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New approach to the simultaneous analysis of catecholamines and tyrosines in biological fluids

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Abstract

New high-performance liquid chromatographic (HPLC) methods with amperometric–CoulArray detection were developed for simultaneous analyses of norepinephrine, epinephrine, L-DOPA, dopamine, 3-nitrotyrosine, *m*-, *o*-, and *p*-tyrosines. Overall, detection limit was in the low pmol range with amperometry, and in the low fmol range for the CoulArray method. Linear (r^2=0.99) detector performances were observed in the ranges of 2–200 pmol with amperometry, and 0.2–20 pmol for the CoulArray method. Analytical precision values were better than 80 and 95% for HPLC–amperometry and HPLC–CoulArray method, respectively. These methods offer sensitivity, specificity, minimal sample requirement, and especially the HPLC–CoulArray method allows simultaneous assessment of various similar biomolecules.

Keywords: Detection, LC; Amperometric detection; CoulArray detection; Catecholamines; Tyrosines

1. Introduction

The area of reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation during oxidative stress conditions has sparked interest due to its relevance to various pathological conditions such as acute lung diseases [1], atherosclerosis [2,3], Parkinson's disease [4] and inflammatory bowel diseases [5]. Also, activated oxygen and nitrogen radicals are formed under normal physiological conditions as by-products of cellular respiration [6], and are involved in cell signaling [7] and in defence mechanisms [8].

Formation of ROS and RNS during oxidative stress in vivo can be assessed by analysis of their reaction products. For instance, generation of hydroxyl radical, a reactive oxygen species is assessed by the analysis of tyrosine isomers (Fig. 1) that are formed as a result of hydroxylation of phenylalanine [9–11]. Similarly, presence of peroxynitrite, a reactive nitrogen species is followed by analysis of 3-nitrotyrosine (Fig. 1) in biological systems [12]. Catecholamines (Fig. 1) are another class of compounds studied with respect to oxidative stress-related pathology [13,14]. For instance, catecholamines have been associated with oxidative stress related-pressor responses [15], and L-DOPA and dopamine have been implicated in oxidative stress-mediated apoptosis [16].

It is crucial that sensitive and selective analytical methods with minimal experimental artifacts are developed for the estimation of very low levels of markers of oxidative stress-mediated patho-

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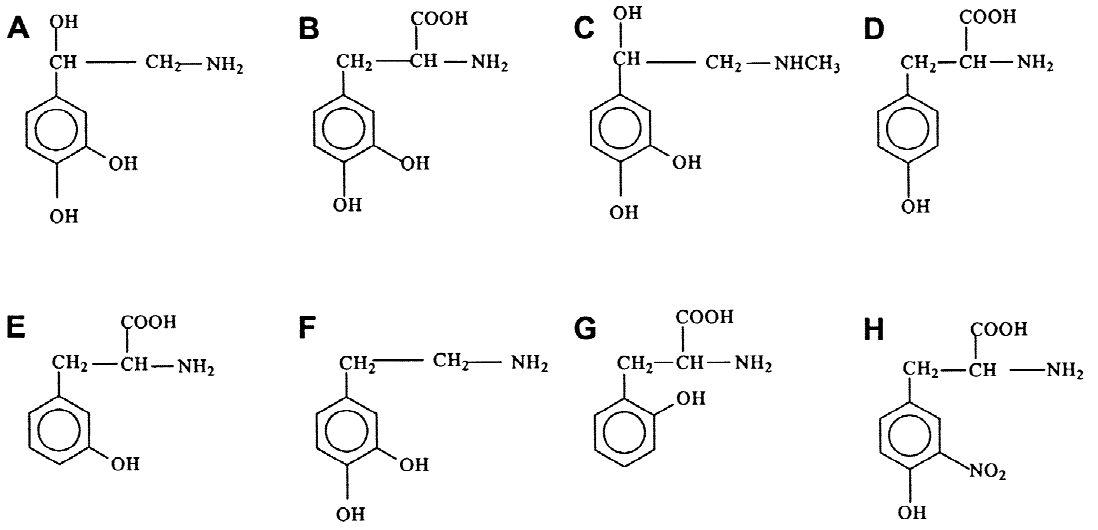

Fig. 1. Chemical structures of (A) norepinephrine, (B) L-DOPA, (C) epinephrine, (D) *p*-tyrosine, (E) *m*-tyrosine, (F) dopamine, (G) *o*-tyrosine and (H) 3-nitrotyrosine.

physiological changes. Existing HPLC methods for the analyses of plasma samples for catecholamines involve detection by radioactive immunoassay [17], electrochemical [18,19], or fluorescence methods [20,21]. Similarly, tyrosines and 3-nitrotyrosine in plasma samples have been analyzed by various HPLC methods [22]. These analysis methods for catecholamines, tyrosine and 3-nitrotyrosine involve separate sample preparation procedures and analysis conditions and, therefore, require increased plasma volumes, will be more labour intensive and costly. The main objective of this study was to develop a sensitive, specific, rapid and simple HPLC method that will allow us to simultaneously measure these oxidative stress markers in biological samples for routine assessment of physiological or various pathological processes.

