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Determination of catecholamines in human plasma by high-performance liquid chromatography with electrochemical detection

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Abstract

In the present study, assays were improved for the determination of catecholamines in human plasma. High-performance liquid chromatography with electrochemical detection was employed for quantitative analysis. The influence of various parameters on chromatographic performance, such as the composition and the pH of the mobile phase, and the detection potential, was investigated. An accurate solid-phase extraction procedure, after catecholamine complexation with diphenylborate, was developed. The efficiency yield for all catecholamines was in the range 92–98%. Relative standard deviation values for repeatability and for intermediate precision were less than 2% and 3%, respectively, for all three analytes.

Keywords: Catecholamines

1. Introduction

Catecholamines (CAs) are important natural molecules containing a catechol ring, which act as neurotransmitters or hormones. The main endogenous catecholamines are epinephrine, norepinephrine and dopamine (Fig. 1). The interaction of one of these substances with the relevant receptors initiates on the cell membrane a cascade of biochemical events, which ends in one or many physiological

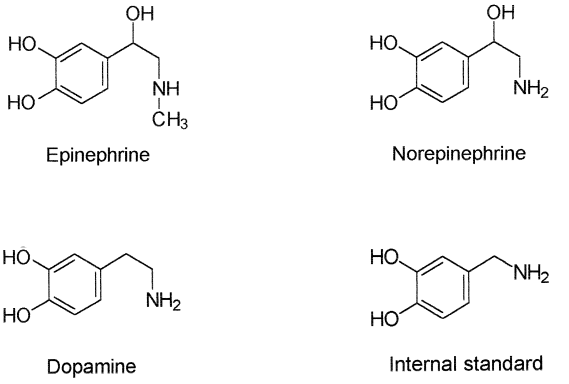

Fig. 1. Chemical structures of the catecholamines.

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responses (secretion, contraction, relaxation, metabolic alteration, etc.).

The plasma level determination of catecholamines and their metabolites is necessary in studies which aim to evaluate neuroendocrine disorders [1] and the role of the autonomic nervous system in several physiological and pathological situations, in animal models [2] and in humans [3].

Several analytical methods, high-performance liquid chromatography (HPLC) above all, have been reported for the analysis of catecholamines and their metabolites in biological fluids. Interesting HPLC procedures [4,5] with micellar mobile phases, precolumn CA oxidation to aminochromes and spectrometric [6] or coulometric detection [5] are reported and applied to urine samples [7]. Recently published papers [3,5,8–16] use a HPLC procedure with either amperometric [3,10] or coulometric [5,8,9] detection and a C_{18} (octadecyl column) as the stationary phase for the analysis of catecholamines in urine or plasma samples.

HPLC with electrochemical detection seems to be particularly suitable for the assay of these important biomolecules, due to its high sensitivity and selectivity. In fact, the amounts of epinephrine, norepinephrine and dopamine to be determined in plasma are in the submicro analysis range, i.e., few parts per billion or a fraction thereof (hundreds of parts per trillion). This is a difficult analytical problem, especially if one takes into account the chemical instability of these compounds, the numerous metabolites present, and the complex matrix from which they have to be extracted.

Despite the large number of papers published about this issue, there are still problems associated with the plasma sample clean-up procedure. The CAs from human plasma are usually purified by absorption on alumina [3,14,17–20] or on boric acid gel [21] or by extraction with organic solvents [22,23]. However, these methods are not selective enough, and in addition they often do not give high extraction yields.

The aim of this paper is to propose a new method, rapid and sensitive, for the determination of catecholamine plasma levels. It is based on the use of an HPLC procedure with coulometric detection. In order to optimize the procedure, a solid-phase extraction (SPE) method for plasma sample preparation was accurately implemented. It provided a high extraction yield and other advantages, such as selectivity and precision, that made it superior to other purification procedures reported in the literature.

