 ng/mL (Figure 3). For the batch, the correlation coefficient (r) value was 0.998 and the equation was y=0.005938* x-0.053491. With adequate sensitivity and accuracy, LOQ and LLOD were chosen as a 100 µg/L (level 2) subcalibration point for DA. The LOQ and LOD of DA were 1.215 ng/mL and 0.36 ng/mL, respectively.

Precision and accuracy

The maximum values of intra-day and inter-day precisions (RSD %) were 5.87% and 2.81%, respectively (Table 1). In addition, the intra-day and inter-day CV % maximum levels were 10.55% to 7.57% (Table 2) respectively, indicating that this method was accurate and precise for DA quantification in urine.

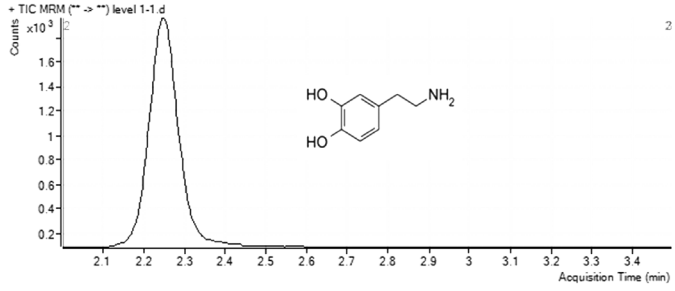

Figure 4. Representative chromatogram of dopamine in urine

Table 1. The intra-day and inter-day precision values of the dopamine in artificial urine (n=5)

		Intra-day precision		Inter-day precision	
	Nominal concentrations (ng/mL)	Concentration found (mean ± SD, ng/mL)	*RSD %	Concentration found (mean ± SD, ng/mL)	*RSD %
LLQC	100	96.45±2.92	3.03	106.519±2.42	2.27
LQC	200	196.29±11.53	5.87	216.943±5.01	2.31
MQC	400	403.63±14.04	3.47	440.063±10.61	2.41
HQC	1000	954.36±42.89	4.48	1058.594±29.75	2.81

*RSD %: The intra-day and inter-day precisions, SD: Standard deviation, LQC: Low-quality control, MQC: Medium-quality control, HQC: High-quality control

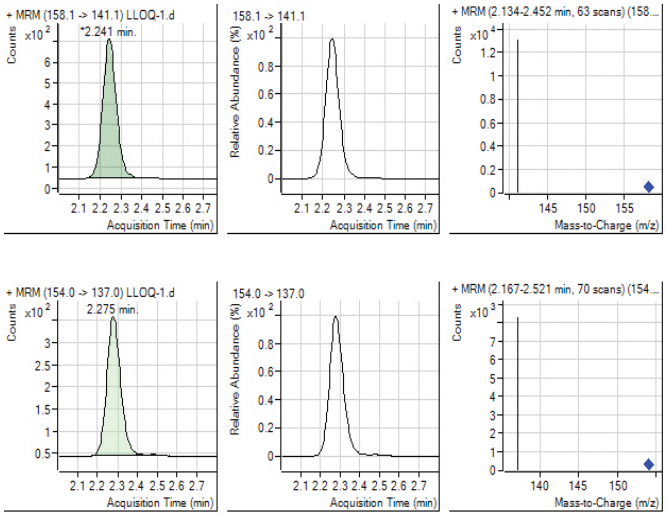

Figure 5. Dopamine and the internal standard peaks

Extraction recovery and matrix effects

The extraction recovery results were listed in Table 3 and found to be within the range of 95.622-106.147%.

Matrix effects of DA are shown in Table 4. The matrix effect values obtained were matrix 1 (CV %, 1.5%), matrix 2 (CV %, 5.5%), matrix 3 (CV %, 2.7%), matrix 4 (CV %, 7.2%), matrix 5 (CV %, 4.3%), and matrix 6 (CV %, 14.3%). It has been determined that there was no effect of urinary matrix variability in the presence of IS in DA quantification and the CV% value was less than the acceptance criterion for matrix effect (15%) as shown in Table 4. DA and IS did not show a matrix effect in urine.

Stability

Six replicates of DA samples were measured for stability using freeze-thaw cycles (frozen 7 days at -20˚C). The CV % did not exceed 15%, proving the freeze–thaw stability of DA in artificial urine (Table 5).