2. Experimental

2.1. Chemicals

Dulbecco's phosphate-buffered saline (PBS, calcium and magnesium free), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DETPA), phenylmethylsulfonyl fluoride (PMSF), sodium acetate, trisodium salt of citric acid, Trizma hydrochloride, Trizma base, molecular mass cut-off filters (M_r 30 000) and standards of norepinephrine, L-DOPA, epinephrine, dopamine, *m*-tyrosine, *o*-tyrosine, *p*-tyrosine and 3-nitrotyrosine were from Sigma (St. Louis, MO, USA). Analytical reagent-grade sodium chloride, alumina (acidic) and HCl were from BDH (Toronto, Canada). Acetone and methanol were of reagent-grade purity and were obtained from common commercial suppliers. Butylated hydroxytoluene (BHT) was purchased from United States Biochemical Corporation (Cleveland, OH, USA). Perchloric acid was obtained from Aldrich (Milwaukee, WI, USA). Deionized water was obtained from a Super-Q Plus High Purity Water System (Millipore, Bedford, MA, USA). Compressed gaseous nitrogen was UHP grade quality, and was supplied by Matheson Gas Products (Whitby, Canada).

2.1.1. Standards

Aqueous stock solutions of *m*-, *o*-, *p*-tyrosines, 3-nitrotyrosines and catecholamines such as norepinephrine, epinephrine, L-DOPA, and dopamine were diluted with acidified water (0.2 *M* HCl) to obtain working standard solutions at required concentrations. All working standard solutions were stored at −4 °C for about a week. Typically new stock and working standard solutions were made every week.

2.1.2. Blanks

Acidified deionized water samples served as blanks in the analyses.

2.2. Animals

Wistar male rats (200–250 g) and C57BL/6 mice (15–25 g), were all barrier raised, specific pathogen free, and were obtained from Charles River (St. Constant, Canada). Animals were kept in accordance with the standard operating procedures of the Animal Resources Division and as set forth in the guidelines of the Canadian Council of Animal Care. Procedures pertaining to the use of experimental animals were reviewed by the Animal Care Committee of Health Canada. The animals were housed in plexiglass cages on wood chip bedding, under charcoal-filtered and 0.2-μm filtered air stream, in a 12-h dark/light cycle. Food and water were provided ad libitum.

2.3. Plasma sample preparation

Blood samples were received in vacutainer tubes containing the sodium salt of ethylenediaminetetraacetic acid (10 mg/ml) and phenylmethylsulfonyl fluoride (1.7 mg/ml), mixed gently, and placed on ice. Samples were then centrifuged at 2000 rpm for 10 min to obtain plasma. Aliquots of plasma samples were transferred into 1.5-ml Eppendorf tubes and were treated with 200 μl of aqueous DETPA solution and 200 μl BHT solution in isopropanol at strengths of 0.1 and 0.3 *M*, respectively, per 1 ml of plasma sample, and were vortexed to prevent any autoxidation processes. Plasma samples were then cleaned up by either the alumina or molecular mass cut-off method prior to the analysis by HPLC.

2.4. Sample clean up by alumina method

The following plasma clean up procedure was based on the commonly adapted procedure for extraction of plasma catecholamines [23,24] with some modifications. Thirty mg portions of alumina (acidic) in 5 ml conical tubes were treated with either aliquots of 1 ml of plasma samples containing BHT and DETPA, or aliquots of a working standard mix solution containing catecholamines, tyrosines and 3-nitrotyrosine standards in PBS. One ml aliquots of Tris buffer (pH 8.6) containing EDTA at 0.20 m*M* and $NaHSO_3$ at 10 m*M* were then added to these sample tubes, vortexed for 5 min and allowed to stand for 15 min. All samples were then centrifuged at 2800 rpm for 10 min, and supernatants were isolated by decanting. Residues were washed with 1-ml aliquots of ice cold deionized water, and were centrifuged. The washing procedure was carried out three times. Supernatants were discarded. Aliquots of 125 μl of ice cold 0.1 *M* perchloric acid containing 5 m*M* EDTA were then added to the alumina residues, vortexed for 10 min, and samples were centrifuged again. Supernatants were filtered via Acrodisc LC13 poly(vinylidene difluoride) (PVDF) syringe filters (Gelman Sciences, Ann Arbour, MI, USA). Filtrates were analyzed by the HPLC–amperometry method after dilution with acidified water.

2.5. Sample clean up by molecular mass cut-off method

Plasma samples (250 μl) containing BHT and DETPA were treated with 1.5-fold, by volume, of ice-cold acid–acetone mix (acetone–1 *M* HCl–water, 40:1:5, v/v) to precipitate proteins. Samples were vortexed and centrifuged at 9000 *g* for 10 min to obtain supernatants and were concentrated to 150 μl by evaporation under nitrogen flow. The samples were deproteinized and concentrated once again. Molecular weight cut-off filters (M_r 30 000) were washed with 50 μl of deionized water by centrifugation at 5000 *g* for 10 min. Concentrated samples were then loaded into the washed molecular mass cut-off filters and centrifuged at 5000 *g* for 30 min. After complete drainage of samples, the molecular mass cut-off filters were washed with 75 μl aliquots of a methanol–water (50:50, v/v) mix. Filtrates were then dried under a flow of nitrogen. Samples were reconstituted with 200 μl of acidified water, vortexed gently and were diluted as required for the analysis by HPLC.

