A Rapid, Precise, And Sensitive LC–MS/MS Method for Quantitative Determination of Urinary Dopamine by Simple Liquid-liquid Extraction Technique

Basit Sıvı-sıvı Ekstraksiyon Tekniği ile İdrardaki Dopaminin Kantitatif Tayini İçin Hızlı, Kesin ve Hassas Bir LC-MS / MS Yöntemi

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ABSTRACT
INTRODUCTION: Dopamine (DA) is a prominent neurotransmitter and immunomodulator with extensive biochemistry. The quantitative determination of DA can pave way for better understanding of the regulation of endocrine system, cardiovascular and renal functions. The purpose of this study was to develop a rapid, precise, and extremely sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the routine clinical quantitation of DA in urine.

METHODS: Urine samples were prepared via a simple and rapid liquid-liquid extraction technique followed by a sensitive LC-MS/MS method developed using multiple reaction monitoring mode (MRM).

RESULTS: The retention times of dopamine and internal standard were found to be 2.28 and 2.24 min, respectively. Mean extraction recovery of DA and DA-IS in urine was above 95.62%. DA calibration curve in urine was linear (r² ≥ 0.998) ranging from 20 to 1000 ng/mL. The intra- and inter-day precisions (RSD%) and coefficient of variation (CV%) maximum levels are 5.87 and 2.81; 10.55% and 7.57%, respectively.

DISCUSSION AND CONCLUSION: A rapid, precise, sensitive, and quantitative LC-MS/MS detection of dopamine without the use of derivatisation, evaporation, and reconstitution or use of ion-pairing reagents has been developed with a non-invasive sample and easy preparation technique for clinical laboratory applications, basic neuroscience research and drug development studies.

Keywords: Dopamine, LC-MS/MS, urine, method validation

ÖZ

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Dopamine determination by LC-MS/MS
GİRİŞ ve AMAÇ: Dopamin (DA), kapsamlı biyokimyaya sahip 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 kromatografi-tandem kütle spektrometresi (LC-MS / MS) yöntemi geliştirmektir.

YÖNTEM ve GERECİLER: İdrar numuneleri, basit ve hızlı bir sıvı-sıvı ekstraksiyon tekniği ve ardından çoklu reaksiyon izleme modu (MRM) kullanılarak geliştirilen hassas bir LC-MS / MS yöntemi ile hazırlanır.

BULGULAR: Dopamin ve iç standardın alıkonma zamanları sırasıyla 2.28 ve 2.24 dakika olarak bulundu. İdrarda DA ve DA-IS'nin ortalama ekstraksiyon geri kazanımı %95.62'nin üzerindeydi. İdrardaki DA kalibrasyon eğrisi, 20 ila 1000 ng/mL arasında değişen doğrusaldır (r² ≥ 0.998). Gün içi ve günler arası kesinlik (%RSD) ve varyasyon katsayısı (%CV) maksimum seviyeleri 5.87 ve 2.81; sırasıyla %10.55 ve %7.57'dir.

TARTIŞMA ve SONUÇ: Türetme, buharlaştırma ve yeniden yapılandırma veya iyon eşleştirme reaktifleri kullanılmadan, dopaminin 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, metot validasyonu

1. Introduction

Dopamine is a simple chemical neurotransmitter responsible for a plethora of neurological activities, especially in the brain (Figure 1). These pathways, collectively called “dopamine pathways” or “dopaminergic pathways” are intricately implicated in reward response.¹ Dopaminergic pathways are commonly activated in response to, or in anticipation of reward, and dopamine is thought to be deeply involved in motivation and reward-associated satisfaction.² This extensive relationship between dopaminergic signalling and reward response results in the large number of psychotropic pharmaceuticals and illicit drug substances having significant crosstalk, and even direct influence over dopaminergic signalling. Therefore, malfunctions in the dopaminergic system may result in a wide variety of neurological disorders. Dopamine is a poor penetrator of the blood-brain barrier; therefore, its neurological and non-neurological syntheses are thought to be largely independent of each other. Most of blood dopamine is thought to be synthesised in the mesentery or acquired from the digestion of food, about 95% of which is circulated as the biologically inactive dopamine sulphate.⁴ Thus, the biological activity of blood dopamine is chiefly due to the unconjugated (“free”) dopamine. Such functions include vasodilation and noradrenaline release inhibition. There are indications, however not well illuminated, that dopamine may be released into the bloodstream in response to hypoxic conditions.⁵ The immune system is also responsive to dopamine, with most pronounced effects on lymphocytes. Some of these cells may in turn synthesise and release dopamine themselves. Although the function of dopamine with regards to these cells is largely unclear, it is thought to be an integral component of immunogenesis through the modulation of lymphocyte activation.⁶⁷ The potential, through dopamine, of interactions between the nervous and immune systems has been touted as a potential route of crosstalk between the two systems and malfunctions of this interaction have been suggested to be relevant to autoimmune disorders.⁷⁸

