Analysis of Catecholamines in Urine by Positive-Ion Electrospray Tandem Mass Spectrometry

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Background: Determination of urinary catecholamines (CATs) is considered important for clinical diagnosis of pheochromocytoma, paraganglioma, and neuroblastoma. The major disadvantages of existing tests include relatively long instrumental analysis time and potential interference from drugs and drug metabolites that are structurally similar to CATs.

Methods: CATs were extracted from a 300-μL aliquot of urine by a two-step liquid-liquid extraction method specific for compounds containing a catechol group. Chromatographic separation did not require the use of ion-pairing reagents, which typically hinder MS detection but are frequently used in HPLC analysis of CATs. Instrumental analysis was performed by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in the multiple-reaction monitoring mode. Stable-isotope-labeled CATs were used as internal standards.

Results: Epinephrine (E), norepinephrine (NE), and dopamine (D) were measured within 3.5 min instrumental run time. Quantification limits were 2.5 μg/L for E and D and 10 μg/L for NE. The total imprecision (CV) was ≤9.6%; extraction recoveries were 71% ± 12%.

Conclusions: HPLC with ESI-MS/MS in combination with sample preparation specific to catechol group-containing compounds allows rapid testing for disorders associated with increased CAT concentrations. The method is free of interferences from drugs and drug metabolites, which commonly interfere with HPLC methods.

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Catecholamines (CATs) are natural molecules that act as neurotransmitters and hormones. Each of the primary endogenous CATs has characteristic physiologic actions. Epinephrine (E) is quantitatively the most important substance produced by the adrenal medulla; dopamine (D) and norepinephrine (NE) are important neurotransmitters in the central nervous system (1). Determination of CATs in body fluids is used to help identify neuroendocrine disorders and other physiologic and pathologic conditions. Abnormally high urinary or plasma concentrations may indicate a CAT-producing tumor, such as pheochromocytoma, paraganglioma, or neuroblastoma.

Because of the low physiologic concentrations of CATs, the time-consuming analysis, and the tendency of the catechol group to be oxidized, measurement of CATs presents numerous difficulties. A commonly accepted method of catecholamine analysis in biological fluids is liquid chromatography (LC) using a reversed-phase LC column in conjunction with ion-pairing reagents with electrochemical (ECD) or fluorescent detection (2–4). Because of potential interference from a large number of endogenous and exogenous compounds, analysis commonly requires extensive sample preparation in addition to an extended LC separation time. The most common sample preparation methods are based on utilization of Al2O3 or cation-exchange adsorbents (5–7). Commonly used methods for CAT analysis suffer from interference caused by several drugs and dietary constituents. One way to overcome the interference is by avoiding intake of these substances during sample collection. Methods for CAT analysis that use LC combined with fluorescent detection suffer from less interference and usually are more reliable than those that use ECD.

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Chen et al. (8) developed a procedure for CAT analysis that uses 9-fluorenlymethoxyoxycarbonyl (FMOC) derivatization and a quadrupole time-of-flight tandem mass spectrometer. The method uses selective multiple-reaction monitoring (MRM) transitions and therefore is more specific than other LC detection modes. The disadvantages of the method include a high concentration of byproduct (FMOC acid), present after the derivatization reaction, that is highly retained by the reversed-phase column. The presence of the byproducts makes gradient LC elution necessary and increases instrument run time to 20 min per sample. Recently Chan et al. (9) developed a method for analyzing free CATs and metanephrines by LC–mass spectrometry (MS). Sample preparation is performed using Bio-Rex 70 cation-exchange resin followed by analysis on a single-quadrupole LC-MS instrument. The instrumental analysis time is 7 min, and the method is not fully specific. Currently there are no methods available that allow rapid analysis of CATs for clinical applications that are free of interference from drugs and drug metabolites having structures and properties similar to CATs. The goal of this work was to develop a rapid, reliable method for the selective analysis of E, NE, and D in urine samples by LC–tandem MS (MS/MS).

