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In Matrix Derivatization Combined with LC-MS/MS Results in Ultrasensitive Quantification of Plasma Free Metanephrines and Catecholamines

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ABSTRACT: Plasma-free metanephrines and catecholamines are essential markers in the biochemical diagnosis and follow-up of neuroendocrine tumors and inborn errors of metabolism. However, their low circulating concentrations (in the nanomolar range) and poor fragmentation characteristics hinder facile simultaneous quantification by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Here, we present a sensitive and simple matrix derivatization procedure using propionic anhydride that enables simultaneous quantification of unconjugated L-DOPA, catecholamines, and metanephrines in plasma by LC-MS/MS. Dilution of propionic anhydride 1:4 (v/v) in acetonitrile in combination with 50 µL of plasma resulted in the highest mass spectrometric response. In plasma, derivatization resulted in stable derivatives and increased sensitivity by a factor of 4−30 compared with a previous LC-MS/MS method for measuring plasma metanephrines in our laboratory. Furthermore, propionylation increased specificity, especially for 3-methoxytyramine, by preventing interference from antihypertensive medication (β-blockers). The method was validated according to international guidelines and correlated with a hydrophilic interaction LC-MS/MS method for measuring plasma metanephrines ($R^2 > 0.99$) and high-performance liquid chromatography with an electrochemical detection method for measuring plasma catecholamines ($R^2 > 0.85$). Reference intervals for L-DOPA, catecholamines, and metanephrines in $n = 115$ healthy individuals were established. Our work shows that analytes in the subnanomolar range in plasma can be derivatized in situ without any preceding sample extraction. The developed method shows improved sensitivity and selectivity over existing methods and enables simultaneous quantification of several classes of amines.
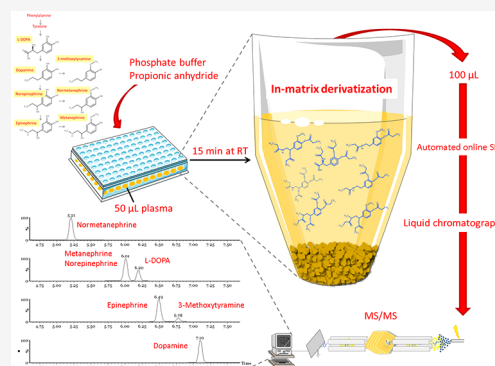
Plasma catecholamines including L-DOPA, epinephrine, norepinephrine, dopamine, and their metabolites the metanephrines, which comprise metanephrine, normetanephrine, and 3-methoxytyramine, are important diagnostic markers (Figure S-1). Plasma-free metanephrines and catecholamines are quantified for the diagnosis of catecholamine-producing tumors like pheochromocytoma, paraganglioma, and neuroblastoma as well as inborn errors of monoamine neurotransmitter synthesis, metabolism, and transport.[1−4]

Simultaneous analysis of metanephrines and catecholamines remains an analytical challenge because of their low concentrations in plasma, the oxidation-prone catechol moiety, potential chromatographic interferences, and poor fragmentation characteristics in the mass spectrometer.[5,6] Several analytical methods have been described to measure metanephrines or catecholamines in plasma, including immunoassays, high-performance liquid chromatography (HPLC) with electrochemical detection (ECD), gas chromatography coupled with mass spectrometry, and liquid chromatography with tandem mass spectrometry (LC-MS/MS).[7−10] However, most of these described methods are laborious, relatively imprecise, and use large sample volumes.[6,8] There is only one method to date that describes the simultaneous analysis of plasma metanephrines and catecholamines by LC-MS/MS.[11] However, this method involves an offline extraction and evaporation step, which can be delicate with oxidation-sensitive catecholamines, and still needs 200 µL of plasma, which is not ideal for volume-limited studies, such as those using samples from a biobank or preclinical studies using samples from animals. In addition, the catecholamine precursor L-DOPA is not analyzed as it is not extracted by the weak cation exchange sorbent employed in this method. To improve the sensitivity of mass spectrometric detection of catecholamines, several derivatization strategies have been
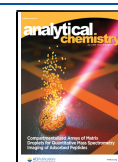
proposed. However, these are time consuming and use toxic chemicals which cannot easily be used in a clinical laboratory.[12,13] We have previously shown that in situ or direct-matrix derivatization can be performed directly in plasma without any preceding sample cleanup, improving chemical stability during sample preparation.[14] Other possible advantages of derivatization are higher sensitivity by improved ionization efficiency, improved fragmentation characteristics, and more uniform and reproducible extraction and chromatographic properties.