In the detection and continued monitoring of relatively recent xenobiotic exposure, urine retains its seat as the primary matrix of choice due to several advantages, among which can be listed its relatively wide temporal envelope of detection, lasting as long as several days, an increased xenobiotic concentration due to urine’s nature as a concentrating waste carrier, as well as the ease and non-invasiveness of sample procurement, the urination of the patient into a sterile container being the sole requirement. Using the aforementioned non-invasive techniques, urine can be used as the medium of detection of dopamine in the diagnosis and continued surveillance of several...
diseases, such as stress-induced diseases and sympathoadrenal system dysfunction. Urine is chemically vastly simpler than comparable media of detection, circumventing the necessity of tedious, effort-intensive and complicated preparatory steps. Thus, the pre-treatment of the sample was as simple as dilution in mycellar solution, followed by filtration and direct injection. There exist a number of previously published studies on the analysis of dopamine and associated species in the biological fluids. This generates a landscape containing a variety of methods engineered to detect and quantify dopamine including spectrophotometry, LC-fluorometry, enzyme immunoassays, and LC-electrochemical detection. LC-MS-based methods are considered the frontrunner as tandem LC-MS methodologies can provide increased selectivity while retaining only minimal reliance on chromatographic separation. These methods, while providing a good enough combination of selectivity, sensitivity and ease of utilisation, are ill-equipped to provide the same quality of output for multi-target analysis, which forms the backbone of the present study, thus necessitating the development of novel methods. Optimal result generation for all relevant analytes in a simultaneous manner required substantial method modification and optimisation. As a result of these modifications, the method discussed herein has succeeded in the delivery of what can be considered top-of-the-line quality in sensitivity, selectivity and robustness as it applies to the detection of dopamine.

The simplicity, robustness, sensitivity and specificity aspects were all at the centre of the consideration criteria for this study, with the main objective being the development of an LC-MS/MS method that incorporates and satisfies all of these conditions. In addition, a cost-effective solution was developed, with simplified and highly effective liquid-liquid extraction step utilising a minimised sample volume, which will prove invaluable for considerations of routine clinical testing.

To the best of the authors’ knowledge, an LC-MS/MS platform, while recognised for its superiority in both selectivity and specificity over other contenders, has not been extensively developed in the past to the degree discussed herein. Thus, this study also serves as a testbed that demonstrates and establishes the method discussed herein in terms of its applicability and reliability to a larger sample population, and the scarcely-discussed clinical applications.

2. Materials and methods

2.1. Chemicals and materials

Analytical grade chemicals were used in all steps of the method. Artificial urine, dopamine and creatinine were procured from Sigma-Aldrich (St. Louis, MO, USA). 13C12-Dopamine (99%), and creatinine-d3 standards (both isotopically labelled) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). HPLC-grade methanol, formic acid, and Hydrochloric acid fuming 37% was obtained from Merck (KGaA, Darmstadt, Germany). Deionized water (18.2 MΩ) treated with Millipore (Simplicity, 185) Milli-Q water purification system (Elga Labwater Veolia, Anthony, France) was used for all aqueous solutions.

2.2. Preparation of calibration standards and QC samples

2.2.1. Internal Standard solution

First of all; dilution solution (DS) was performed with 50 mL 1N hydrochloric acid and 1 liter water to obtain for stock solution. After that preparation for stock solution (500 µg/L); 5 mg dopamine-d4 internal standard (IS) was diluted with DS. Secondly for dilute stock solution (5 ng/mL); 0.1 mL stock solution diluted with 10 mL DS. Finally, for internal standard solution to be used in analyzes; 200 µL dilute stock solution (100 ng/mL) diluted with 10 mL DS.

2.2.2. Standard solutions

50 mg dopamine diluted with 10 mL DS (4000 ng/mL). Preparation for stock standard solution (100 ng/mL); 200 µL of 5000 ng/mL solution was diluted with 10 mL dilution solution. For the
intermediate standard stock solution (2000 µg/L); 100 µL of 100 ng/mL solution was diluted with 10 mL DS. Preparation of stock standard solution levels were presented in figure 2.

