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A sensitive and selective quantification of catecholamine neurotransmitters in rat microdialysates by pre-column dansyl chloride derivatization using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and sensitive liquid chromatography tandem mass spectrometry method for simultaneous quantification of catecholamine neurotransmitters in microdialysates was developed. The catecholamine neurotransmitters dopamine (DA) and norepinephrine (NE) were pre-column derivatized with dansyl chloride and analyzed. A gradient elution method was used to separate the analytes from the interferences on an Agilent Poroshell 120 EC-C18 outer porous micro particulate column. The method was robust and sensitive to determine with the lower limit of quantification value of 0.068 pmol/mL and 0.059 pmol/mL for DA and NE, respectively. It has acceptable precision and accuracy for concentrations over the standard curve range. The method was successfully applied for simultaneous quantitation of DA and NE in the prefrontal cortex (PFC) dialysates of rats obtained from a microdialysis study dosed with vehicle and atomoxetine through intra peritoneal (i.p.) route at a dose of 3 mg/kg to monitor the change in extracellular concentrations. Thus, accomplishment of this method would facilitate the neurochemical monitoring for discovery of new chemical entities targeted for the treatment of attention deficit hyperactivity disorder (ADHD).

1. Introduction

Dopamine (DA) and norepinephrine (NE) are two prominent catecholamine neurotransmitters in the extracellular fluid of the brain. They play a vital role in many functions of the brain and critical in attention and focus [1,2].

Attention-deficit/hyperactivity disorder (ADHD) is a psychiatric disorder that affects both children and adults. However, little is known about the mechanisms of drugs to treat the symptoms. Modulation of DA and NE in cortical (prefrontal cortex, PFC) and subcortical (nucleus accumbens, NACb) extracellular fluid is critical in controlling the impulsive behavior associated in ADHD [1,2]. Adequate levels of DA and NE are vital in PFC function, and insufficient levels can lead to PFC dysfunction and symptoms similar to ADHD [2–5]. Moreover, the extracellular concentrations of these neurotransmitters are significant for communication between neurons, thus monitoring of neurochemicals in extracellular fluids are essential in a variety of CNS disorders [3]. The in vivo brain microdialysis in preclinical species is the practical way to monitor the modulation of catecholamines in PFC by new drugs designed to treat ADHD. It offers to measure the neurotransmitters from conscious and freely moving animal over the time [6–9]. However, the samples from brain dialysis results small sample volumes (20–30 μL) and pico molar range of dialysate concentrations of neurotransmitters necessitate the sensitive analytical methods.

In the literature, various analytical methods are reported for the quantification of dialysates DA, NE and their metabolites. Among them, the most frequently used methods are high performance liquid chromatography (HPLC) coupled with electrochemical detection (HPLC–ECD) [10–15], HPLC coupled with fluorescence detection (HPLC–FLD) [16–20] and HPLC coupled with tandem mass spectrometry (HPLC–MS/MS) [7–9,21–26]. HPLC–ECD methods have limitations like high background noise, low sensitivity and poor separation [10–15]. Similarly, the HPLC–FLD methods suffer with low detection limits, lengthy derivatization process and longer run times [16–20]. Although several HPLC–MS/MS methods are reported for neurochemical quantification, these methods have limitations like elution of analytes of interest in void time, noise interferences, peak resolution, lengthy derivatization processes and longer run times. Cai et al. [21] have reported a HPLC–MS/MS method for the quantification of dansylated monoamine, amino acid neurotransmitters and their metabolites in human plasma. In

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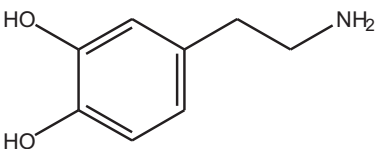
this method, the lower limit of quantification (LLOQ) was 0.57 or 0.40 pmol/mL for DA and NE, respectively, with a 300 μL of plasma.

The purpose of the present investigation was to explore the high selectivity of triple quadrupole tandem mass spectrometry (MS–MS) with electrospray ionization (ESI) interface for the development and validation of a robust reversed-phase method to quantitate DA and NE in rat brain dialysates. The developed method offer several advantages over reported analytical methods like: (1) it requires low matrix volume (20 μL) – as the in vivo microdialysis permits low sample volumes and additionally it can provide better time resolution for microdialysis study design; (2) LLOQ concentrations of 0.068 pmol/mL for DA and 0.059 pmol/mL for NE – adequate to determine the basal levels in dialysates from most commonly studied rat brain regions; (3) a satisfactory resolution and separation from the noise, and interfering peaks due to high salt concentration present in the matrix. The method was validated using artificial CSF (aCSF) and successfully applied to study the modulation of DA and NE levels in rats treated with atomoxetine. Therefore, development of such a sensitive and selective analytical method for catecholamine in rat microdialysates will be useful in discovering the new chemical entities for the treatment of ADHD and other CNS disorders.