Materials and Methods

CHEMICALS

E, NE, D, tetraoctylammonium bromide (TOAB), diphenylboronic acid 2-aminoethyl ester (PBA), NH4Cl, disodium EDTA, formic acid, sodium metabisulfite, ammonium hydroxide, ammonium formate, 1-octanol, and glacial acetic acid were purchased from Sigma. The certified deuterated analogs d3-E, d3-NE, and d4-D were prepared from the stock solutions at a concentration of 1.5 mg/L in methanol. The deuterated CATs d3-E, d3-NE, and d4-D were purchased from Cambridge Isotope Laboratories. Methanol, heptane, tetrahydrofuran (THF), water (all HPLC grade), microcentrifuge tubes, glass autosampler vials, vial inserts, and caps were obtained from Fisher Scientific. All chemicals were of the highest purity available.

CALIBRATORS AND SOLUTIONS

Stock solutions of calibrators were prepared at a concentration of 1 g/L for E, NE, and D, and contained 1 g/L sodium metabisulfite in aqueous 83 mmol/L acetic acid solution. The combined working calibration solution containing E, NE, and D was prepared from the stock solutions at a concentration of 0.5 mg/L in methanol. The deuterated CATs d3-E, d3-NE, and d4-D were prepared at concentrations of 1 g/L and contained 1 g/L sodium metabisulfite in aqueous 83 mmol/L acetic acid solution. The combined working internal standard solution was prepared from the stock solutions at a concentration of 1.5 mg/L d3-E and d3-NE, and 3 mg/L d4-D. We confirmed that the internal standard solution was free of nondeuterated analogs by analyzing CAT-free urine supplemented with working internal standard. The buffer solution for the first extraction was prepared by dissolving 5 g of EDTA and 0.5 g of PBA in 1 L of 2 mol/L NH4Cl adjusted to pH 8.95 ± 0.1 with ammonium hydroxide. The extraction solvent was prepared by dissolving 6 g of TOAB in 1 L of 77 mmol/L 1-octanol in heptane.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

The PE series 200 HPLC system consisted of two pumps, a vacuum degasser, and an autosampler (Perkin-Elmer Analytical Instruments). The LC column was an Allure Basix™ column [50 mm × 2 mm (i.d.); 5-μm particles; Restek]. The mobile phase was THF–6.5 mmol/L aqueous formic acid (3:2 by volume). All tubing in the HPLC system was made of stainless steel. The mobile phase flow rate was 0.4 mL/min, and the LC column effluent split flow was 0.25–0.3 mL/min. The column temperature was ambient, and the injection volume was 5 μL. The injection syringe was washed eight times between injections with 100 μL of water. The injection interval was 3.5 min.

The API 2000 (Applied Biosystems/MDS SCIEX) tandem mass spectrometer was used in the positive-ion mode with a TurboIonSpray™ interface. Quantitative analysis was performed in the MRM mode. The collision gas was nitrogen with a cell pressure of 1.1 Pa. The TurboIonSpray capillary voltage was 6.0 kV, the orifice voltage was 15 V, and the mass transition-dependent collision energy was 50 V for transitions m/z 184→107, 170→107, 154→91, 187→110, 173→110, and 158→95, and 65 V for transitions m/z 184→77, 170→77, and 154→65.

MRM transitions monitored were m/z 184→107 and 184→77 for E, m/z 170→107 and 170→77 for NE, and m/z 154→91 and 154→65 for D. The quantitative product mass ions for E, NE, and D were (m/z) 107, 107, and 95, and the qualitative product ions were (m/z) 77, 77, and 65, respectively. The MRM transitions monitored for the internal standards d3-E, d3-NE, and d4-D were (m/z) 187→110, m/z 173→110, and m/z 158→95, respectively. The qualitative product ion response ratio acceptability range was established as ± 50% of the mean value of the product ion ratios obtained from the calibrators. The response ratios used for the analytes (m/z) were (184→107)/(184→77) for E, (170→107)/(170→77) for NE, and (154→91)/(154→65) for D. Quantitative data analysis was performed using TurboQuan™ software (Applied Biosystems/MDS SCIEX).

SAMPLE PREPARATION

Aliquots of working internal standard (30 μL) were added to microcentrifuge tubes. To this, 300 μL of urine specimen, 750 μL of PBA containing NH4Cl buffer solution, and 900 μL of TOAB solution in heptane were added. The tubes were vortex-mixed for 3 min and centrifuged at 15 000g for 3 min. The supernatant was transferred to a second set of tubes containing 50 μL of 166 mmol/L aqueous acetic acid and 500 μL of 1-octanol. The tubes were vortex-mixed for 3 min and centrifuged at 15 000g for 3 min. The organic phase was discarded, and the
aqueous portion was transferred to labeled autosampler vials.