Table 2. The intra-day and inter-day accuracy values of the dopamine in artificial urine (n=5)

		Intra-day		Inter-day	
	Nominal concentrations (ng/mL)	Concentration found (mean ± SD, ng/mL)	*CV %	Concentration found (mean ± SD, ng/mL)	*CV %
LLQC	100	91.42±2.69	8.58	106.39±3.09	6.38
LQC	200	186.14±5.18	6.92	215.16±4.76	7.57
MQC	400	379.10±5.81	5.22	427.51±2.57	6.87
HQC	1000	894.49±19.10	10.55	1064.594±18.10	6.40

*CV %: Accuracy, coefficient of variation, SD: Standard deviation, LQC: Low-quality control, MQC: Medium-quality control, HQC: High-quality control

Table 3. Extraction recoveries of spiked artificial urine samples

Spiked urine standards (n=6)	*Extraction recoveries			Recoveries (mean ± SD)	CV %
1	101.38	98.39	103.83	101.20±2.22	2.17
2	96.84	95.24	94.79	95.62±0.87	0.90
3	97.91	96.91	97.37	97.39±0.40	0.40
4	105.54	105.13	107.78	106.15±1.16	1.09
5	99.99	103.29	99.11	100.80±1.79	1.77
6	97.94	96.66	98.57	97.73±0.79	0,80

*Extraction recovery (%): [(Response of extracted sample/response of post extracted spiked sample) x 100]. CV: Coefficient of variation, SD: Standard deviation

Table 4. The matrix effect of dopamine

Number of spiked samples	Matrix response results (n=6, %)	Correlation coefficient %
Matrix-1	107.00	5.63
Matrix-2	101.80	
Matrix-3	106.46	
Matrix-4	103.94	
Matrix-5	98.15	
Matrix-6	115.69	

Table 5. Stability of dopamine in artificial urine

	Nominal concentration (ug/L)	Concentration found (mean ± SD, ng/mL)	Correlation coefficient %
LLQC	100	88.05±5.51	7.01
LQC	200	188.13±5.89	3.51
MQC	400	395.86±4.87	1.38
HQC	500	987.40±17.76	2.01

SD: Standard deviation, LQC: Low-quality control, MQC: Medium-quality control, HQC: High-quality control

DISCUSSION

With further research clarifying the specific role of DA in the development and maintenance of these systems, urine DA testing could become a mainstay regular test in clinical settings, in addition to neurological analyses. Such testing may also provide unique insight into the biochemical processes behind the disorders in such systems, providing for a better understanding of how such systems develop, maintain, and sustain themselves, as well as their pathological situations.

By measuring DA concentration in other bodily fluids, such as the cerebrospinal fluid (for which this study will be of pioneering significance in terms of method development), neurological disorders related to DA biosynthesis or utilization can also be characterized. Theoretically, such novel techniques may allow for a more effective evaluation of illnesses treatment, as well as early-stage diagnosis of these illnesses.

The method achieved functional sensitivity requirements and quantified analytes over a wide dynamic range. Precise quantification of DA concentrations in urine could help researchers better understand the pathophysiology and pathogenesis of many neuropsychiatric disorders [e.g., drug addiction, schizophrenia, Parkinson's and Alzheimer's disease, and attention deficit hyperactivity/hyperkinetic disorder (ADHD)] and pharmaceutical research on novel drugs. The method developed in this study demonstrates its viability for determining DA levels in urine. The complexity of methodology is greatly reduced by simple precipitation and dilution directly leading to direct injection without intervening steps, a feat that can be considered the primary strength of this procedure, giving it an advantage over its alternatives. Previously published methodologies all have long and tedious sample preparation and derivatization steps, making them unsuitable for high-throughput applications (for example; a busy clinical laboratory serving many patients at the same time). These drawbacks are eliminated in the method described herein, with its simple and straightforward sample preparation and a short-duration chromatographic run, which makes it more applicable and desirable in such high-volume operations (Table 6).

In terms of its speed, reliability, quantitative potency, and cost-effectiveness, the method developed and validated in this study is an improvement over the microextraction methodology developed and employed by El-Beqqali et al.[19] Such advantages are particularly prevalent in a clinical setting, where fast and reliable detection of low DA levels in biological matrices may be crucial.[28] Moriarty et al.[8] developed an SPE/LC-MS/MS method for urinary DA detection in the diagnosis of ADHD. Li et al.[28] discovered that using solid-phase extraction (SPE) can have unforeseen and detrimental effects on the results obtained through LC-MS/MS analyses. The proposed method, by utilizing liquid-liquid extraction, demonstrably prevents the aforementioned problems. The selectivity and specificity attained in the method discussed herein significantly exceed those reported by Woo et al.[32], who utilized SPE as the extraction technique.[32,38,39] Significantly improved analytical reliabilities can be achieved by eliminating variance related to the extraction and analysis methodologies, allowing for widespread implementation of such methods in relevant fields. To prove our point, Zhanga et al.[38] and van de Merbel et al.[39] used SPE to isolate DA from blood plasma. Catecholamines, such as DA, are unstable in alkaline conditions, such as blood, which is a cause of concern. Thus, acidification and antioxidant addition were used to treat the extracts to provide a more amenable and permissive environment for inhibiting DA degradation in such matrices. Our method, which makes use of the naturally acidic urine, prevents these pH-associated effects and allows DA concentrations to be preserved considerably more effectively. When urine is used as the biological matrix of analysis, pH-related effects and their mitigation become a more manageable, if not outright ignorable, inconvenience.