In this study, we describe the development and validation of an automated LC-MS/MS method using direct-matrix derivatization for the simultaneous quantification of free fractions of L-DOPA, catecholamines, and metanephrines in plasma at the picomolar level.

■ MATERIALS AND METHODS

Reagents. LC-MS grade acetonitrile, isopropanol, methanol, formic acid, and ammonium acetate were purchased from Biosolve BV (Valkenswaard, The Netherlands). Glutathione (reduced), ascorbic acid, dipotassium hydrogen phosphate, pyridine, and hydrochloric acid (32%) were from Merck Millipore (Darmstadt, Germany). Ammonium hydroxide solution (28−30%), propionic anhydride, K_2EDTA dihydrate, L-DOPA, dopamine-HCl, norepinephrine, epinephrine, 3-methoxytyramine, DL-metanephrine-HCl, DL-normetanephrine-HCl, and L-DOPA-d_3, all of analytical purity, were purchased from Sigma-Aldrich (MI, USA). Stable deuterated isotopes for dopamine-d_4-HCl, norepinephrine-d_6-HCl, and epinephrine-d_3 were from CDN Isotopes (Pointe-Claire, Canada), 3-methoxytyramine-d_4-HCl and DL-metanephrine-d_3-HCl from Cambridge Isotopes (MA, USA), and DL-normetanephrine-d_3-HCl from Medical Isotopes (NH, USA). Ultrapure water was produced using an in-house purification system (Merck Millipore, MA, USA).

Preparation of Stock Solutions, Calibrators, and Internal Standard Solutions. Stock solutions were prepared in 0.08 mol/L acetic acid. Stock solutions were serially diluted in ascorbic acid in water 0.04% (w/v) to their respective working solutions (mix of all analytes). Stock solutions were kept at −80 °C. Working solutions were prepared fresh on the day of analysis to prevent degradation due to oxidation. Eight calibrators were prepared by adding different volumes of working solution into a surrogate matrix (dialyzed plasma; for detailed description, see Supporting Information, section Dialyzed plasma). Calibration curves ranged from 6.8 to 680 nmol/L (L-DOPA), from 0.070 to 7.0 nmol/L (dopamine), from 0.31 to 31 nmol/L (norepinephrine), from 0.091 to 9.1 nmol/L (epinephrine), from 0.066 to 6.6 nmol/L (3-methoxytyramine), from 0.26 to 26 nmol/L (normetanephrine), and from 0.10 to 10 nmol/L (metanephrine). An internal standard working solution was prepared in ascorbic acid in water 0.04% (w/v) containing L-DOPA-d_3 (270 nmol/L), dopamine-d_4 (1.7 nmol/L), norepinephrine-d_6 (3.5 nmol/L), epinephrine-d_3 (2 nmol/L), 3-methoxytyramine-d_4 (0.7 nmol/L), normetanephrine-d_3 (1.2 nmol/L), and metanephrine-d_3 (0.7 nmol/L).

Optimization of the Derivatization Reaction. The volume of plasma (50 and 100 μL) and the ratio of propionic anhydride to acetonitrile (v/v) were optimized. The ratio of propionic anhydride to acetonitrile was varied between an undiluted, 1:1, 1:4, and 1:10 dilution in acetonitrile (v/v %). Other experimental conditions, like buffer strength, buffer pH,

and incubation time, were as previously described.[14] The optimal incubation time for derivatization of the catecholamines and metanephrines was verified to be 15 min (Figure S-3).[14] The experiment was performed with six different plasma pools obtained from anonymous patient samples that were screened for plasma metanephrines at our laboratory. The internal standard peak area was used to evaluate which combination of variables gave the highest signal. The derivatization procedure was performed as described below.

Derivatization Procedure. Aliquots of thawed plasma samples (50 μL) and calibrators were mixed with 50 μL of internal standard working solution, 250 μL of 0.5 mol/L dipotassium phosphate, and 4 mol/L K_2EDTA, pH 8.5 in a 2.0 mL 96-deep well plate (Greiner Bio-One). Then 50 μL of 25% (v/v) propionic anhydride in acetonitrile was added, and the plate was vortexed for 15 min. Water was added to all wells to a total volume of 0.5 mL. The plate was vortexed and centrifuged for 30 min at 1500g. Then 100 μL of each calibrator and sample was injected onto the online solid-phase extraction (SPE) LC-MS/MS system.

Online SPE and LC-MS/MS. Online SPE and liquid chromatography were performed with an automated system as previously described (Symbiosis Pharma system, Spark Holland, Emmen The Netherlands).[15] The online SPE procedure was carried out on 1 × 10 mm Oasis HLB 30 μm SPE cartridges. For a detailed description of the online SPE procedure, see the Supporting Information (section Online SPE and LC-MS/MS, and scheme in Figure S-2).