**EFFECT OF LC ELUENT COMPOSITION ON CAT IONIZATION AND MRM FRAGMENTATION**

A series of test eluents were prepared for a study of the organic modifier and electrolyte additive influence on the CAT IonSpray-MS/MS response. The eluents contained E, NE, and D at 0.01 g/L in 50:50 (by volume) organic solvent–buffer solution. Measurements above pH 8.5 were not investigated because this pH exceeds the recommended stability limit for most of the commonly used silica-based columns. Data were acquired in the positive and negative ionization full-scan mode throughout the m/z 50–250 range. The signals for the [M + H]⁺ and [M – H]⁻ adducts were extracted from the full-scan data, and the peak height was recorded for each compound. The MRM fragmentation of the CAT molecular ions [M + H]⁺ was evaluated at several collision energies at a concentration of 0.01 g/L in a mobile phase composed of methanol–6.5 mmol/L aqueous formic acid (1:1 by volume).

**RECOVERY STUDIES**

Experiments to evaluate absolute extraction recovery were performed with supplemented human urine samples containing CATs at concentrations of 100 and 200 μg/L each of E, NE, and D (n = 6). Internal standard was added to the first group of samples before the extraction and to the second group after the extraction. The extracts were analyzed at the same time. The percentage of recovery was determined by comparing the CAT concentrations in the samples to which the internal standard was added before extraction to the results observed in the samples to which the internal standard was added after the extraction.

**PRECISION, LINEARITY, AND SENSITIVITY STUDIES**

Method imprecision was determined from the results of analyses of controls observed during the method routine utilization (analyzed in duplicate within 15 days). Instrument imprecision was determined by repetitive injections from the same vial of an extracted sample containing 30, 110, and 440 μg/L of E, NE, and D, respectively.

Linearity was evaluated by analyzing samples containing E, NE, and D at concentrations of 1, 2000, 4000, 5000, 6000, 8000, and 10 000 μg/L. The limit of quantification was determined by analyzing calibrators containing progressively lower concentrations of CATs. The upper limit of linearity and the limit of quantification were determined based on criteria of maintaining accuracy within 85–115%, the qualitative branching ratio within ± 50%, and imprecision (CV) <10%. Each sample was analyzed in duplicate over a 2-day period.

**PATIENT SAMPLE COMPARISON STUDIES**

The method comparison was performed on 120 patient specimens submitted for analysis of free CATs. Samples used for analysis were aliquots from 24-h urine collections with the pH adjusted to 1–5. All studies with samples from humans were approved by the Institutional Review Board of the University of Utah. After analysis by the comparative HPLC-ECD method, aliquots of specimens were stored for 1–3 weeks at −70 °C before analysis by LC-MS/MS. To account for imprecision in both the comparative and the evaluated methods, the results were evaluated by Deming regression (10).

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**Fig. 1.** Ionization efficiency (Relative abundance of the [M + 1]⁺ molecular ion) of CATs with different mobile phase compositions in the positive-ion mode.

Buffer: 0.005 mol/L ammonium acetate adjusted to pH 3 with 0.01 mol/L formic acid.
INTERFERENCE STUDIES

Some common drugs and compounds with structures similar to those of the CATs were evaluated for potential interference in the evaluated method. Evaluated compounds were acetaminophen, caffeine, cimetidine, diazepam, isetharine, isoproterenol, labetalol, levodopa, 3-methoxytyramine, metachlopromide, metanephrine, methyldopa, normetanephrine, salsolenol, serotonin, and theophylline. Each individual compound was added to catecholamine-free urine at a concentration of 5000 μg/L. Sample preparation and analysis were performed as described for patient samples.