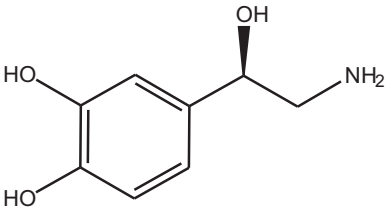
2. Experimental details

2.1. Chemicals

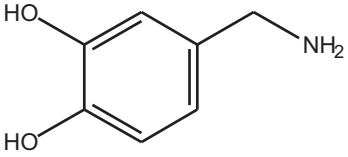
HPLC-grade methanol, acetonitrile and acetone were purchased from Merck (Darmstadt, Germany). Ammonium acetate, formic acid and sodium hydroxide were purchased from Merck (Worli, Mumbai, India). HPLC grade water was generated using Milli-Q system (Millipore, Bedford, MA, USA). Isoflurane was obtained from Baxter Healthcare Corporation (Deerfield, IL, USA). Dansyl chloride, sodium chloride, potassium chloride, calcium chloride dihydrate and magnesium chloride were obtained from Sigma–Aldrich (St. Louis, MO, USA). Atomoxetine hydrochloride standard was obtained from Sigma–Aldrich (St. Louis, MO, USA). Dopamine hydrochloride, norepinephrine bitartrate and 3,4-dihydroxybenzylamine hydrobromide (the internal standard; IS) standards (Fig. 1) were obtained from Sigma–Aldrich (St. Louis, MO, USA).



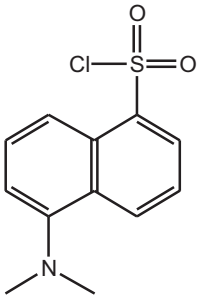
(A) Dopamine [DA]



(B) Norepinephrine [NE]



(C) 3,4-Dihydroxybenzylamine [DHBA] (IS)



(D) Dansyl chloride (derivatizing agent)

Fig. 1. Chemical structures for (A) dopamine, (B) norepinephrine, (C) 3,4-dihydroxybenzylamine (the internal standard) and (D) dansyl chloride (the derivatizing agent).

2.2. LC–MS/MS instrument and conditions

The sample analysis was performed on an API-4000 triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada), which was coupled with the HPLC SIL HTC system (Shimadzu Corporation, Kyoto, Japan). The HPLC system was equipped with LC-AD VP binary pump, a DGU20A5 degasser, and a SIL-HTC auto sampler equipped with a CTO-10AS VP thermostated column oven. An Agilent Poroshell 120 EC-C18 2.7 μm, 4.6 mm × 100 mm column (West Chester, PA, USA) at 25 °C temperature was used for chromatographic separation.

2.3. LC–MS/MS method

The analytes were eluted by a gradient mobile phase system consisting of solvent A (10 mM ammonium acetate adjusted to pH 4.0 with formic acid) and solvent B (acetonitrile). After sample injection, a fixed combination of 58% solvent A and 42% solvent B was held for 0.5 min, then solvent B was steeply changed to 95% until 0.6 min and held up to 4.0 min and after this solvent B was steeply reversed to 42% from 4.0 min to 4.1 min. Finally, the combination of 58% solvent A and 42% solvent B held up to 5.0 min for equilibration of the column. The mobile phase was pumped at a flow-rate of 1.2 mL/min with a split ratio of load to waste 10:90. The high proportion of organic solvent (95% of acetonitrile) eluted the dansylated DA, NE and the IS at retention times of 4.24, 3.82 and 4.12 min, respectively.

The mass spectrometer was operated in positive ionization electrospray multi reaction monitoring (MRM) mode. Typically, source conditions were optimized as follows: the turbo-gas temperature was set at 450 °C, and the ion spray needle voltage was adjusted at 5500 V. The common parameters, viz., ion source gas-1, ion source gas-2, curtain gas and collision gas were set at 30, 25, 15 and 6, respectively. The mass spectrometer was operated at unit resolution for both Q1 and Q3 with a dwell time of 100 ms per MRM channel. The precursor/product ion pairs monitored were

m/*z* 853–170, *m*/*z* 869–170 and *m*/*z* 839–170 for the dansylated DA, NE and the IS, respectively. The collision energy was set at 79 V for dansylated DA, NE and the IS. Data acquisition was performed with Analyst® software version 1.5.2 (MDS-SCIEX, Concord, Ontario, Canada).