2.6. Analysis methods

2.6.1. HPLC–amperometry analyses

The HPLC unit consisted of a BAS 400 solvent delivery system (Bioanalytical Systems, West Lafayette, ID, USA), a Gilson autosampler (model

231 XL; Middleton, WI, USA), a Supelcosil LC18 reversed-phase column (25 cm×4.6 mm I.D., 5 μm particle size; Supelco, Bellefonte, PA, USA) and an electrochemical detector equipped with a glassy carbon working electrode and an Ag/AgCl reference electrode (BAS amperometric detector, model LC-4B). PE NELSON software (Perkin-Elmer Nelson Systems, Cupertino, CA, USA) was used for data collection and analysis. The mobile phase (pH 4.75) was composed of 97.2% sodium citrate (final concentration, 26.3 m*M*) in acetate buffer (final concentration, 10.9 m*M*) and 2.8% methanol. Isocratic elution of analytes was carried out at a flow-rate of 1.0 ml/min, and the analyses were performed at an oxidation potential of +1.2 V. Injection volume was 100 μl. Run time was 38 min. Working standard solutions, plasma samples, and spiked plasma were analyzed along with acidified water blanks.

2.6.2. HPLC–CoulArray analyses

The HPLC unit consisted of a solvent delivery module (model 582; ESA, Chelmsford, MA, USA), a ESA autosampler (model 542), a ESA model 5600A CoulArray detector and a Supelcosil LC18 reversed-phase column (25 cm×4.6 mm I.D., 5 μm particle size; Supelco). The CoulArray detector unit comprised eight electrodes in series. The mobile phase composition was as that used for the HPLC–amperometric system. Isocratic elution of analytes was carried out at a flow-rate of 1.0 ml/min. The applied voltages on the eight channels were +(0, 150, 300, 450, 600, 650, 700, 800) mV. Injection volume was 20 μl. Run time was 38 min for the set of target analytes discussed in this study. Working standard solutions, plasma samples, and spiked plasma were analyzed along with acidified water blanks.

3. Results

HPLC–amperometry conditions were characterized by analysis of working standard mix solutions containing catecholamines (norepinephrine, epinephrine, L-DOPA, dopamine), 3-nitrotyrosine, and tyrosine isomers. Fig. 2 illustrates the chromatographic profiles of these analytes by HPLC–amperometry. Retention times of norepinephrine, L-DOPA, epinephrine, *m*-tyrosine, dopamine, *o*-tyrosine and 3-nitrotyrosine are 3.5, 4.5, 5.7, 8.7, 10.7, 12.3, 27.1, respectively. Analysis of standards were carried out at +0.8 and +1.2 V to optimize the applied potential for the simultaneous determination of analytes (Table 1). Working standard mix 1 (analyte concentration in nmol/100 μl: norepinephrine 0.088, L-DOPA 0.184, epinephrine 0.068), *m*-tyrosine 0.072, dopamine 0.056, *o*-tyrosine 0.119 and 3-nitrotyrosine 0.015) and mix 2 (analyte concentration in nmol/100 μl: norepinephrine 0.109, L-DOPA 0.238, epinephrine 0.097, *m*-tyrosine 0.085, dopamine 0.090, *o*-tyrosine 0.160 and 3-nitrotyrosine 0.025 were used for these analysis. Results indicated that at +0.8 V, tyrosine isomers and 3-nitrotyrosine were not detected, and the catecholamines, although detected, exhibited low peak area values. At an applied voltage of +1.2 V, all analytes yielded maximum responses (Table 1).

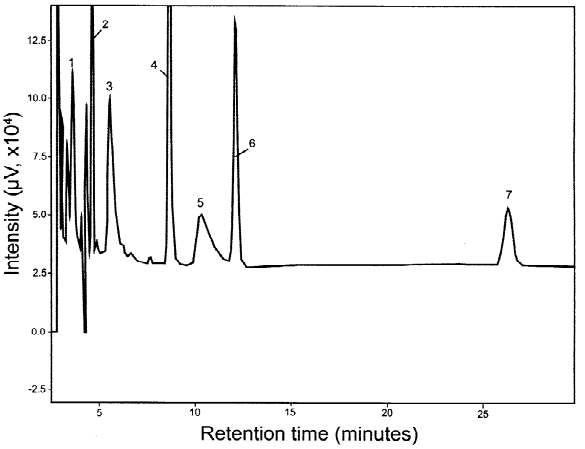

Fig. 2. HPLC–amperometry chromatographic profile of analyte standards (100 pmol/100 μl): 1=norepinephrine, 2=L-DOPA, 3=epinephrine, 4=*m*-tyrosine, 5=dopamine, 6=*o*-tyrosine and 7=3-nitrotyrosine.

Under optimized instrument conditions, the overall detection limit values by analysis of standards were in the low pmol range. Linear relationships (r^2= 0.99) between detector performance and analyte concentrations were established over a range of 2–200 pmol per 100 μl, for all analytes by analysis of working standard mix solutions.