Results

The ionization efficiencies of E, NE, and D were studied utilizing electrospray (ES) MS using several organic solvents and buffer compositions. The [M + H]⁺ molecular ions were observed for each of the three CATs at pH 2–8, whereas the deprotonated [M – H]⁻ ions were observed only above pH 6 in the presence of ammonium formate buffer. The relative abundance of the [M + H]⁺ molecular ions of the CATs were comparable to one another in the presence of ammonium formate buffer at acidic pH. When the buffer was replaced with formic acid, the abundance of the molecular ions of NE and D increased two- to threefold, whereas the abundance of E increased 20–30% (Fig. 1). Secondary fragmentation of the CATs was investigated under various conditions to determine whether detection specificity could be gained through the utilization of different collision energies. The MS/MS product ion spectra of the [M + H]⁺ molecular ions are presented in Figs. 2–4.

Because the sample preparation is specific to compounds containing the catechol group, LC separation plays a lesser role in the method. A short LC column was used primarily to separate compounds from the solvent front to eliminate possible signal suppression. Comparison of recoveries between an extracted sample and CATs added to the mobile phase at the same concentrations showed that ion suppression did not occur under the LC conditions used. The chromatograms for the monitored MRM transitions for an extracted urine sample are presented in Fig. 5.

The absolute extraction recovery of E, NE, and D for the method was 71% ± 12% (n = 6). The within-run,
between-run, and total imprecision (CV) for the results obtained in the experiments are presented in Table 1. The CV for instrument imprecision, determined by re-injecting a sample from the same vial (n = 15), was 5.5%, 1.9%, and 2.3% for E, NE, D, respectively. The assay was linear up to 10 000 μg/L for each of the CATs, with an accuracy better than 90–110% for each CAT at the highest evaluated concentration of 10 000 μg/L (r = 0.998). The limit of quantification for the method was 2.5, 10, and 2.5 μg/L for E, NE, D, respectively.

Bland–Altman plots for the results of the correlation study with a comparative HPLC-ECD method for urine samples are presented in Fig. 6. Samples included in the study contained CATs at concentrations ranging from within the reference interval to significantly increased. The correlation coefficient, $S_{xy}$, and regression equations for the comparison are presented in Table 2. The regression line slope values for E were lower than those for NE and D. This can be explained by the greater instability of

<table>
<thead>
<tr>
<th>CAT</th>
<th>Concentration, μg/L</th>
<th>CV, %</th>
<th>Within-run</th>
<th>Between-run</th>
<th>Total</th>
<th>Accuracy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>12</td>
<td></td>
<td>6.4</td>
<td>7.1</td>
<td>9.6</td>
<td>100.8</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td></td>
<td>6.2</td>
<td>4.2</td>
<td>7.5</td>
<td>108.5</td>
</tr>
<tr>
<td>NE</td>
<td>29</td>
<td></td>
<td>6.2</td>
<td>6.8</td>
<td>9.2</td>
<td>111.7</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td></td>
<td>5.4</td>
<td>6.9</td>
<td>8.7</td>
<td>101.4</td>
</tr>
<tr>
<td>D</td>
<td>66</td>
<td></td>
<td>5.8</td>
<td>6.4</td>
<td>8.6</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td></td>
<td>3.5</td>
<td>5.1</td>
<td>6.2</td>
<td>107.0</td>
</tr>
</tbody>
</table>

* Two replicates per day over 15 days.

<table>
<thead>
<tr>
<th>CAT</th>
<th>Concentration range, μg/L</th>
<th>Regression equation</th>
<th>r</th>
<th>$S_{xy}$</th>
</tr>
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<tr>
<td>E</td>
<td>0–380</td>
<td>$y = 0.90x - 0.4$</td>
<td>0.983</td>
<td>14.4</td>
</tr>
<tr>
<td>NE</td>
<td>0–600</td>
<td>$y = 1.12x - 1.1$</td>
<td>0.977</td>
<td>18.7</td>
</tr>
<tr>
<td>D</td>
<td>0–7000</td>
<td>$y = 1.05x - 21.7$</td>
<td>0.991</td>
<td>154.4</td>
</tr>
</tbody>
</table>
E. The qualitative ion response ratios in all of the analyzed patient samples with concentrations corresponding to positive results were consistently within the acceptance limits established by the calibration. The data indicated that the bias between the methods was not significant.