Liquid chromatography was performed on a Luna Phenyl-Hexyl 2.0 × 150 mm, 3 μm column (Phenomenex, Torrance, CA), with a binary gradient system that consisted of 10 mM ammonium acetate with 0.1% formic acid (mobile phase A) and 0.1% formic acid in 100% acetonitrile (mobile phase B). Initial conditions were 80:20 (v/v) mobile phase A:mobile phase B at a flow rate of 0.3 mL/min followed by a linear increase of mobile phase B to 60% over 8.25 min. Thereafter, mobile phase B was increased to 80% over 15 s, where it was kept constant for 1 min. The mobile phase was then returned to the starting conditions and kept constant for a further 1.5 min, giving a total run time of 11.5 min.

All analytes were analyzed in positive electrospray ionization mode on a triple-quadrupole mass spectrometer (Waters Xevo TQ-MS). Mass spectrometer transitions and settings were optimized by tuning the derivatives in the selective reaction monitoring (SRM) (Table S-1). The following settings were applied throughout: capillary voltage 0.5 kV, desolvation temperature 600 °C, nitrogen desolvation gas flow 1000 L/h, nitrogen cone gas flow 50 L/h, and argon collision gas flow 0.20 mL/min. Analytes were quantitated using the peak-area response ratios of the quantifier transitions for the analyte and the corresponding internal standard. Calculations were performed with Targetlynx version 4.1 (Waters, Milford, MA, USA).

Evaluation of Assay Performance. The method was validated by evaluating imprecision, limit of quantification (LOQ), linearity, carryover, recovery, and ion suppression and by comparing with other methods.[16] The stability of derivatives was tested by analyzing 38 derivatized plasma samples at $T = 0$ and after $T = 72$ h in the autosampler. Detailed information on the procedures for method validation are provided in the Supporting Information (section Method validation).

Quality control (QC) plasma samples containing low, medium, and high levels of the respective analytes were prepared (see Table 1 for concentrations) using pooled

Table 1. Intra- and Interassay Imprecision and LOQ[a]

sample	mean (nmol/L)	intra-assay (n = 20) (%)	interassay (n = 20 days) (%)	LOQ (nmol/L)
L-DOPA				1.0
QC low	10.2	2.1	7.1	
QC med	46.8	2.3	4.1	
QC high	329	2.1	3.9	
dopamine				0.011
QC low	0.095	6.9	5.7	
QC med	0.451	2.2	3.5	
QC high	3.33	1.8	3.5	
norepinephrine				0.010
QC low	2.26	2.3	3.3	
QC med	4.48	1.7	3.3	
QC high	17.1	1.7	2.4	
epinephrine				0.030
QC low	0.216	4.2	0.5	
QC med	0.652	4.5	5.8	
QC high	4.37	2.4	2.3	
3-methoxytyramine				0.010
QC low	0.110	3.5	3.1	
QC med	0.326	1.6	3.6	
QC high	3.10	1.3	2.4	
normetanephrine				0.050
QC low	0.534	5.2	3.2	
QC med	1.68	3.1	3.1	
QC high	12.1	2.5	2.6	
metanephrine				0.040
QC low	0.220	7.1	5.9	
QC med	0.667	3.3	3.4	
QC high	4.80	3.6	3.0	

[a]Abbreviations: LOQ, limit of quantification; QC, quality control

anonymized human EDTA plasma collected during routine patient care for plasma- free metanephrines. This was classified as non-WMO research (Dutch law on Research Involving Human Subjects Act) and received an exemption from the Medical Ethical Committee of our hospital. QC samples were stabilized with glutathione (~5 mg/mL) and stored at −80°C until analysis. Methods for norepinephrine and epinephrine were compared with the routine HPLC-ECD method in our laboratory in 58 patient plasma samples.[17] Methods for 3-methoxytyramine, normetanephrine, and metanephrine were compared with a hydrophilic interaction chromatography (HILIC) LC-MS/MS method in our laboratory in 40 patient plasma samples.[15] Certain antihypertensive medications are known to interfere in the LC-MS/MS analysis of metanephrines.[18] Our HILIC LC-MS/MS method for measuring 3-methoxytyramine suffered from analytical interference from the β-blocker metoprolol, which had been taken by the patient. To check for analytical interferences, we analyzed plasma samples from a previous study in which blood was collected from patients before and 1 month after the start of antihypertensive medication (β-blockers, thiazide diuretics, and angiotensin-converting enzyme inhibitors).[19] 3-O-Methyldopa was also tested as it can interfere with the analysis of plasma 3-methoxytyramine.[5] 3-O-Methyldopa is fragmented in-source,

resulting in 3-methoxytyramine, which produces the same fragment ions.[5]

Reference Interval Study. A reference interval study was performed by analyzing 115 plasma samples from apparently healthy individuals who gave informed consent. Detailed information on the inclusion criteria and blood sampling can be found in the Supporting Information (section Reference interval study). The study was approved by the medical ethics committee of the University Medical Center Groningen (Netherlands trial register number NTR5066).