2.4. Surgical procedure

Stereotaxic surgery and microdialysis procedures used here were modified from previous procedures described by Nirogi et al. [27]. Rats were anesthetized using isoflurane (induction: 5%; maintenance: 2%) and mounted in a stereotaxic apparatus (Stoelting, IL, USA) with the incisor bar set at 3.2 mm below the horizontal plane passing through the interaural line. An incision was made to reveal bregma from which coordinates were taken. Holes were then drilled for anchor screws and another for placement of a guide cannula (BASi, IN, USA) into the PFC (Anterior Posterior +3.2 mm, Medial Lateral +0.5 mm, Dorsal Ventral −1.0 mm). Coordinates were taken according to Paxinos and Watson [28] with reference points taken from bregma and vertical from the skull. Cannula was secured to the skull using dental cement (DENTALON® plus, AgnTho's AB, Sweden) and anchor screws (CMA Microdialysis, Stockholm, Sweden). The wound was sutured and the animals were left to recover for 5 days in round.

2.5. Microdialysis experiment

One day prior to microdialysis experiment, rats were connected to a dual quartz lined two-channel liquid swivel (Instech, UK) on a counter balance lever arm, which allows unrestricted movements of the animal. Pre-equilibrated microdialysis probe (surface lengths: 4 mm for PFC, BASi, IN, USA) was inserted into guide cannula of the unrestrained rat 16 h prior to the initiation of the experiment.

On the day of study, the probe was perfused at a constant rate of 1.5 μL/min with aCSF (NaCl 150 mmol, KCl 3.0 mmol, $MgCl_2$ 0.9 mmol, and $CaCl_2{\cdot}2H_2O$ 1.7 mmol). A stabilization period of 2 h was maintained, and four basal samples were collected at 30 min intervals. Test compound/vehicle was administered and dialysate samples were collected at 30 min interval for an additional 4 h period. All samples were stored below −70 °C until quantification of DA and NE.

2.6. Sample preparation

2.6.1. Preparation of standards

Standard stock solutions (1 mg/mL) of DA, NE and the IS were individually prepared in methanol (100%, v/v). Working solutions for calibration and controls were prepared by appropriate dilution in aCSF (100% v/v; diluent). The IS working solution (5 μg/mL) was prepared by diluting its stock solution with diluent. Working solutions (0.020 mL) of aCSF were added to test tubes, to obtain DA concentration levels of 0.068, 0.136, 0.340, 0.681, 1.361, 3.404, 6.807 and 13.614 pmol/mL and NE concentration levels of 0.059, 0.119, 0.297, 0.595, 1.189, 2.973, 5.947 and 11.893 pmol/mL as a single batch at each concentration. Quality control (QC) samples were prepared as a bulk on an independent weighing of standard drugs, at concentrations of 0.068 (LLOQ), 0.204 (low), 6.807 (medium) and 10.892 pmol/mL (high) for DA and 0.059 (LLOQ), 0.178 (low), 5.947 (medium) and 9.514 pmol/mL (high) for NE as a single batch at each concentration.

2.6.2. Sample preparation in aCSF/dialysates (derivatization of sample)

20 μL of aCSF/dialysate sample was pipetted into a test tube, then 2 μL of the IS working solution (5 μg/mL) was added and mixed well. To this 10 μL of 0.05 M sodium hydroxide solution was added and vortex mixed for 30 s, then added 50 μL of 1 mg/mL dansyl chloride (derivations agent) and vortex mixed for 30 s. Then samples were kept in a hot water bath (Julabo Labortechnic GMBH, Seelbach, Germany) for 20 min at 50 °C. Then the derivatized sample was removed from the water bath, transferred into labeled vials and 5 μL aliquot was injected into the chromatographic system.

2.7. Bioanalytical method validation

All the validation parameters were performed using aCSF utilizing similar HPLC and mass spectrometric conditions. The within-batch precision and accuracy were determined by analyzing four sets of QC samples (LLOQ, low, medium and high concentrations) each comprised of six replicates in a batch and the between-batch precision and accuracy as three different batches. The acceptance criteria for within- and between-batch precision were 20% or better for LLOQ and 15% or better for the other concentrations, and the accuracy were 100 ± 20% or better for LLOQ and 100 ± 15% or better for the other concentrations.