2.3. Sample pre-treatment
200 µL of internal standard solution (100 µg/L), 800 µL of dilution solution (DS) and 100 µL of artificial urine sample was mixed and vortex the sample 3-4 minutes.

2.4. LC–MS/MS conditions
The LC system consisted of Agilent 1200 Series 6460 triple quadrupole mass spectrometry with Jet-Stream atmospheric pressure electrospray ionization source and Mass Hunter data acquisition/Quantitation software (Santa Clara, USA). Chromatographic separation was carried out on an Zorbax SB-C18 3.0 x 50 mm 3.5-micron 600 BAL. Mobile phases consisting of (A) 50% formic acid aqueous and (B) Methanol (HPLC gradient grade). The flow rate was set at 5 mL/min. The injection volume was 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 Dopamine IS (positive) and at m/z 154.0→137.00 for dopamine (positive). Dwell time was set at 150 ms for each analyte.

2.5. Method validation
The validation of the LC–MS/MS method was based on the FDA Guidance for Industry: Bioanalytical Method Validation27 (selectivity, carryover, linearity, LLOD and LLOQ, accuracy, precision, matrix effect, extraction recovery, and stability). Artificial urine was used during the validation step due to the lack of genuine blank urine samples without all targeted analytes.

2.5.1. Preparation of calibration curve
The linearity of the dopamine method was quantified with a calibration curve constructed in the range of 20–2000 ng/mL (Figure 3), which included the lower limit of quantitation (LLOQ). Acceptance criteria for each recalculated standard concentration should not exceed 15% of the nominal values and 20% in the lower quantification limit (LLOQ). Each validation run consisted QC samples at three concentrations (n=6, at each concentration). Such validation studies should be created in three consecutive days.

2.5.2. Accuracy and precision
The accuracy and precision studies should be evaluated and reported as intra-day/within-run accuracy with single injection and as between-run accuracy. Intra-day and inter-day accuracy and precision should be determined with quality control samples at each level of at least five samples and at least 4 different concentration levels of dopamine at low (LQC: 200 ng/mL), medium (MQC: 400 ng/mL) and high-quality control (HQC: 100 ng/mL) samples including LLOQ (100 ng/mL). The mean concentration value should not exceed 15% except at LLOQ, of the nominal concentration 20% and coefficient of variation (CV%) values should be <15% over the calibration range, except at the LLOQ.

2.5.3. Selectivity
The analysis matrix should be examined as 6 different lots, it should be proved that the CV% should not exceed 20% of the lower quantification limit (LLOQ) in terms of the substance to be analyzed, and that in the case of the internal standard, it is not affected by not exceed 5% of the internal standard response.

2.5.4. Recovery
The recovery of dopamine, six concentration levels and IS in artificial urine were specified by equating the peak area of each analyte.

2.5.5. Stability
To determine the stability of dopamine were carried out by using six replicates of the all QC samples (LLQC, LQC, MQC and HQC) stored at +4°C after one week. For the acceptance criteria of stability should not exceed 15% of the nominal concentration.

2.5.6. Matrix effect
The matrix effect for dopamine and its internal standard is the ratio of the response of the metabolite to be analyzed, added at certain concentrations to in 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 should not exceed 15%.

3. Results
3.1. Selectivity and optimization of chromatographic conditions
Six different artificial urine matrices were employed to obtain and tested to evaluate the selectivity of the method. In addition, interference at the analyte and IS retention times were also quantified at this time. Dopamine and IS separation from the artificial urine were both observed to be well satisfactory in the aforementioned chromatographic parameters with retention times of ~2.09 and ~1.08 min, respectively (figure 4). No significant interfering peaks were observed from the retention time corresponding to dopamine and the internal standard (Figure 5). The data obtained thus corroborated that the method developed herein is highly specific and possesses high selectivity towards dopamine in urine samples.

3.2. Linearity, LOQ and LOD
The method was validated over the nominal concentration range of 20–2000 ng/mL for dopamine (figure 3). The correlation coefficient ($r$) value was 0.998 for the batch and the equation was $y=0.005938\times -0.053491$. LOQ and LLOD were chosen as 100 µg/L (Level 2) subcalibration point for dopamine with acceptable sensitivity and accuracy. The values were 1.215 ng/mL for LOQ and 0.36 ng/mL for LOD of dopamine.