Statistics. Method comparisons and autosampler stability were calculated by passing and Bablok regression using cp-R, an interface to R.[20] Reference ranges were calculated by parametric analysis for 3-methoxytyramine and metanephrine and log-transformed parametric analysis for L-DOPA, dopamine, norepinephrine, epinephrine, 3-methoxytyramine, and normetanephrine using Analyze-it (Analyze-it Software, Ltd., Leeds, UK). Results were expressed as mean ± standard deviation or median [interquartile range] for normally distributed and non-normally distributed data, respectively.

RESULTS AND DISCUSSION

Derivatization. Optimization of the derivatization conditions showed that a 1:4 propionic anhydride dilution in acetonitrile (v/v) gave the highest internal standard area responses (Figure 1). Undiluted propionic anhydride and the 1:1 propionic anhydride dilution showed a lower response for catecholamines and metanephrines (5−26%, Table S-2) because the pH drops below pH 7 almost immediately after propionic anhydride is added, which stops the reaction. The pH falls below 7 approximately 10 min after a 1:4 or 1:10 propionic anhydride dilution is added, which allows the reaction to continue for longer. This decrease in pH combined with in situ derivatization facilitated protein precipitation. After centrifugation, the supernatant could be injected directly. We tested if the addition of acetonitrile without propionic anhydride also caused proteins to precipitate, but it did not. The pI or tertiary structure of certain proteins is modified by propionylation (derivatization of lysine side chain, hydroxyl groups, and N-terminal amine groups). This, together with the drop in pH after derivatization, causes proteins to precipitate. The plasma volume was set at 50 μL as this gave consistently higher responses compared with 100 μL of plasma. This may be related to suppressed ionization or incomplete derivatization when 100 μL of plasma is used. A 1:4 ratio of propionic anhydride together with 50 μL of plasma was chosen for the remaining experiments. Addition of stable isotope-labeled internal standards before derivatization is pivotal in correcting for any difference in derivatization efficiency between samples. One can argue that there could be differences in derivatization efficiency at different concentrations. The concentration of the internal standard is fixed, whereas the concentrations of the endogenous analytes vary. However, the