The stability of the analytes and the IS in stock solutions and as well as in aCSF were evaluated at different temperatures (~4 °C temperature, ~25 °C temperature, −20 °C temperature and at −50 °C). QC samples were subjected to short-term room temperature conditions, to long-term storage conditions. All the stability studies were conducted at two concentration levels (low and high QC values) with six replicates for each. All these stability samples were compared against freshly prepared samples.

3. Results and discussion

3.1. Method development

Catecholamine neurotransmitters are the low molecular weight primary amines containing one amino group connected to the catechol ring by a two carbon side chain. These amines are highly polar in nature and have poor retention times on reverse phase (RP) liquid chromatography (LC) columns. This poor retention behavior of catecholamines on RP columns and their low molecular weights results to higher interferences from the mobile phase used, sample matrix and the environment occur in the low *m*/*z* regions of MS. The ion suppression due to the matrix interference can be avoided by employing sample clean-up procedure along with the use of gradient elution programs. However, these steps may not be possible for dialysis samples obtained from rat brain, due to their low volume. In addition to the low sample volume, dialysates contain high salt concentrations, leading to higher noise in the low *m*/*z* region of MS. In-order to overcome these challenges, we have used pre-column derivatization of monoamines to retain polar compounds in the column by changing their polarity. Thus, we have selected dansyl chloride for the derivatization of primary amines and the phenolic hydroxyl groups present in the catechol nucleus. Moreover, dansyl chloride derivatized compounds give a common product ion of *m*/*z* 170 which is more helpful in MS quantification. As DA, NE and the IS being had a total of three derivatization sites including one primary amine and two phenolic hydroxyl groups; the molecular mass of 233 was added from each dansyl part resulting the $[M+H]^+$ of 853, 869 and 839 for DA, NE and the IS, respectively (Fig. 2A–C).

A 20 μL aCSF sample containing DA, NE and the IS was derivatized using 50 μL of 1 mg/mL dansyl chloride in acetone in the presence 10 μL of 0.05 M sodium hydroxide buffer as basic medium. The derivatized monoamines have retained in the Water symmetry C18, 4.6 mm × 100 mm, 3.5 μm column (Waters, Ireland) for a sufficient period. Further, this method was applied for brain dialysates, but it was found that peaks in study samples were merging in the