The analytical paper is part of a broader interdisciplinary study on the psychobiological responses to experimental psychological stress and experimental aggressiveness in healthy humans.

2. Experimental

2.1. Chemicals

Norepinephrine (NE), dopamine (DA) hydrochloride, epinephrine (E), tetrabutylammonium bromide (TBA), diphenylboric acid 2-aminoethyl ester (DPB), 3,4-dihydroxybenzylamine hydrobromide (DHBA) and bovine albumin were purchased from Sigma (St. Louis, MO, USA). Citric acid, 1 mol l^{-1} sodium hydroxide, ethylenediaminetetraacetic acid sodium salt (EDTA), potassium chloride (KCl), sodium chloride (NaCl), potassium dihydrogenphosphate (KH_2PO_4), sodium hydrogenphosphate (Na_2HPO_4), ammonium chloride (NH_4Cl), sodium metabisulfite, concentrated ammonia, 30% (m/m), concentrated phosphoric acid, 85% (m/m), concentrated hydrochloric acid, 37% (m/m), water PLUS for HPLC and methanol for HPLC were purchased from Carlo Erba (Milan, Italy); 1-octanesulfonic acid sodium salt monohydrate (OSA) was purchased from Fluka (Buchs, Switzerland).

2.2. Solutions

Stock solutions (1 mg ml^{-1}) of E, NE and DA were prepared by dissolving 20 mg of pure substance, 20 mg of sodium metabisulfite, 160 mg of NaCl and 100 μl of 37% HCl in 20 ml of ultrapure water. Standard solutions were obtained by diluting stock solutions with ultrapure water.

Buffer solution A was prepared as follows: 100 mg of DPB, 600 mg of TBA, 250 mg of EDTA and 5.35 g of NH_4Cl were dissolved in about 30 ml of water and brought up to pH 8.5 with 30% ammonia.

The solution was brought up to 50 ml with ultrapure water.

Buffer solution B was prepared as follows: 200 mg of TBA, 25 mg of EDTA and 535 mg of NH_4Cl were dissolved in about 30 ml of water and brought up to pH 8.5 with 30% ammonia. The solution was brought up to 50 ml with ultrapure water.

''Reconstituted'' plasma was prepared by dissolving 20 mg of KCl, 800 mg of NaCl, 20 mg of KH_2PO_4, 115 mg of Na_2HPO_4 and 4 g of bovine albumin in 100 ml of ultrapure water.

2.3. Patients and sample collection

The study was performed on volunteer young boys with drug or alcohol addicted parents. Before the collections they were submitted to stressing video games.

Blood samples were drawn into test tubes, containing EDTA, 100 μg ml^{-1} sodium metabisulfite added, and centrifuged at 3000 rpm for 20 min. The supernatant plasma was transferred into test tubes and frozen at −80°C until analysis.

2.4. Apparatus and chromatographic conditions

The HPLC apparatus consisted of a Beckman Instruments (Palo Alto, CA, USA) 168 chromatographic pump and an ESA (Milford, MA, USA) Coulochem II coulometric detector. The conditioning cell was set at 0.00 V; the analytical cell was set at detector 1=+0.45 V; detector 2=−0.35 V.

Chromatographic separation was achieved on a Jones (Mid-Glamorgan, UK) Apex reversed-phase column (C_8, 150×4.6 mm I.D., 5 μm) with a Varian (Harbor City, CA, USA) pre-column (C_8, 30×4.6 mm I.D., 5 μm). The mobile phase was a mixture of methanol (2.5%) and an aqueous solution (97.5%) of 10.5 g l^{-1} citric acid, 20 mg l^{-1} EDTA and 20 mg l^{-1} OSA buffered to pH 2.9 with 1 mol l^{-1} NaOH. The flow-rate was 1 ml min^{-1} and the samples were injected by means of a 20-μl loop. Data were analyzed by means of Beckman ''Gold noveau'' software installed on a Intel Pentium processor. The cartridges employed for the SPE step were Waters (Milford, MA, USA) Oasis HLB (30 mg, 1 ml).