interassay imprecision experiments showed no considerable increase in variation at lower analyte concentrations compared with higher concentrations. For example, when looking at 3-methoxytyramine, the interassay imprecision was 3.1% at 0.110 nmol/L, 3.6% at 0.326 nmol/L, and 2.4% at 3.1 nmol/L (Table 1). Figure 2 illustrates the derivatization reaction of dopamine with propionic anhydride. Derivatization resulted in precursors at the theoretically predicted m/z for L-DOPA, dopamine, and 3-methoxytyramine (Figure 3). For norepinephrine, epinephrine, normetanephrine, and metanephrine, the most intense
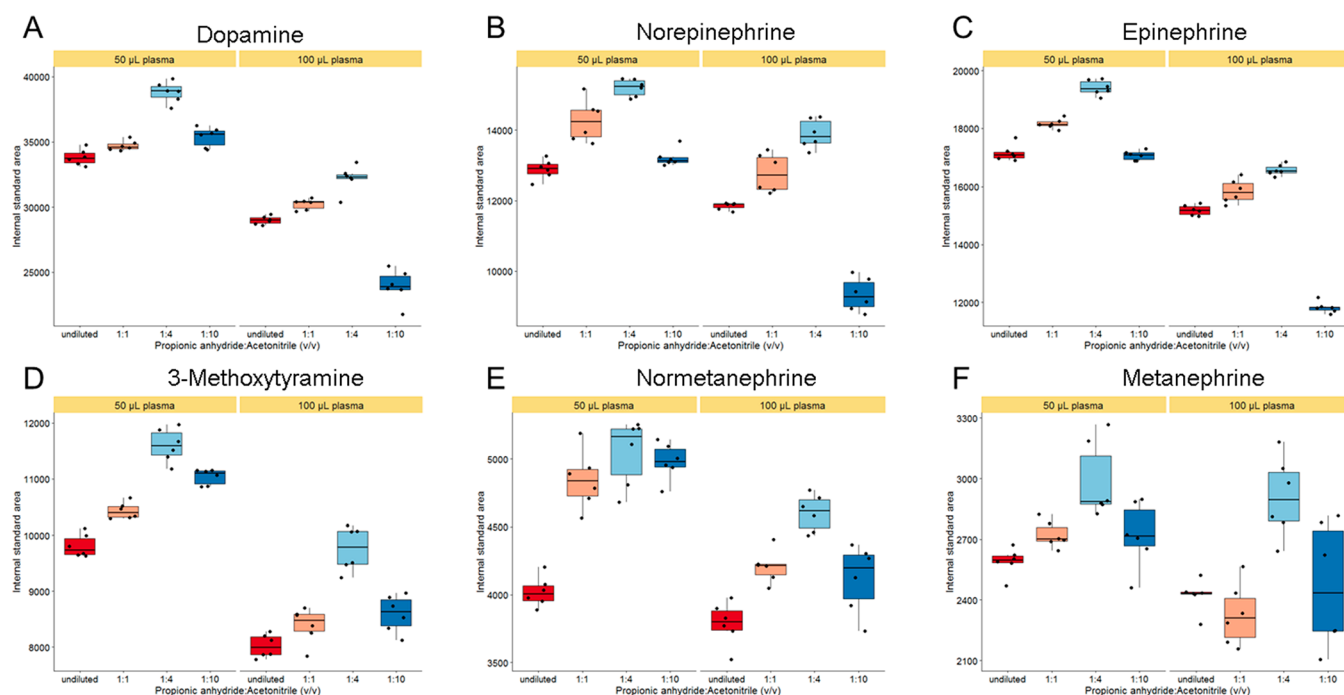
Figure 1. Effect of different derivatization reaction conditions on the internal standard peak-area response for the three different catecholamines. Internal standard peak area is shown on the *y* axis, and ratio of propionic anhydride to acetonitrile (v/v) is on the *x* axis. (A) Results for dopamine-d_4 for 50 and 100 μL of plasma. (B) Results for norepinephrine-d_6 for 50 and 100 μL of plasma. (C) Results for epinephrine-d_3 for 50 and 100 μL of plasma. (D) Results for 3-methoxytyramine-d_4 for 50 and 100 μL of plasma. (E) Results for normetanephrine-d_3 for 50 and 100 μL of plasma. (F) Results for metanephrine-d_3 for 50 and 100 μL of plasma. Results for L-DOPA-d_3 are shown in Figure S-4.
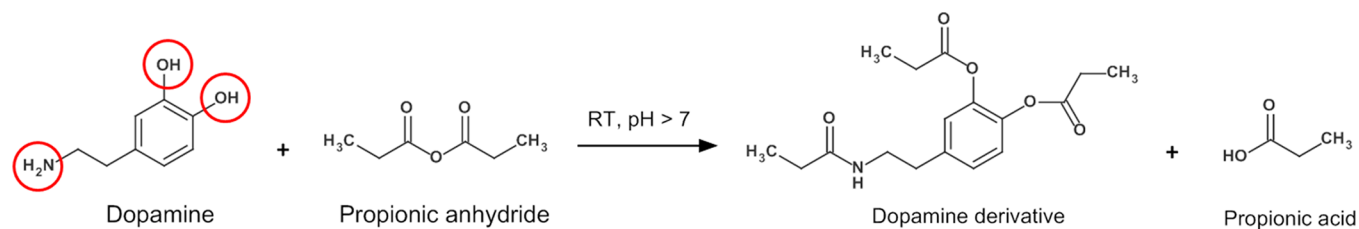

Figure 2. Derivatization reaction of dopamine with propionic anhydride. Formed derivative product is shown on the right side.

precursor *m/z* was that without the derivatized β-hydroxyl group (hydroxyl group on the side chain). This group was easily lost by in-source fragmentation, which could be avoided by adjusting the cone voltage (see norepinephrine example in Figure S-5). The fragmentation patterns of formed derivatives were comparable for all analytes (Figure 3). Dependent on the applied collision energy, the propionyl groups can be lost, resulting in high intensity, analyte-dependent product ions. This is illustrated for dopamine in Figure S-6, which shows the product ion spectrum at collision energies of 15 and 30 eV. Propionylation was shown to be effective in aqueous medium for gas chromatographic analysis of biogenic amines and showed advantages over the more reactive fluorinated reagents.[21−23] However, in this study, we did not perform in situ derivatization in plasma but only in neat solutions and with pyridine as a catalyst.