Table 2 summarizes HPLC–amperometry results obtained for a working standard mix solution (analyte concentration in nmol/100 μl: norepinephrine

Table 1
Effect of applied voltage on the HPLC–amperometry analysis

Working standard mix	Applied voltage	Peak area (μV×min)						
		Norepinephrine	L-DOPA	Epinephrine	*m*-Tyrosine	Dopamine	*p*-Tyrosine	3-Nitrotyrosine
1	0.8	589 353	294 403	534 403	BDL	713 280	BDL	BDL
	1.2	1 515 655	1 361 093	1 599 038	1 011 490	1 225 249	1 082 788	243 807
2	0.8	755 089	328 036	796 805	BDL	1 023 935	BDL	BDL
	1.2	1 797 909	1 758 615	2 177 708	1 201 592	1 932 352	1 424 327	363 896

BDL=below detection level.

0.093, L-DOPA 0.166, epinephrine 0.127, dopamine 0.162, *o*-tyrosine 0.177 and 3-nitrotyrosine 0.178) and a rat plasma subjected to the commonly used alumina extraction procedure for catecholamines, and to the procedure employing initial acid–acetone extraction followed by molecular mass cut-off filtration method. Results from Table 2 indicate that with the alumina extraction procedure, tyrosines were not detected, and furthermore, all catecholamines were measurable only with the use of the working standard mix solution. However, with the molecular mass cut-off method, all analytes exhibited increased responses than seen with the alumina extraction method, for both the standard and for the plasma sample (Table 2).

Analytical precision values for duplicate plasma samples cleaned up using the molecular mass cut-off method and analyzed under optimal HPLC–amperometry conditions were better than 80% for all analytes. Fig. 3A and B exhibit the chromatographic profiles of unspiked and spiked rat plasma samples. Spiked recovery values by analysis of unspiked and spiked plasma samples for norepinephrine, epinephrine, L-DOPA, dopamine, *o*-tyrosine and 3-nitrotyrosine were 94, 78, 71, 95, 103 and 78%, respectively.

HPLC–CoulArray detection was optimized for analyte responses by variation of channel voltages. Fig. 4 illustrates the HPLC–CoulArray profiles of a working standard mix solution containing norepinephrine, epinephrine, L-DOPA, dopamine, 3-nitrotyrosine, and the *m*-, *o*-, *p*-tyrosine isomers at the applied voltages, +(0, 150, 300, 450, 600, 650, 700, 800) mV. Retention times of norepinephrine, L-DOPA, epinephrine, *p*-tyrosine, *m*-tyrosine, dopamine, *o*-tyrosine and 3-nitrotyrosine are 3.6, 4.7, 5.6, 6.5, 9.1, 10.9, 12.8, 28.2, respectively. Under optimized HPLC–CoulArray method conditions, detection limits for all analytes were in the low fmol range (≈5). Linear detector performances (r^2=0.99) were established in the individual analyte concentration range of 0.2–20 pmol per 20 μl, for all analytes, by analysis of working standard mix solutions.

HPLC–CoulArray chromatographic profiles of a rat plasma sample at increasing applied potentials are given in Fig. 5A. Fig. 5B clearly illustrates the chromatographic profiles at the two channel voltages where catecholamines, tyrosine isomers and 3-nitro-

Table 2
Comparison of extraction methods

Sample	Method	Peak area (μV×min)					
		Norepinephrine	L-DOPA	Epinephrine	Dopamine	*p*-Tyrosine	3-Nitrotyrosine
Standard mix	Direct injection	1 579 668	1 229126	2 816 898	3 408 220	1 566 325	2 211 433
	M_r cut-off	1 555 133	1 212 560	2 552 407	3 281 864	1 629 202	2 152 600
	Alumina	1 234 757	842 609	1 937 532	1 593 259	BDL	BDL
Plasma	M_r cut-off	1 368 801	184 785	74 566	214 414	61 303	48 125
	Alumina	234 298	88 126	BDL	BDL	BDL	BDL

BDL=below detection level.