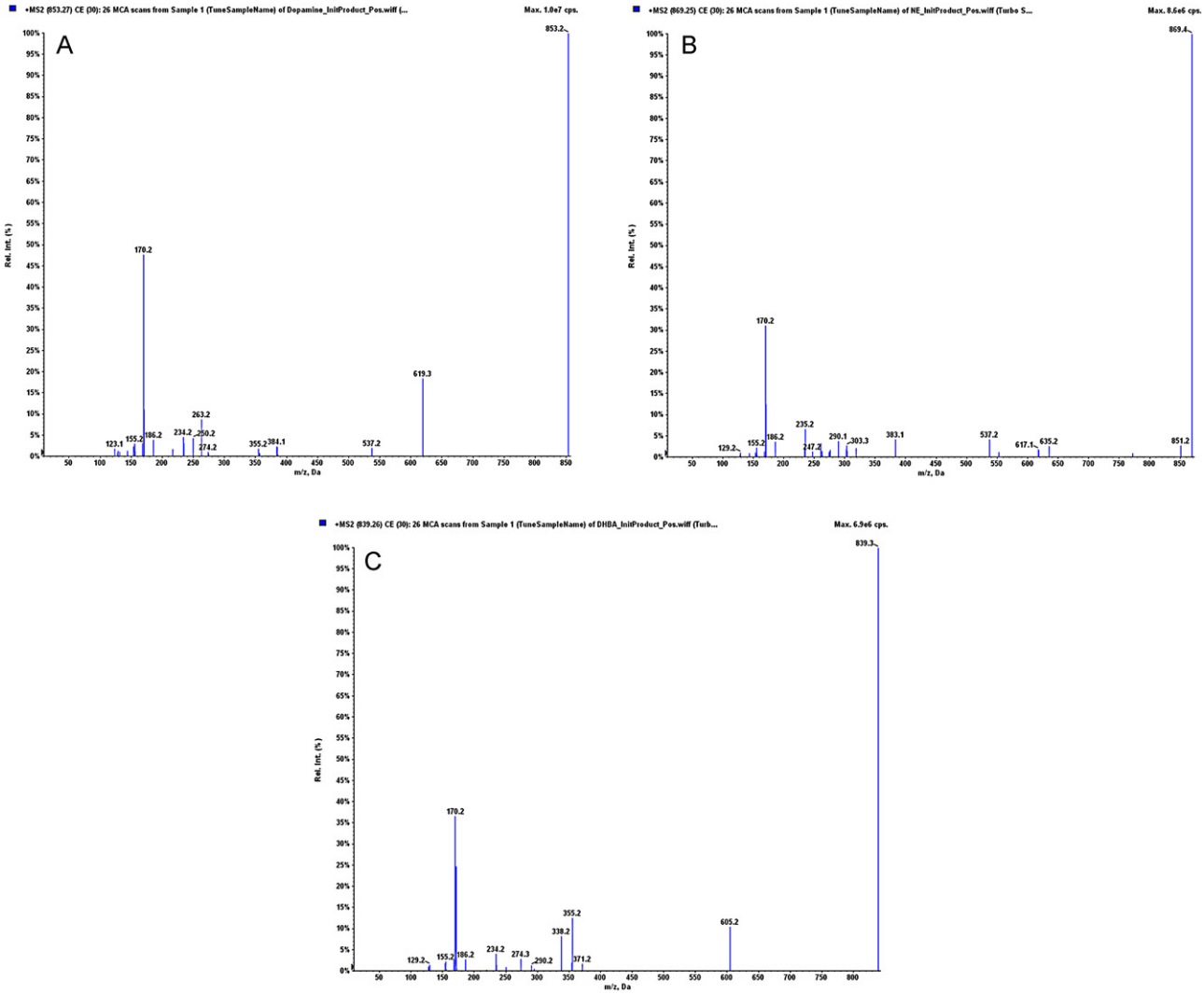


Fig. 2. Full scan product ion mass spectra of dansylated: (A) dopamine, (B) norepinephrine and (C) 4-dihydroxybenzylamine (internal standard).

noise for NE at lower concentrations. Even after testing various gradient programs we were unsuccessful in separating NE in dialysate samples from interfering peaks. In order to address this, series of experiments were conducted using different columns including Zorbax XDB®, YMC-Pack ODS-AQ®, Waters Atlantis® dC18, Waters Atlantis® HILIC, Chromolith Performance® and Agilent Poroshell 120 EC-C18 to optimize the good peak shape and resolution from the noise and the interfering peaks. It was observed that a mixture of 10 mM ammonium acetate buffer (pH adjusted to 4 with formic acid) and acetonitrile could achieve this purpose under gradient program. A smaller particle size column with 2.7 μm (Agilent Poroshell 120 EC-C18, 4.6 mm × 100 mm) efficiently separated the analyte peaks from the interfering peak with high resolution may be due to inner solid core and a porous silica outer layer applied with an EC-C18 bonded phase. The peaks were efficiently separated from the interference with a signal-to-noise ratio of more than 10 for both DA and NE.

In order to prevent contamination of mass spectrometer from higher salt concentrations, the effluent from the column was diverted to waste using a valco valve (Valco Instruments Co. Inc., Houston, TX, USA) system except retention time of analytes. Thus, mobile phase was allowed into the mass spectrometer only during 2.6–4.5 min and remaining was diverted to waste.

3.2. Assay performance and validation

The eight-point calibration curve was linear over the concentration range of 0.068–13.614 pmol/mL for DA and 0.059–11.893 pmol/mL for NE. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ($1/x$, $1/x^2$ and none). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighting factor, giving a mean linear regression equation for the calibration curve.

As shown in Fig. 3A, no significant, direct interference in the aCSF was observed at the retention time of DA, NE and the IS. Fig. 3B depicts a representative ion-chromatogram for the LLOQ (0.068 pmol/mL for DA and 0.059 pmol/mL for NE) in aCSF.

The between-batch percent relative standard deviation (RSD) for DA at the LLOQ, low, medium and high concentrations were 5.0, 7.9, 5.8 and 7.0%, respectively. The between-batch accuracy for DA at the LLOQ, low, medium and high concentrations were 100.8, 99.3, 100.3 and 100.7%, respectively (Table 1). The within-batch RSD for DA at the LLOQ, low, medium and high concentrations were 6.6, 8.7, 3.5 and 6.6%, respectively. The within-batch accuracy for DA at the LLOQ, low, medium and high concentrations were 100.5, 103.0, 103.4 and 105.6%, respectively.