Assay Performance. For all analytes, intraassay and interassay coefficients of variation were <8.2% (Table 1). Only one other study analyzed catecholamines and meta-nephrines (but not L-DOPA) in one analysis, and the imprecision observed in the present study for the respective analytes was comparable to or better than 11. Mean recovery of the added analytes was 97−101% for L-DOPA, 100−104%
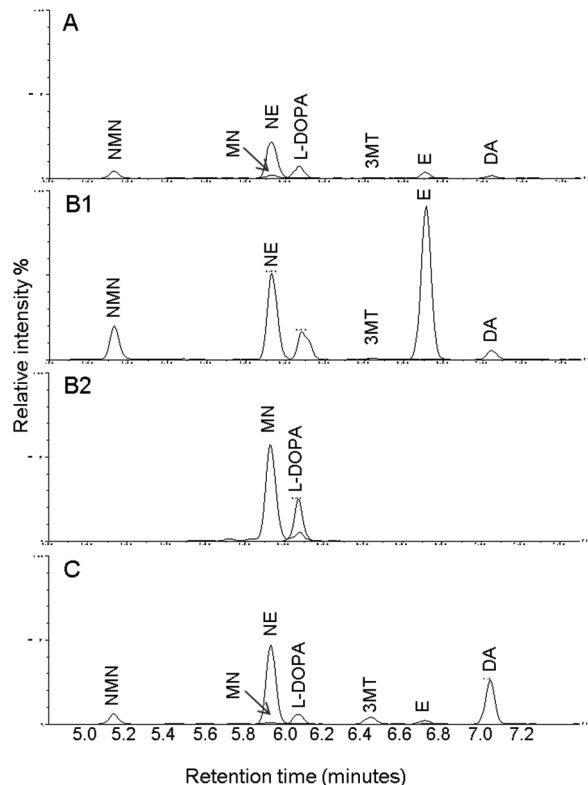
for dopamine, 95−105% for norepinephrine, 98−100% for epinephrine, 97−100% for 3-methoxytyramine, 97−99% for normetanephrine, and 95−99% for metanephrine (Table S-3). Carryover was <0.1% for each analyte, and no significant ion suppression was observed.

In situ derivatization resulted in stable derivatives, with an autosampler stability of at least 72 h (Figures S-6 and S-7). The increase in stability after derivatization for the catechol-amines is critical, as catecholamines are prone to oxidation due to their vicinal phenolic OH groups.[24] Quantification limits for most analytes were in the lower picomolar range (Table 1), which corresponds to 100−500 amol on column. The derivatization procedure resulted in an unprecedented LOQ for each analyte compared with those reported in the literature, especially when the sample volume is considered. Compared with the HILIC LC-MS/MS method we use for plasma metanephrines, sensitivity increased 30 times for 3-methoxytyr-amine, 5 times for normetanephrine, and 4 times for metanephrine.[15] When examining the analytes with the lowest plasma concentrations, namely, epinephrine, dopamine, and 3-methoxytyramine, two previous reports showed comparable LOQs for epinephrine (0.03 and 0.05 nmol/L, respectively) but used 5−10 times more plasma than our method does.[13,25]
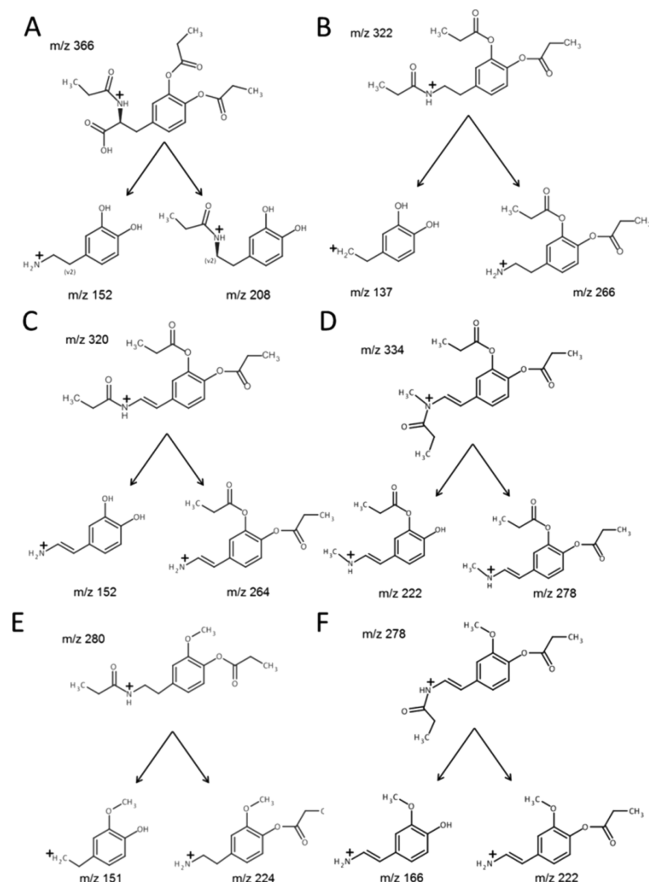
Figure 3. Proposed fragmentation scheme for each analyte: (A) L-DOPA; (B) dopamine; (C) norepinephrine; (D) epinephrine; (E) 3-methoxytyramine; (F) normetanephrine. Scheme for metanephrine is shown in Figure S-10.