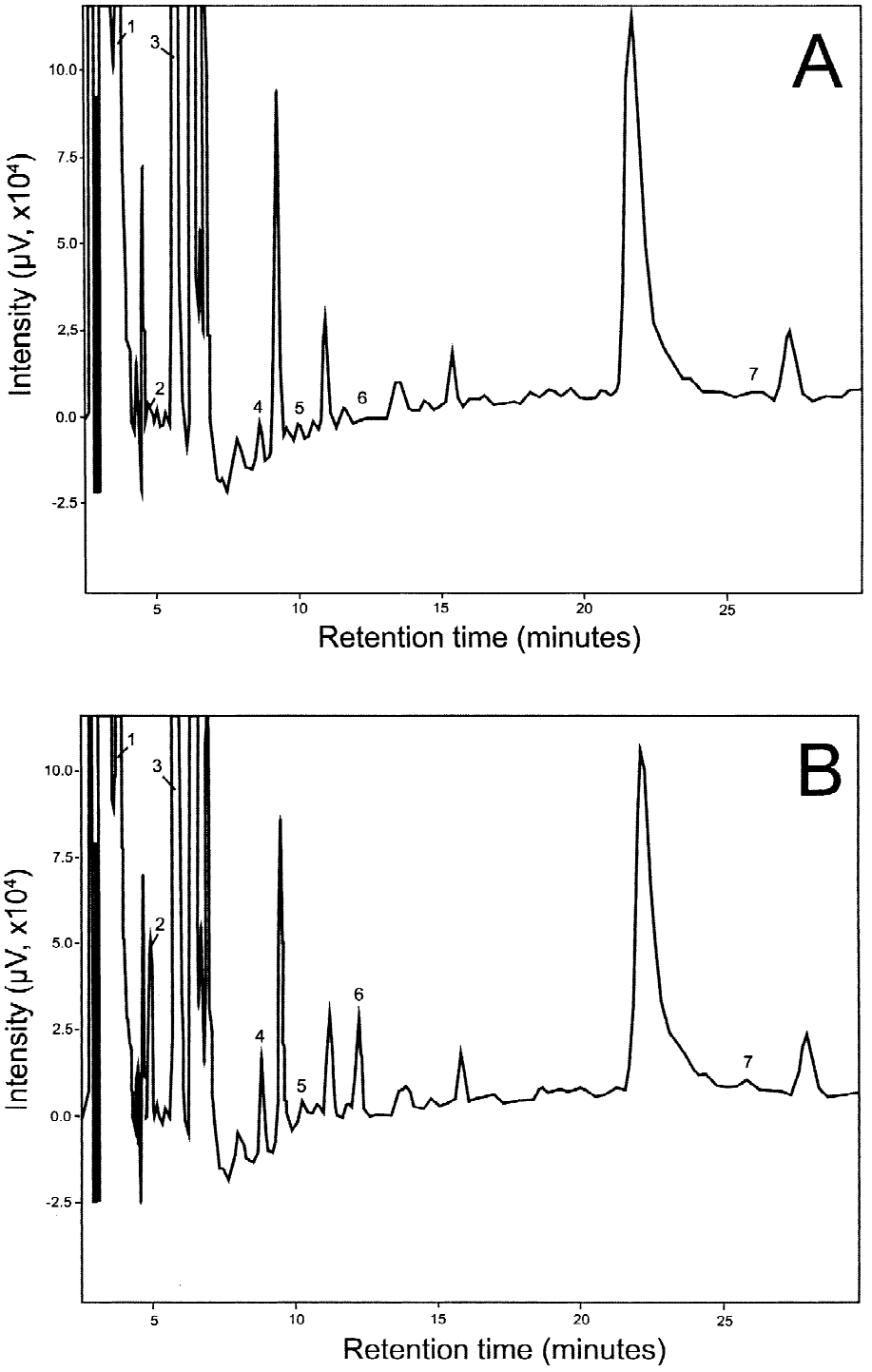

Fig. 3. HPLC–amperometry chromatograms of (A) unspiked (B) spiked (with analyte standards) rat plasma sample: 1=norepinephrine, 2=L-DOPA, 3=epinephrine, 4=*m*-tyrosine, 5=dopamine, 6=*o*-tyrosine and 7=3-nitrotyrosine.

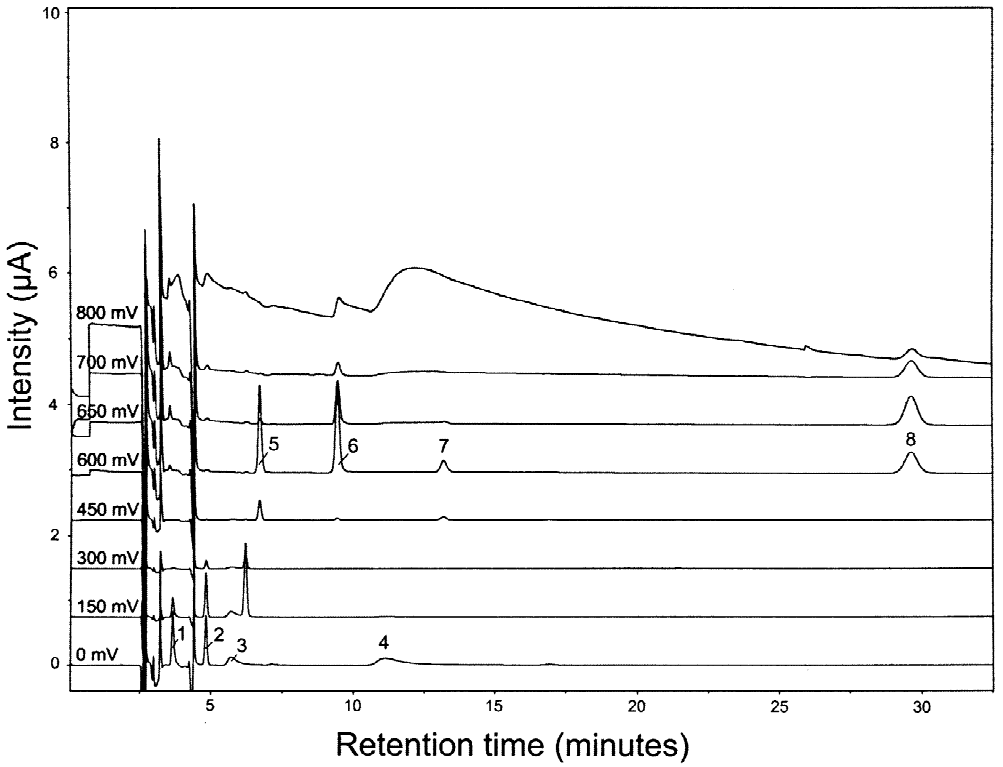

Fig. 4. HPLC–CoulArray chromatographic profiles of analyte standards (each analyte in nmol/20 μl): 1=norepinephrine (0.02), 2=L-DOPA (0.02), 3=epinephrine (0.02), 4=*p*-tyrosine (0.02), 5=*m*-tyrosine (0.02), 6=dopamine (0.02), 7=*o*-tyrosine (0.02) and 8=3-nitrotyrosine (0.04).

tyrosine yield high responses. Analytical precision values for duplicate plasma sample analysis were better than 90% for all analytes. Spiked recovery values determined by analysis of unspiked and spiked plasma samples were 101, 74, 78, 77, 75, 106, 76, 78% for norepinephrine, epinephrine, L-DOPA, dopamine, 3-nitrotyrosine and *m*-, *o*-, *p*-tyrosine isomers, respectively.