This study aims at demonstrating that a high-speed, low-complexity, robust, sensitive, and specific LC-MS/MS method for urinary DA analysis and quantification can be developed at an affordable rate. To ensure extensive and repeatable reliability, this method was validated following standard laboratory protocols and guidelines. During the validation steps, artificial urine was used. The development of the assay was made possible by the robustification of the method discussed herein, which allowed for the emergence of novel features, such as easy sample preparation, rapid LC-MS/MS detection of DA without the use of derivatization, evaporation, and reconstitution, as well as the use of ion-pairing reagents.

Table 6. Comparison of the proposed method with previous studies

Analyte	Linearity (ng/mL)	LLOQ (ng/mL)	Sample preparation	Equipment	Reference
NE	10-210	6.0	LLE	LC-MS/MS	Diniz et al.[29]
E	3.0-53	1.0	-	-	-
DA	15-1015	11	-	-	-
NMN	30-2130	8.0	-	-	-
MN	20-1420	4.4	-	-	-
NE	10-10000	10	LLE	ESI-MS/MS	Kushnir et al.[24]
E	2.5-10000	2.5	-	-	-
DA	2.5-10000	2.5	-	-	-
NE	1.69-203	1.69[a]	SPE (Bond Elut Plexa)	LC-MS/MS	Whiting[30]
E	1.83-110	1.83[a]	-	-	-
DA	0.77-306	0.77[a]	-	-	-
NMN	0.92-769	0.92[a]	-	-	-
NE	0.39-345	0.39	SPE (WCX µElution)	LC-MS/MS	Peitzsch et al.[31]
E	0.24-500	0.24	-	-	-
DA	0.49-100	0.49	-	-	-
NMN	0.24-125	0.24	-	-	-
MN	0.24-250	0.24	-	-	-
NE	7.4-2359	7.4	SPE (Strata-X-CW)	LC-MS/MS	Woo et al.[32]
E	3.8-2163	3.8	-	-	-
DA	5.4-2825	5.4	-	-	-
NMN	3.7-2569	3.7	-	-	-
MN	3.5-2466	3.5	-	-	-
NE	2.5-500	2.5	SPE (PBA-HLB plate)	LC-MS/MS	Li et al.[33]
E	0.25-250	0.25	-	-	-
DA	2.5-1000	2.5	-	-	-
NE	2.5-500	2.5	SPE (PBA-HLB µElution)	LC-MS/MS	Li et al.[34]
E	0.5-500	0.5	-	-	-
DA	2.5-1250	2.5	-	-	-
NMN	2.5-1250	2.5	-	-	-
NE	5-500	15	SPE (CAT-PBA)	HPLC	Rozet et al.[35]
E	5-500	5	-	-	-
DA	5-500	50	-	-	-
NE	1-150	1[a]	Bio-Rex	HPLC	Manickum[36]
E	3-50	3[a]	-	-	-
DA	3-625	3[a]	-	-	-
A	0.5-20	0.16	SPME	HPLC	Kossakowska et al.[37]
NA	0.25-20	0.08	-	-	-
DA	0.5-20	0.16	-	-	-
L-Tryp	0.25-20	0.09	-	-	-
L-Tyr	0.5-20	0.16	-	-	-
DA	50-4000	1.0	MEPS	LC-MS/MS	El-Beqqali et al.[19]
5-HT	50-4000	1.0	-	-	-
DA	20-2000	0.36[a]	LLE	LC-MS/MS	This study

[a]LOD value was used since LLOQ was not reported. NE: Norepinephrine, E: Epinephrine, DA: Dopamine, NMN: Normetanephrine, MN: Metanephrine, L-Tryp: L-Tryptophan, L-Tyr: L-Tyrosine, 5-HT: Serotonine, MEPS: Microextraction in the packed syringe, LC-MS/MS: Liquid chromatography-tandem mass spectrometry

Urine sampling, as a non-invasive and less expensive sample procurement approach, surpasses preexisting methods, in situations like clinical laboratories conducting neuroscience research or pharmaceutical companies conducting drug development since it is non-invasive and less expensive.

CONCLUSION

This study presents an LC-MS/MS-based methodology that eliminates derivatization, evaporation, reconstitution, and the use of ion-pairing reagents while having considerable speed and simplicity advantages. The proposed method also has the advantages of non-invasive sample procurement and a simplified preparation technique, which are useful in clinical laboratory applications, neuroscience research, and drug development studies.

Conflict of interest: No conflict of interest was declared by the authors. The authors are solely responsible for the content and writing of this paper.

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