Figure 4. LC-MS/MS analyses of plasma from a healthy volunteer (A), from one patient with pheochromocytoma (B1/B2), and from one patient with HNPGL (C) in the SRM mode. Chromatograms were normalized to the same intensity. (A) Calculated concentrations in plasma were as follows: L-DOPA, 12 nmol/L; DA, 0.063 nmol/L; NE, 2.3 nmol/L; E, 0.20 nmol/L; 3-MT, 0.010 nmol/L; NMN, 0.47 nmol/L; MN, 0.16 nmol/L. Calculated concentrations in plasma were (for B and C, respectively) as follows: L-DOPA, 38 and 11 nmol/L; DA, 0.26 and 1.3 nmol/L; NE, 5.2 and 5.9 nmol/L; E, 5.4 and 0.14 nmol/L; 3-MT, 0.033 and 0.22 nmol/L; NMN, 2.5 and 0.67 nmol/L; MN, 5.2 and 0.084 nmol/L. Abbreviations: DA, dopamine; NE, norepinephrine; E, epinephrine; 3-MT, 3-methoxytyramine; NMN, normetanephrine; MN, metanephrine; HNPGL, head and neck paraganglioma.

Two prior reports on LC-MS/MS methods for dopamine show similar LOQs to our method, respectively, 0.039 and 0.065 nmol/L versus 0.020 nmol/L for our method.[26,27] The authors from the first report used 25 μL of plasma, an evaporation step, and a derivatization step for the amine group.[26] However, with this method, the LOQs for epinephrine and norepinephrine were considerably higher than for our method (0.10 and 0.39 nmol/L versus 0.03 and 0.010 nmol/L).[26] The LOQ for epinephrine in particular was not sufficient to measure endogenous epinephrine in human plasma samples. The other report also used propionic anhydride for the derivatization of dopamine but performed the derivatization after the SPE step.[27] For 3-methoxytyramine, two earlier reports state LOQs for 3-methoxytyramine close to our LOQ of 0.010 nmol/L, respectively 0.0375 and 0.024 nmol/L.[28,29] However, these methods used 3−18 times more plasma as well as high-end, more sensitive triple-quadrupole mass spectrometers compared with our lower end, less sensitive triple-quadrupole mass spectrometer. Another advantage of our method is that we only use 50 μL of plasma; so less blood needs to be taken from the patient or from biobanks. Figure 4 displays chromatograms obtained from a healthy volunteer, a patient with pheochromocytoma, and a patient with head and neck paraganglioma. Chromatographic selectivity was achieved on a phenyl-hexyl column, which baseline separates the analytes, except for metanephrine and norepinephrine—these two analytes can be discriminated

based on their *m/z* transitions and did not show cross-talk. The LC-MS/MS analyses show distinctive profiles for the healthy volunteer and the two patients (each chromatogram is normalized to the same intensity for comparison). Figure 4B1/B2 (patient with pheochromocytoma) shows increased norepinephrine, epinephrine, normetanephrine, and metanephrine concentrations compared with the healthy volunteer (Figure 4A), whereas Figure 4C (patient with head and neck paraganglioma) shows increased dopamine and 3-methoxytyramine concentrations. These findings are in line with the literature on pheochromocytoma and paraganglioma.[3,30,31] No analytical interferences were detected in the samples from the antihypertensive medication study (β-blocker, thiazide diuretic, or angiotensin-converting enzyme inhibitor).[19] β-Blockers did not interfere with the 3-methoxytyramine transitions in the present study. This is in contrast to our previous HILIC LC-MS/MS method, where we could not analyze 3-methoxytyramine in patients using metoprolol because the β-blocker interfered at the retention time of 3-methoxytyramine. In addition to possible interference in conventional LC-MS/MS assays, recent literature suggests that 3-O-methyldopa could have a role in the diagnosis of neuroblastoma.[5,32] We found

that 3-*O*-methyldopa was easily incorporated in the assay and did not interfere with other transitions. To validate the accuracy of our new method, samples from the quality assurance program for plasma-free metanephrines of the Royal College of Pathologists of Australasia (RCPA) were analyzed.[33] Six samples, between January and March 2019, were analyzed. They revealed excellent agreement with target values reported by the RCPA Quality Assurance Program. Errors ranged from −8.3% to 4% for 3-methoxytyramine, from −12% to 3.7% for normetanephrine, and from −6.6% to 6.5% for metanephrine (Table S-4).