For preliminary assays, other kinds of cartridges were used, namely: International Sorbent Technology (Mid-Glamorgan, South Wales, UK), Isolute C_8 (50 and 100 mg, 1 ml), Varian BondElut C_8 (50 and 100 mg, 1 ml) and Varian BondElut CH (100 mg, 1 ml).

2.5. Analytical procedures

2.5.1. Standard solutions

CA standard solutions at several concentrations were injected into the HPLC system. The peak area values at 4.1, 5.3 and 8.9 min were plotted against the concentrations of NE, E and DA, respectively, to obtain calibration curves. Standard solutions of the same concentration were injected several times over the same day in order to obtain the repeatability of the assay, and over several days to obtain the intermediate precision of the assay.

2.5.2. SPE procedure

2.5.2.1. Plasma calibration curves. To aliquots of 500 μl of reconstituted plasma, several amounts of catecholamine (as standard solutions), 5 ng ml^{-1} of internal standard and 0.5 ml of buffer A were added, and the resulting mixtures were loaded onto previously conditioned cartridges. The conditioning was effected by passing sequentially through the cartridge 1 ml of methanol two times, 1 ml of water two times, then 1 ml of buffer A two times. The washing of the cartridge after the loading consisted of 1 ml of buffer A two times, then 1 ml of a buffer B–methanol (1:1) mixture two times. The elution was performed by passing through the cartridge 500 μl of mobile phase. The eluate was injected into the HPLC system by means of a 20 μl loop. The absolute recovery (or extraction efficiency) was evaluated as the percentual ratio between the amount of catecholamine found from extraction and the corresponding standard solution. Calibration curves were set up by plotting the catecholamine peak area against the respective concentration of each catecholamine.

2.5.2.2. Method precision. Repeatability was evaluated by adding the same quantities of catecholamines and of I.S. to six aliquots of reconstituted plasma, by

subjecting the resulting mixture to SPE and to HPLC analysis within the same day and by calculating the relative standard deviation (RSD, %) of the results.

Intermediate precision was evaluated by performing the same procedure on one aliquot each day, for six consecutive days.

2.5.2.3. Sample analysis. To an aliquot of 0.5 ml of plasma, 0.5 ml of buffer A were added, and the resulting mixture was loaded onto the previously conditioned cartridge. The SPE procedure was carried out in the same manner as indicated in Section 2.5.2.1, above.

2.5.2.4. Recovery studies. Known amounts of catecholamines and 5 ng ml^{-1} of I.S. were added to 0.5 ml of previously analyzed human plasma, and the resulting mixture was subjected to SPE procedure and to HPLC analysis.

3. Results and discussion

Several HPLC procedures with coulometric detection about the analysis of cathecolamines in human plasma are reported in the literature [3,9,24–26].

We have studied and notably improved the analytical procedure by introducing important modifications both in the chromatographic conditions and the SPE procedure. The modifications in the chromatographic conditions led to a more rapid and selective method, while the modifications in the SPE procedure led to a more sensitive and accurate assay of plasma catecholamine levels.

3.1. Chromatographic conditions

Instead of the C_{18} columns used by other authors [9,24–26], we used a C_8 column as the stationary phase, in order to obtain a good separation in less time. The electrochemical conditions reported by other authors [3] (conditioning cell set at +0.35 V; analytical cell set at: detector 1=+0.05 V; detector 2=−0.30 V) were modified as follows: conditioning cell set at 0.00 V; analytical cell set at: detector 1=+0.45 V; detector 2=−0.35 V. These changes in the potential values allowed one to obtain better sensitivity.

3.2. Choice of the mobile phase

The optimization of the mobile phase for the chromatographic analysis is very important. Preliminary experiments were performed using a mobile phase at pH 3 containing: 5% methanol, 10.5 g l^{-1} citric acid, 4.9 g l^{-1} phosphoric acid, 20 mg l^{-1} EDTA and 100 mg l^{-1} OSA as reported in the literature [25].