With the HPLC–amperometry method, rat plasma (mean of $n=3$ Fisher 344 rats) levels were, for norepinephrine 6.80 ng/ml, L-DOPA 1.05 ng/ml, epinephrine 1.00 ng/ml, *p*-tyrosine 0.671 μg/ml, *m*-tyrosine 4.16 ng/ml, dopamine 3.60 ng/ml, *o*-tyrosine 6.4 ng/ml and 3-nitrotyrosine 11.5 ng/ml. When assessed by the HPLC–CoulArray method, rat plasma (mean of $n=3$ Fisher 344 rats) levels of these analytes were, for norepinephrine 0.676 ng/ml, L-DOPA 0.500 ng/ml, epinephrine 0.367 ng/ml, *p*-tyrosine 3.69 μg/ml, *m*-tyrosine 2.83 ng/ml, dopamine 0.704 ng/ml, *o*-tyrosine 9.84 ng/ml and 3-nitrotyrosine 4.9 ng/ml.

4. Discussion

Although several HPLC methods with different detection methods are reported [12,15,17,18,20,21] for the analysis of catecholamines, 3-nitrotyrosine and tyrosines separately, there are no methods known to date for the analysis of all these analytes concurrently. As there was a need for measurement of the above mentioned analytes in limited volumes of plasma, for instance, from mice blood samples, cannula blood samples, and where the samples also had to be split for other endpoints, the development of a HPLC method for simultaneous analysis of these endpoints was essential. Our goal was to develop a non-radioactive detection method. Furthermore, we