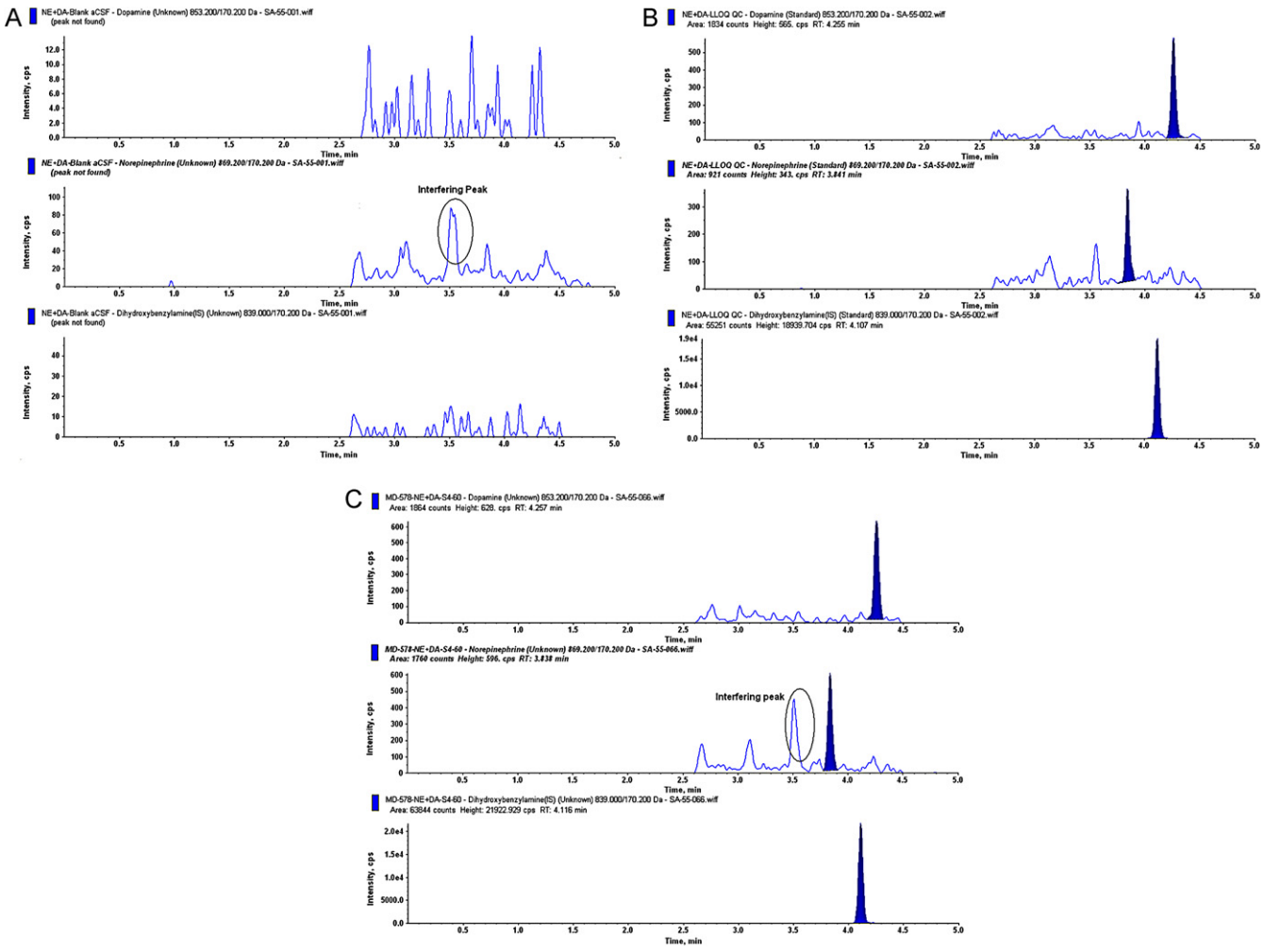


Fig. 3. MRM chromatograms for dopamine and norepinephrine resulting from analysis of (A) blank in aCSF, (B) LLOQ in aCSF and (C) sample chromatogram at the basal level concentrations of 0.069 pmol/mL for DA and 0.113 pmol/mL for NE in PFC of freely moving rats.