In order to increase the selectivity of the method, and to obtain shorter analysis times, some parameters of the mobile phase, such as pH and organic modifier content, were studied. The following results were obtained.

(a) Voltammetric experiments, performed in the 2–5 pH range showed that the optimal pH of the mobile phase was equal to 2.9, with regard to the height and the potential of the anodic wave, corresponding to the oxidation of catecholamines. This pH value was in agreement with literature data [27].

(b) An increase in the amount of OSA from 10 to 40 mg l^{-1} did not produce appreciable changes; a decrease did produce a worse chromatographic performance, which is in agreement with the literature [28].

(c) The presence of phosphoric acid was found to be unnecessary therefore, for the sake of convenience, only citric acid was used.

(d) Changes in the concentration of citric acid, from 5.2 to 21.0 g l^{-1}, were studied: an increase of citric acid caused a shift in the chromatographic peaks to higher times.

(e) The influence of the organic modifier content was found to be very significant: an increase in the amount of methanol from 0 to 10% produced shorter retention times; however for methanol >5%, an overlap of the peaks of analytes was found.

For these reasons we decided to compromise: we chose to use 2.5% methanol and 10.5 g l^{-1} citric acid to obtain a good separation of the analytes in a satisfactory time. Moreover, 20 mg l^{-1} OSA, 20 mg l^{-1} EDTA and pH 2.9 were used.

Varying the flow ratio of the mobile phase between 0.5 and 1.5 ml min^{-1}, resulted in an optimal flow-rate of 1 ml min^{-1}. Under these optimal

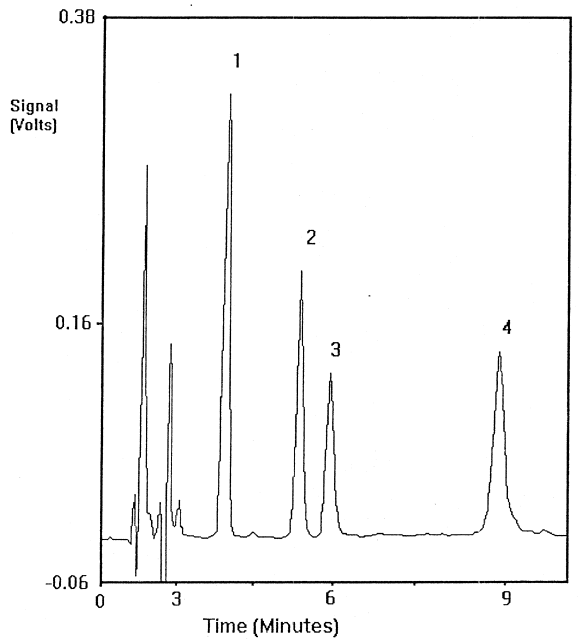


Fig. 2. Typical chromatogram of a catecholamine standard mixture (20 ng ml^{-1}), containing sodium metabisulfite. Peaks: 1=NE, 2=E, 3=DHBA, 4=DA.