3.3. Precision and accuracy
The intra- and inter-day precisions (RSD%) maximum levels were 5.87 and 2.81, respectively (Table 1). Also, the intra- and inter-day CV% maximum levels were 10.55% to 7.57% (Table 2) respectively, suggesting that this method was accurate and precise for quantification of dopamine in urine.

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

| Nominal concentrations (ng/mL) | Intra-day precision | Inter-day precision |
|-------------------------------|---------------------|---------------------|
|                               | 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%              |                     |     |
|                               |                     |                     |                     |     |

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

| Nominal concentrations (ng/mL) | Intra-day | Inter-day |
|-------------------------------|-----------|-----------|
|                               | Concentration found | *CV% | Concentration found | *CV% |
|                               |                     |       |                     |       |
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### 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)x100].

Matrix effects of dopamine was 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%), matrix 6 (CV%, 14.3%). It has been determined that there was no effect of urinary matrix variability in the presence of internal standard in dopamine quantification and the CV% value was less than the acceptance criterion for matrix effect (15%) as shown table 4. Dopamine and IS did not show a matrix effect in urine.

### Table 4. Matrix effect of the dopamine.

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

### 3.5. Stability

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

|        | Nominal concentration (ug/L) | Concentration found (mean ± SD, ng/mL) | Correlation coefficient (CV%) |
|--------|-------------------------------|----------------------------------------|------------------------------|
| 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                         |

4. Discussion
In addition to neurological analyses, the intricate implication of dopamine in the endocrine and immune systems, with further research illuminating the precise role played by dopamine in the development and maintenance of these systems, can result in urinary dopamine measurement becoming a mainstay regular test in clinical settings. Such testing may also provide a unique insight to the biochemical pathways underlying the pathologies in such systems, allowing for a deeper understanding of how such systems develop, maintain, and sustain themselves, in addition to the mechanisms through which such systems may pathologically malfunction.

Through the measurement of dopamine in other bodily fluids, such as the cerebrospinal fluid, for which this study will prove to have pioneer value in way of method development, neurological disorders contingent on or related to dopamine biosynthesis or utilisation may also be characterised. Such novel techniques may, in principle, not only allow for more effective evaluation of the treatment of illnesses, but also early-stage diagnosis of these disorders as well.

The method achieved the required functional sensitivity, and quantitated analytes over a sufficiently wide dynamic range. Precise quantification of dopamine concentration levels in urine may contribute to a better understanding of the pathophysiology and pathogenesis of many neuropsychiatric disorders (e.g. drug addiction, schizophrenia, Parkinson’s and Alzheimer’s disease, attention deficit hyperactivity/hyperkinetic disorder) and to pharmaceutical research on novel drugs. The method developed in this study manifestly demonstrates its usefulness as a viable method for the determination of dopamine in urine. Complexity of methodology is greatly reduced by the possibility of a simple precipitation and dilution directly leading to direct injection without intervening steps, a feat that can be considered the primary strength of this procedure, one that gives it a significant advantage over its alternatives. Previously published methodologies universally suffer from extended and arduous sample preparation and derivatisation steps, which collectively make them highly undesirable in high-throughput applications, such as a busy clinical laboratory that needs to serve many patients simultaneously. These disadvantages are entirely eliminated in the method this study describes, with its simple and straightforward sample preparation and a short-duration chromatographic run, thus making it inordinately more applicable and desirable in such high-volume operations (Table 6).

Table 6. Comparison of the proposed method to previous studies.

| Analyte | Linearity (ng/mL) | LLOQ (ng/mL) | Sample preparation | Equipment | Ref.          |
|---------|-------------------|--------------|--------------------|-----------|---------------|
| 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          |                    |           |               |
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| NE          | 10-10000 | 10 | LLE             | ESI-MS/MS | Kushnir et al. (30) |
|------------|----------|----|-----------------|-----------|---------------------|
| E          | 2.5-10000 | 2.5 | SPE (Bond Elut  | Whiting et al. (31) |
|            |          |    | Plexa)         |           |                     |
| DA         | 2.5-10000 | 2.5 |                 |           |

| NE          | 1.69-203  | 1.69^a | SPE (WCX μElution) | Peitzsch et al. (32) |
|------------|----------|--------|-------------------|----------------------|
| E          | 1.83-110  | 1.83^a | SPE (Strata-X-CW) |                       |
| DA         | 0.77-306  | 0.77^a |                   |                       |
| NMN        | 0.92-769  | 0.92^a |                   |                       |