None of the evaluated compounds interfered with the analysis except normetanephrine, which produced product mass ions \( m/z \) 107 and 77 of E. Normetanephrine at a concentration of 5000 \( \mu g/L \) produced a signal equivalent to 11 \( \mu g/L \) E with a substantially increased product ion response ratio (\( m/z \) 184→107/184→77). This may be attributable to an impurity in the normetanephrine reagent (the compounds are structural isomers) or rearrangement of a methyl group within the molecule. Normetanephrine in patient specimens is not expected to interfere with the analysis of free CATs because >85% of normetanephrine is present in a conjugated form (11) and the concentration of free normetanephrine rarely exceeds 500 \( \mu g/L \).

Injection syringe wash and LC system carryover for CATs were evaluated by analyzing a negative control after injection of a sample containing 10 000 \( \mu g/L \) each of E, NE, and D. No carryover to the following sample or to the following injection was detected. Between injections, the autosampler injection syringe was washed eight times with water.

CATs are unstable during storage and tend to decompose, especially at increased pH. This presents a difficulty for method standardization. The decomposition rate depends on the solvent used, the pH, storage conditions, and other constituents present in the solution. Raggi et al. (4) reported degradation of CAT calibrators after storage, with formation of an additional peak detectable by ECD. The size of the degradation product peak increased as the calibrator aged. To estimate the extent of the decomposition and to optimize the stability of the calibrators, we

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Table 3. Recovery (%) of CATs in the calibration solution* prepared in different matrices after 2 months of storage at different conditions.

<table>
<thead>
<tr>
<th>Storage temperature, °C</th>
<th>20</th>
<th>4</th>
<th>−20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>31</td>
<td>68</td>
<td>46</td>
</tr>
<tr>
<td>Methanol containing 500 ( \mu g/L ) sodium metabisulfite</td>
<td>48</td>
<td>58</td>
<td>82</td>
</tr>
</tbody>
</table>

* The calibrators were prepared at concentration of 0.5 mg/L.
evaluated CAT degradation after storage at different conditions. The stability was evaluated by analyzing the calibrators prepared in methanol, with and without sodium metabisulfite, and stored at room temperature, 4, and −20 °C for 2 months (Table 3). The results indicate improved stability of the calibrators when sodium metabisulfite was added to the solution and the calibrators were stored at 4 °C.

**Discussion**

Use of PBA-based extraction specific to catechol group-containing compounds has been reported to be more effective than cation-exchange and Al₂O₃-based solid-phase extraction (7). The specificity of this sample preparation method is based on the difference in affinity for PBA between the CATs and potentially interfering compounds present in the sample matrix. CATs react with PBA only in an alkaline solution that promotes the formation of the phenylboronate ion \([\text{PhB(OH)}₂⁻]\), a reactive form of PBA. Compounds containing vicinal cis-diol groups, such as those present in CATs, covalently bind to the boronate ion with the concomitant release of a molecule of water. The product PBA–catecholamine complex is polar and must be coupled with an ion-pairing reagent, such as TOAB, to be extracted into a nonpolar solvent. The complexation reaction requires a pH above 8.5, whereas release of CATs from the complex takes place in acidic solution.

Among the conditions that most markedly affected the extraction were the pH of the solution, an excess of PBA over CAT concentrations, and an excess of TOAB over the phenylboronate–CAT complex. We observed that even
within a pH range sufficient for consistent recovery of the CATs, minor variations in pH during the extraction led to a major difference in recovery between E, NE, and D. Because of the difficulty in maintaining pH within a very narrow range between samples, we decided that it was more practical to use individual deuterated internal standards for each of the CATs rather than restricting the target pH range. Use of individual deuterated internal standards for each of the CATs compensated for the effect of the pH difference, and a substantial improvement in the accuracy and precision of the method was observed.

The sensitivity achieved in the LC-MS/MS system is related to the efficiency of the ionization and desolvation within an interface, and it is critical to balance conditions optimal for sensitivity with the requirements for the chromatographic separation. All published methods of CAT analysis by reversed-phase LC use ion-pairing reagents for the separation because of the highly polar nature of the molecules. Ion-pairing reagents are harmful to ES ionization and cause signal suppression. The Allure Basix LC column, with a polar group imbedded in a hydrophobic chain, was suitable for the separation of basic polar compounds without ion-pairing reagents and produced good peak shape for CATs with a simple mobile phase. A high percentage of organic modifier in the mobile phase improved the ionization/evaporation efficiency in the MS interface and translated into increased sensitivity. We observed improved retention of CATs when THF was used in the mobile phase compared with methanol. This can be explained by the strong proton acceptor properties of THF, which promote pairing with protonated CAT molecules. Under the reported conditions, CATs exhibited good peak shape and sufficient separation from the solvent front.