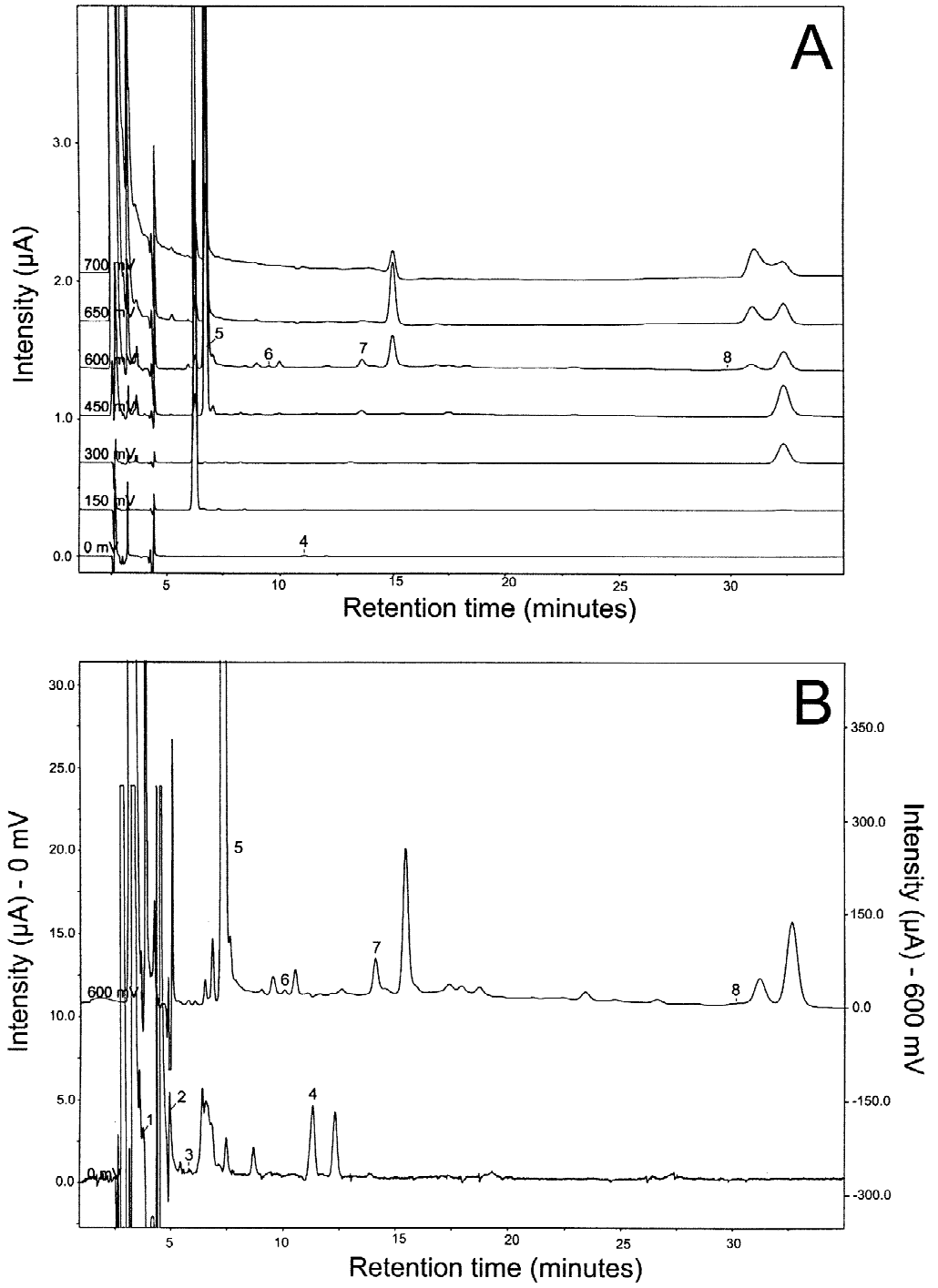

Fig. 5. HPLC–CoulArray chromatograms of rat plasma: (A) display of eight channels, (B) display of two channels of overall maximum response (channel 0 mV, catecholamines; channel 600 mV, tyrosine isomers and 3-nitrotyrosine): 1=norepinephrine, 2=L-DOPA, 3=epinephrine, 4=*p*-tyrosine, 5=*m*-tyrosine, 6=dopamine, 7=*o*-tyrosine and 8=3-nitrotyrosine.

were interested in developing HPLC–electrochemical methods as the target analytes were compounds that participated in in vivo oxido-reductive reactions. As the biomolecules mentioned in this study are typically engaged in interacting mechanistic pathways in vivo, employing one analysis for all analytes can maintain the integrity of the determination of associations among them under specific biological conditions, by eliminating the influence of analytical methods on the end result. For example, inter-analysis method variations can be removed.

Initially, we established a HPLC–amperometry method for the simultaneous analysis of norepinephrine, epinephrine, L-DOPA, dopamine, 3-nitrotyrosine, and the isomers of tyrosines. The information obtained from the HPLC–amperometry analysis was utilized to develop the HPLC–CoulArray method, as we gained access to this unit. We report both the HPLC methods here, so that the users of both these techniques will benefit from them.

Plasma samples were stabilized initially to prevent any postmortem changes such as autoxidation by treatment with BHT and DETPA. Also, the sample clean up procedure was selected to be less labour intensive, and to yield improved recovery for all analytes investigated in this study. As shown by the optimized HPLC–amperometry analysis results (Table 2) for both the analyte standard mix as well as plasma sample, the recovery values were better for all analytes with the acid–acetone precipitation followed by molecular mass cut-off filtration compared to the alumina extraction procedure. Alumina extraction procedure has been reported previously for catecholamines and other similar biogenic amines from plasma [23–25]. In this study, especially, with tyrosines and 3-nitrotyrosine, purification by alumina resulted in loss of detection of these analytes, both from the standard solution and from the plasma sample (Table 2). Alumina has only affinity for hydrocarbons with *cis*-diol groups, and only at a pH value of approximately 8.6. The molecular mass cut-off method was therefore, chosen as the sample clean up procedure as all analytes were recovered better from plasma, compared to the alumina extraction method. Solid phase extraction of catecholamines from plasma samples have been reported previously [26]. However, in terms of analysis of numerous samples and especially, when oxidation of samples is of concern, as this analysis is directed towards the assessment of oxidative stress end points, use of solid-phase extraction for sample clean up may be limited. Furthermore, a solid-state extraction procedure is comparably labour intensive and expensive with respect to the molecular mass cut-off method

Both HPLC–amperometry and –CoulArray methods exhibited the capability to concurrently analyze catecholamines and tyrosines, and these methods were sensitive especially for tyrosines and 3-nitrotyrosine. Blanks were inserted in between every four sample analysis to determine system cross contamination due to sample analysis so that appropriate corrections could be made. In general, there was no cross contamination or sample carry over noticed. Spiked plasma samples analyses were used to confirm the identity of the analyte peaks and also to verify spiked recoveries. Spiked recovery values were in the range of 70–100% for both the HPLC methods. Although in general under these analysis conditions one would expect HPLC–amperometry method to be subjected to interferences, in the vicinity of norepinephrine and epinephrine peaks, the results suggest that in the rat plasma analysis, all analytes determined by HPLC–amperometry were in the same order of magnitude as the levels observed with the HPLC–CoulArray method. Also, we would like to note here that the sets of rats used in the HPLC–amperometry and HPLC–CoulArray analysis were from different cohorts. However, if any interferences arose at the norepinephrine and epinephrine peaks by the HPLC–amperometry method they can be removed by the use of ion-pair reagents [27]. With HPLC–CoulArray method, the norepinephrine and epinephrine peaks did not show any interferences due to the two-dimensional resolution.