The between-batch RSD for NE at the LLOQ, low, medium and high concentrations were 7.0, 8.3, 4.9 and 7.3%, respectively. The between-batch accuracy for NE at the LLOQ, low, medium and high concentrations were 103.5, 101.7, 102.4 and 101.6%, respectively (Table 1). The within-batch RSD for NE at the LLOQ, low, medium and high concentrations were 9.4, 9.6, 3.3 and 5.6%, respectively. The within-batch accuracy for NE at the LLOQ, low, medium and high concentrations were 101.7, 100.0, 100.1 and 101.3%, respectively. A representative MRM chromatogram for DA and NE resulting from analysis of spiked sample at the medium quality control concentrations of 6.807 pmol/mL for DA and 5.947 pmol/mL for NE, respectively, was shown in Fig. S1.

For between-batch experiments and the within-batch experiments, the RSD and accuracy for the analytes met the acceptance criteria (<±15%). The other validation parameters such as selectivity, carryover, recovery and matrix effect met the acceptance criteria from validation guidelines.

Table 1
Precision and accuracy of the method for determining dopamine and norepinephrine concentrations in aCSF samples.

Analyte name	Concentration added (pmol/mL)	Between-batch ($n = 18$)			Within-batch ($n = 6$)		
		Concentration found (mean ± S.D.) (pmol/mL)	Precision (%)	Accuracy (%)	Concentration found (mean ± S.D.) (pmol/mL)	Precision (%)	Accuracy (%)
Dopamine	0.068	0.069 ± 0.003	5.0	100.8	0.068 ± 0.005	6.6	100.5
	0.204	0.203 ± 0.016	7.9	99.3	0.210 ± 0.018	8.7	103.0
	6.807	6.828 ± 0.398	5.8	100.3	7.036 ± 0.249	3.5	103.4
	10.892	10.968 ± 0.771	7.0	100.7	11.502 ± 0.763	6.6	105.6
Norepinephrine	0.059	0.061 ± 0.004	7.0	103.5	0.060 ± 0.006	9.4	101.7
	0.178	0.181 ± 0.015	8.3	101.7	0.178 ± 0.017	9.6	100.0
	5.947	6.087 ± 0.297	4.9	102.4	5.950 ± 0.198	3.3	100.1
	9.514	9.670 ± 0.704	7.3	101.6	9.634 ± 0.541	5.6	101.3

Table 2
Stability results for dopamine and norepinephrine in aCSF.

Analyte name	Sample conc. (pmol/mL)	Calculated conc. (mean ± S.D.) (pmol/mL)	Precision (%)	Accuracy (%)
Autosampler stability for 26 h at 25 °C (n = 6)				
Dopamine	0.204	0.209 ± 0.012	5.7	102.4
	10.892	11.066 ± 0.614	5.6	101.6
Norepinephrine	0.178	0.179 ± 0.012	6.9	101.6
	9.514	10.079 ± 0.456	4.5	105.9
Short-term stability for 4 h at ~25 °C (n = 6)				
Dopamine	0.204	0.187 ± 0.004	2.0	91.5
	10.892	9.549 ± 0.312	3.3	87.7
Norepinephrine	0.178	0.161 ± 0.004	2.3	90.3
	9.514	8.670 ± 0.332	3.8	91.1
Long-term stability for 7 days at below −50 °C (n = 6)				
Dopamine	0.204	0.208 ± 0.014	7.0	101.9
	10.892	10.583 ± 0.489	4.6	97.2
Norepinephrine	0.178	0.178 ± 0.011	6.5	99.8
	9.514	9.667 ± 0.669	6.9	101.6

3.3. Ion suppression

The ion suppression was evaluated using the post column infusion system, as outlined by Nirogi et al. [29]. This problem was evaluated by injecting analytes at higher concentrations through post column infusion. Ion suppression was observed at 0.8 min near to the void volume, whereas, no ion suppression was observed at the retention times of analytes and the IS. Fig. S2 depicts a representative chromatogram for the evaluation of ion suppression in aCSF.

3.4. Stability studies

For short-term stability determination, stored aCSF aliquots were thawed and kept at room temperature for a period exceeding that expected to be encountered during routine sample preparation (~4–24 h). Samples were processed and analyzed as described above and the results indicated reliable stability behavior only up to ~4 h under the experimental conditions of the regular analytical procedure (Table 2). The results for the short-term stability samples up to 24 h do not meet the acceptance criteria of ±15% (data not shown). The stability of processed QC samples kept in the autosampler for 26 h was assessed and the results (Table 2) indicate that reliable stability of the analytes and the IS can remain in the autosampler for at least 26 h without showing significant loss in the quantified values, indicating that processed samples should be analyzed within this period.