Method Comparison. The LC-MS/MS method for analyzing plasma norepinephrine and epinephrine was compared with the HPLC-ECD assay routinely used in our laboratory.[17] Passing and Bablok regression demonstrated no proportional or systematic bias for norepinephrine and a systematic bias of −0.11 nmol/L for epinephrine (Figure S-9). Epinephrine may have shown systematic bias because the HPLC-ECD method uses a one-point calibration and a structural analog as an internal standard (dihydroxybenzylamine). Passing and Bablok regression revealed excellent agreement for 3-methoxytyramine, normetanephrine, and metanephrine with our previously reported HILIC-MS/MS method (Figure S-9).[15] For 3-methoxytyramine, several plasma samples had to be excluded as 3-MT concentrations were below the HILIC LC-MS/MS LOQ of 0.06 nmol/L and β-blockers interfered with the HILIC LC-MS/MS method, as previously mentioned. There was a proportional bias for normetanephrine of 5% (95% CI 1.03−1.11) and for metanephrine of 9% (95% CI 1.05−1.12) but not for 3-methoxytyramine (2%; 95% CI 0.94−1.12). L-DOPA and dopamine data could not be compared because our HPLC-ECD method could not reliably detect endogenous levels of these markers in plasma.

Reference Intervals. Reference intervals for all compounds were established in the supine position. Whether blood samples are collected with the patients in a sitting or supine position has a significant influence, particularly on norepinephrine and normetanephrine.[34] Blood sampling in the supine position is recommended by the clinical practice guideline for pheochromocytoma and paraganglioma of the Endocrine Society.[35]

Reference intervals were as follows: L-DOPA, 5.0−34 nmol/L; dopamine, 0.024−0.18 nmol/L; norepinephrine, 0.68−4.0 nmol/L; epinephrine, 0.029−0.32 nmol/L; 3-methoxytyramine, <0.036 nmol/L; normetanephrine, 0.17−0.79 nmol/L; metanephrine, 0.068−0.28 nmol/L. Our reference intervals were comparable to previously reported intervals for plasma collected in a supine position.[36,37]

CONCLUSIONS

We describe a straightforward direct-matrix derivatization procedure that is an improvement on all existing methods for quantitation of plasma metanephrines and catecholamines and that allows simultaneous mass spectrometric analysis of plasma L-DOPA, catecholamines, and metanephrines for the first time. The assay complies with international guidelines on the validation of clinical assays. This study proves that catecholamines and metanephrines can be propionylated directly in plasma, which greatly improves their detection by mass spectrometry. This increase in sensitivity is probably related to the increased lipophilicity of the derivatives and subsequent increase in ionization efficiency.[21,38] Furthermore, the method is highly automated, which reduces possible human errors. It can be improved even more by converting the liquid chromatography method to ultraperformance liquid chromatography. This combination of profiling and enhanced sensitivity represents a considerable improvement and may open new possibilities for research on catecholamine metabolism.

The low plasma volume needed for simultaneous analysis of plasma L-DOPA, catecholamines, and metanephrines preserves precious biobanked samples and is suitable for advanced diagnostic applications that may open new avenues for microsampling. This may improve the diagnosis of neuroblastoma in pediatric patients, as other methods are hindered by their need for large sample volumes. Furthermore, the method may have other preclinical research applications, such as small animal studies, in vivo microdialysis, and cell culture studies.

In conclusion, we present the first method for simultaneous analysis of L-DOPA, catecholamines, and metanephrines that uses direct-matrix derivatization in combination with online SPE LC-MS/MS. The method is more sensitive and selective than existing methods.

ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01263.

MS settings, derivatization optimization experiment, recovery, proficiency study of RCPA, chemical structures and pathway, scheme of thr online SPE procedure, effect of incubation time on the derivatization reaction, L-DOPA results of the derivatization optimization experiment, precursor spectrum of norepinephrine, product ion spectrum of dopamine, plot of the autosampler stability of catecholamines, plot of the autosampler stability of metanephrines, plot of the method comparison of catecholamines, plot of the method comparison of metanephrines, fragmentation scheme of metanephrine (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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