Ionization efficiency for the CATs in mobile phases of different composition was evaluated to optimize the method sensitivity. CAT molecules can produce both positive and negative molecular ion fragments, depending on the group that is ionized \((12, 13)\). Initial experiments showed that in the positive-ion mode, the intensity of the molecular ion was greater when formic acid was used compared with ammonium formate (Fig. 1). Irrespective of the electrolyte used, methanol promoted a stronger signal than acetonitrile, especially in the presence of formic acid. The best response in the positive-ion mode was observed with methanol in the presence of formic acid at concentrations \(<10 \text{ mmol/L}\).

Because of the relatively soft ionization process of the ES interface, at low orifice voltages Q1 mass spectra typically consist of a single prominent molecular ion. Figs. 2–4 show the effect of increasing collision energy on the fragmentation of E, NE, and D in the positive-ion mode. As the collision energy increased, smaller mass ion fragments were observed and different product ions dominated the mass spectra.

The major product ion fragments of E and NE at low collision energies were observed at \(m/z [M + H - 18]\) and presumably arose from a loss of water. The fragmentation pattern was dependent on the collision energy applied for the MS/MS fragmentation. The sensitivity decreased as the collision energy increased, with the greatest selectivity observed with the medium-range collision energies. The collision-induced dissociation spectra of the CATs produced ions attributable to the loss of \(\text{H}_2\text{O}, \text{CO}, \text{NH}_3\), and \((\text{H}_2\text{O} + \text{CO})\) commonly found during fragmentation of anions \((12)\). Figs. 2 and 3 show the mass spectra of E and NE that correspond to different collision energies. With increasing collision energy, smaller mass ion fragments were observed and different product ions dominated the mass spectra.

Fig. 6. Bland–Altman plots for correlation between LC-MS/MS and HPLC-ECD methods.

(A), E; (B), NE; (C), D. The dashed lines represent 2 SD. Mean differences: \(-0.3, -5.9, \text{ and } 7.9 \mu\text{g/L}\) for E, NE, and D, respectively.
diagnostic ions of NE are the same as those for E and result from the following losses: m/z 152 [170 – H2O], m/z 123 [152 – CNH3], m/z 107 [123 – O], and m/z 77 [107 – CH3O]. The most feasible structures of product ions for the MS/MS spectrum of the protonated molecular ion, m/z 154, of D are depicted in Fig. 4. The product ion mass spectra at low collision energies are dominated by the ion m/z 137, probably attributable to loss of the amine group [M + H – NH3] from the terminus of the molecule. The collision-induced dissociation of the molecular ion, m/z 154, also produced product ions corresponding to m/z 119 [137 – H2O], m/z 91 [119 – CO], and m/z 65 [91 – C2H3]. The m/z 91 and 65 cations likely have very stable aromatic structures (shown in Fig. 4).

Collision energies that produced the optimal signal for each product ion were selected, and voltages on the collision cell were alternated accordingly for each MRM channel specified in the acquisition scan. Alternating the collision cell voltages produced consistent product-ion response ratios for qualitative confirmation of the CATs. The conditions used produced satisfactory sensitivity and specificity for the selective detection of CATs.

In conclusion, we present a method for the selective analysis of CATs that uses positive-ion mode ES ionization. The advantages of the method include its high specificity, which allows the selective quantification of E, NE, and D in the presence of drugs that commonly interfere with HPLC analysis. The selectivity of the method is based on sample preparation specific to compounds containing a catechol group and subsequent unique MS/MS fragmentation of CATs. Such selective detection eliminates the necessity for extensive chromatographic separation and shortens the instrumental analysis time. The method provides high selectivity, sufficient accuracy and precision, and high throughput, which give it advantages over other methods reported in the literature.

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References