The long-term stability data of the analytes in aCSF (at below −20 °C and −50 °C) stored for a period of 7 days were also assessed and showed reliable stability behavior only at below −50 °C, as the mean of the results of the tested samples were within the acceptance criteria of ±15% of the initial values of the controls (Table 2). The results for the long-term stability samples at below −20 °C did not meet the acceptance criteria of ±15% of the initial values of the controls (data not shown). These findings indicate that storage of the analytes in aCSF is stable up to 7 days at below −50 °C and should be processed within 4 h from withdrawing.

The stability of the main stock solutions was tested and established at room temperature for 6 h, 24 h, and under refrigeration (~4 °C) for 7 days (data not shown). The results revealed optimum stability for the prepared stock solutions throughout the period intended for their daily use.

Addition of antioxidants to aCSF or dialysates in order to confer the stability to analytes of interest caused a significant reduction in area response. This observation may be due to contraindicated pH value of antioxidant stabilizers, which are terminating derivatization process.

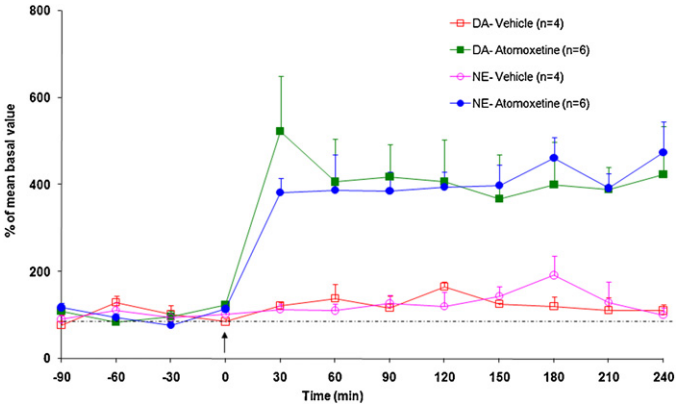


Fig. 4. Representative data showing the mean % change in DA and NE in PFC after the administration of control (vehicle) and atomoxetine at 3 mg/kg i.p. to freely moving rats.

3.5. Application

The validated method was successfully applied to quantify the concentrations of DA and NE in dialysate samples obtained from the rat brain microdialysis studies. Atomoxetine is a non-stimulant; selective NE reuptake inhibitor approved by United States Food and Drug Administration (US FDA) for treatment of ADHD. As atomoxetine increases both DA and NE in PFC, we have administered atomoxetine through intra peritoneal (i.p.) route at a dose of 3 mg/kg to monitor the change in the extracellular concentrations of DA and NE. Four rats have administered with the vehicle and six rats with atomoxetine. The obtained microdialysate samples were analyzed using the validated method. The results with atomoxetine for DA and NE modulation are in agreement with the reported results [2]. Fig. 3C depicts a representative ion-chromatogram for an in vivo sample (0.069 pmol/mL for DA and 0.113 pmol/mL for NE) at the basal concentration. Modulation of DA and NE in PFC of male Wistar rats administered with control (vehicle) and atomoxetine at 3 mg/kg i.p. route is shown in Fig. 4.

4. Conclusions

In summary, a method is described for the quantification of DA and NE in rat microdialysates using LC–MS/MS with dansyl chloride derivatization and validated according to commonly accepted criteria. The present method has shown acceptable precision and adequate sensitivity in quantifying the basal levels of DA and NE in brain extracellular fluids obtained from in vivo rat brain

microdialysis study. Basal levels of DA and NE in PFC are lower than the majority of regions studied with rat brain microdialysis; thus the method demonstrated can be applicable in other regions such as medial PFC, striatum and hippocampus. Moreover, results indicated that the samples should be derivatized with in 4 h from the time of collection and need to be analyzed with in 26 h after completing the derivatization reaction. The current method demonstrated that interference obtained in LC–MS/MS due to high salt concentrations in dialysates can be suitably resolved using low particle size column and gradient programs. Additionally this selective method would facilitate the neurochemical monitoring of discovery of new chemical entities targeted for the treatment of ADHD and other CNS disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.09.034.

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