| NE          | 0.39-345  | 0.39   | SPE (PBA-HLB μElution) | Li et al. (34) |
|------------|----------|--------|-----------------------|---------------|
| 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) | Woo et al. (33) |
|------------|----------|--------|------------------|-----------------|
| 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 μElution) | Li et al. (35) |
|------------|----------|--------|-----------------------|---------------|
| E          | 0.25-250  | 0.25   |                       |               |
| DA         | 2.5-1000  | 2.5    |                       |               |

| NE          | 5-500     | 5      | SPE (CAT-PBA) | Rozet et al. (36) |
|------------|----------|--------|--------------|-------------------|
| E          | 5-500     | 5      | HPLC         |                   |
| DA         | 5-500     | 5      |              |                   |

| NE          | 1-150     | 1^a    | Bio-Rex | Manickum (37) |
|------------|----------|--------|---------|---------------|
| E          | 3-50      | 3^a    |         |                |
| DA         | 3-625     | 3^a    |         |                |

| A           | 0.5–20    | 0.16   | SPME | HPLC | Kossakowska et al. (38) |
|------------|----------|--------|------|-----|------------------------|
| 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 (39) |
|------------|----------|--------|------|---------|----------------------|

| 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; Serotonin, MEPS; Microextraction in packed syringe. The method developed and validated herein is an improvement over the microextraction methodology developed and employed by Beqquali et al., in terms of its speed, reliability, quantitative potency, and cost-effectiveness. Such advantages are especially tangible in a clinical
setting, where fast and reliable detection of low levels of dopamine in biological matrices may be of
crucial importance. 28 Moriarty et al. 8 developed a SPE/LC-MS/MS method for urinary dopamine
detection in the diagnosis of Attention Deficit Hyperactivity/Hyperkinetic Disorder (ADHD). 8 Li et
al. determined that the utilisation of solid phase extraction can have unforeseen and detrimental
effects on the results obtained through LC-MS/MS analyses. 28 The method henceforth developed,
by virtue of its utilisation of liquid-liquid extraction, demonstrably circumvents the aforementioned
confounding circumstances. The selectivity and specificity attained in the method discussed herein
significantly exceeds the selectivity and specificity reported by Woo et al. 33 who utilised SPE as the
method of extraction. 40,41,33 By eliminating variance related to the extraction and analysis
methodologies themselves, greatly improved analytical reliabilities can be attained, enabling more
extensive implementation of such methods in relevant fields. To prove our point, Zhang et al. 40 and
Merbel et al. 41 both employed SPE isolation of dopamine from blood plasma. One point of concern
was the inherent instability of catecholamines, such as dopamine, in alkaline conditions, as blood
naturally is. Thus, it was necessary to treat the extracts to provide a more amenable and permissive
environment to inhibit the degradation of dopamine in such matrices via acidification and/or
antioxidant addition. Our method, via its utilisation of the naturally acidic urine, inherently
circumvents these pH-associated effects and can preserve dopamine concentrations much more
effectively. Thus, pH-associated effects and their mitigation are a much more manageable, if not
outright ignorable, inconvenience if urine is utilised as the biological matrix of analysis.
This study entails the demonstration that a high-speed, low-complexity LC-MS/MS method for
urinary dopamine analysis and quantification can be developed that also proves to be robust,
sensitive and specific at a viable affordability. In order to ensure extensive and repeatable
reliability, the method was validated in accordance with standard laboratory protocols and
guidelines. During the validation steps, artificial urine was used. Robustification of the method
herein discussed allowed for the emergence of novelty of the developed assay, such as easy sample
preparation, rapid LC-MS/MS detection of dopamine without the use of derivatisation, evaporation,
and reconstitution, as well as the use of ion-pairing reagents.
Urine sampling, being a non-invasive sample procurement approach, thus also being cheaper,
significantly improves over the pre-existing methods, especially in settings where such aspects are
important, such as clinical laboratories in the process of neuroscience research or pharmaceutical
industries in the process of drug development.

5. Conclusion
This study provides an LC-MS/MS based methodology with significant disadvantages in speed and
simplicity, while eliminating derivatisation, evaporation, reconstitution or the use of ion-pairing
reagents. Additional benefits of the current method are the non-invasiveness of sample
procurement, and a simplified preparation technique, especially useful in clinical laboratory
applications, neuroscience research and drug development studies.

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![Chemical structure of dopamine](image)

**Figure 1** Chemical structure of dopamine
Figure 2. Preparation for stock standard solution levels

Figure 3. Calibration curve of dopamine

Figure 4. Representative chromatogram of dopamine in urine
Figure 5. Dopamine and the internal standard peaks.