Comparison of both the HPLC methods performance indicated that the CoulArray method was superior in terms of sensitivity, typical of coulometric detection compared to amperometry, which is reflected in method detection limits. This permits the use of smaller volumes of plasma samples, $\approx$50 μl for the concurrent analysis of this set of analytes. The HPLC–CoulArray method also exhibited enhanced specificity due to two-dimensional resolution achieved with retention time and the sequentially increasing potentials set at the coulometric electrodes

in series. This can be performed by following the characteristic potentials at which the analytes exhibit maximum responses and also by examining the analyte profiles at the adjacent potentials. Thus each analyte will exhibit a ratio profile in a series of potentials. Deviation from this ratio of responses is suggestive of a coeluting contaminant. This feature greatly facilitates peak detection and confident assignment. Other similarly biologically important molecules that were detectable and measurable from plasma samples using the HPLC–CoulArray method were, chlorotyrosine, uric acid, vitamin C, glutathione and 8-hydroxydeoxyguanosine (data not shown). The HPLC–CoulArray procedure as described here allows the analysis of all these molecules at once, except in the case of 8-hydroxydeoxyguanosine the run time had to be extended by ≈17 min. The CoulArray method thus provides a basis for the analysis of such biomolecules.

In conclusion, both the HPLC methods permitted simultaneous analysis of catecholamines and tyrosines in biological samples, sensitive, require minimal sample volumes, reproducible, and are less labour intensive, exhibit good recovery values and are cost effective. Especially, the HPLC–CoulArray method in addition grants sensitivity and specificity, and thus has extended applicability towards simultaneous assessment of a number of other similar biologically important molecules as well.

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References

[1] H. Ischiropoulos, A.B. al-Mehdi, A.B. Fisher, Am. J. Physiol. 269 (1995) L158.
[2] T. Heitzer, T. Schlinzig, K. Krohn, T. Meinertz, T. Munzel, Circulation 104 (2001) 2673.
[3] O. Meilhac, S. Ramachandran, K. Chiang, N. Santanam, S. Parthasarathy, Arterioscler. Thromb. Vasc. Biol. 10 (2001) 1681.
[4] D. Offen, A. Hochmaan, S. Gorodin, I. Ziv, A. Shirvan, A. Barzilai, E. Melamed, Adv. Neurol. 80 (1999) 265.
[5] A. D'Odorico, S. Bortolan, R. Cardin, R. D'Inca', D. Martines, A. Ferranato, G.C. Sturniolo, Scand. J. Gastroenterol. 36 (2001) 1289.
[6] B.A. Freeman, J.D. Crapo, J. Biol. Chem. 256 (1981) 10986.
[7] A.U. Khan, T. Wilson, Chem. Biol. 2 (1995) 437.
[8] J. Marcinkiewicz, Immunopharmacology 37 (1997) 35.
[9] L. Liu, P. Kumarathasan, J. Guénette, R. Vincent, Am. J. Physiol. 271 (1996) L995.
[10] P. Kumarathasan, R. Vincent, P. Goegan, M. Potvin, J. Guénette, Biochem. Cell Biol. 79 (2001) 1.
[11] A. van der Vliet, C.A. O'Neill, B. Halliwell, C.E. Cross, H. Kaur, FEBS Lett. 339 (1994) 89.
[12] W. Maruyama, Y. Hashizume, K. Matsubara, M. Naoi, J. Chromatogr. B 676 (1996) 153.
[13] J. Koska, P. Blazicek, M. Marko, J.D. Grna, R. Kvetnansky, M. Vigas, Physiol. Res. 49 (Suppl.1) (2000) S95.
[14] A. Palumbo, A. Napolitano, A. Carraturo, G.L. Russo, M. d'Ischia, Chem. Res. Toxicol. 14 (9) (2001) 1296.
[15] T. Aizawa, N. Ishizaka, S. Usui, N. Ohashi, M. Ohno, R. Nagai, Hypertension 39 (1) (2002) 149.
[16] R. Migheli, C. Godani, L. Sciola, M.R. Dlogu, P.A. Serra, D. Zangani, G. De Natale, E. Miele, M.S. Desole, J. Neurochem. 73 (3) (1999) 1155.
[17] M.J. Sole, M.N. Hussain, Biochem. Med. 18 (3) (1977) 301.
[18] I. Meineke, E. Stuwe, E.M. Henne, G. Rusteberg, E. Brendel, C. De Mey, J. Chromatogr. 493 (2) (1989) 287.
[19] N.R. Musso, C. Vergassola, A. Pende, G. Lotti, Clin. Chem. 35 (9) (1989) 1975.
[20] A.M. Krstulovic, A.M. Powell, J. Chromatogr. 171 (1979) 345.
[21] M. Kamahori, M. Taki, Y. Watanabe, J. Miura, J. Chromatogr. 567 (2) (1991) 351.
[22] R.S. Sodum, S.A. Akerkar, E.S. Fiala, Anal. Biochem. 280 (2) (2000) 278.
[23] P. Maycock, K.N. Frayn, Clin. Chem. 33 (2) (1987) 286.
[24] M. Candito, F. Bree, A.M. Krstulovic, Biomed. Chromatogr. 10 (1) (1996) 40.
[25] A. Bolner, L. Barbato, F. Tagliaro, A. Monge, F. Stocchi, G. Nordera, Forensic Sci. Int. 89 (1–2) (1997) 81.
[26] M.A. Raggi, C. Sabbioni, G. Casamenti, G. Gerra, N. Calonghi, L. Masotti, J. Chromatogr. B 730 (1997) 201.
[27] D.H. Fisher, L.M. Fisher, M. Broudy, J. Liq. Chromatogr. 18 (16) (1995) 3311.