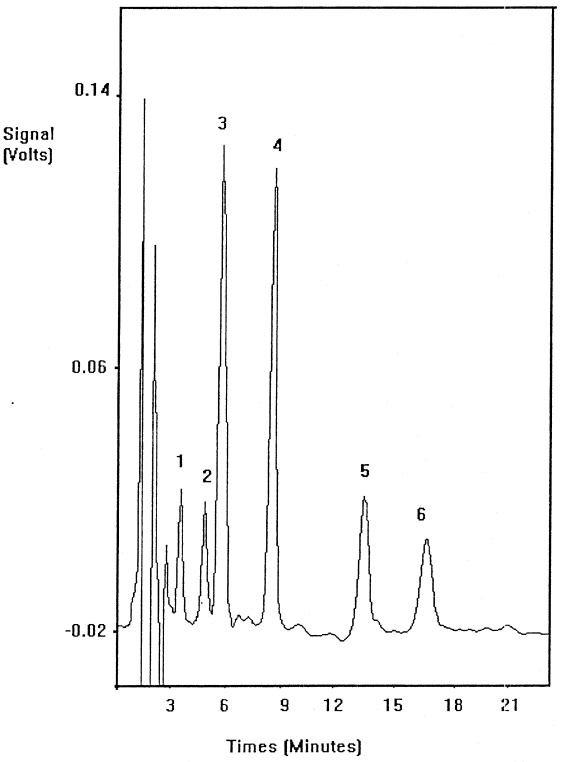


Fig. 3. Chromatogram of a catecholamine standard mixture (10 ng ml^{-1}) without sodium metabisulfite. Peaks: 1=NE, 2=E, 3=DHBA, 4=DA, 5, 6=degradation products.

conditions, the peaks corresponding to catecholamines are well resolved in a short time, within 9 min, as can be seen from Fig. 2. In particular, the first peak, at a retention time of 3.5 min, is due to norepinephrine; the second at 5.2 min is due to epinephrine; the third at retention time 6.1 min is due to the internal standard, and the fourth at retention time 8.8 is due to dopamine.

DHBA was selected as the internal standard (only for the control of retention time) according to literature [24].

Calibration curves were obtained by plotting peak area values against catecholamine concentrations. A good linearity was found in the two following concentration ranges: 40–1600 ng l^{-1} using 5 nA as the full scale value and 0.2–30 ng ml^{-1} using 50 nA as the full scale value, for each analyte. Because the determination of lower levels of analytes for this study was not necessary, only the regression equations and the respective correlation coefficients of higher concentration ranges are reported in Table 1. It should be noted that these satisfactory results were obtained from the analysis of standard catecholamine solutions containing the potassium metabisulfite as an antioxidant. This reagent was added to the stock catecholamine solutions, at the same concentration.

Table 1
Parameters of calibration curves of catecholamines

Analyte	Linear regression equation[a]	Correlation coefficient
Norepinephrine	$y=316\,271x+12\,650$	0.99979
Epinephrine	$y=290\,756x+9601$	0.99981
Dopamine	$y=305\,220x-45\,345$	0.99812

[a] In the linear regression equations, x is expressed as ng ml^{-1} and y is expressed as arbitrary area units.

On the contrary, if the metabisulfite was not added, the peaks corresponding to catecholamines resulted much smaller and additional peaks, corresponding to oxidative degradation products, appeared on the chromatograms. As Fig. 3 shows, two new peaks appeared, with retention times at 13.5 and 17.1 min, which are due to NE and E degradation products, respectively. For this reason, the addition of the antioxidant is very important, for the stability of the catecholamines, and therefore it strongly influences the accuracy of the analysis.

The reproducibility data were also good; in fact, values of RSD less than 2.5% were found for the three catecholamines (intermediate precision: $n=6$, inter-day).

3.3. SPE procedure

For the analysis of plasma samples, a preliminary purification step is necessary for two reasons: (a) to remove interfering compounds, increasing the selectivity of the procedure and (b) to extract as many catecholamines as possible, increasing the sensitivity of the analysis.

This is a very important step which influences the final chromatographic response of the analysis. For this reason it has been studied carefully, using a SPE procedure.

Several experiments were carried out, using different kinds of cartridges; for example Varian BondElut C_8 (50 mg – 1 ml and 100 mg – 1 ml), Step Bio Isolute C_8 (50 mg – 1 ml and 100 mg – 1 ml) and Waters Oasis HLB (30 mg – 1 ml). Furthermore, different loading and elution procedures were tested to obtain the highest recovery yields together with the best selectivity. For this reason, different pH values and volumes of the loading, washing and elution solutions were tried.

In any case, it was necessary to form complexes between CA and diphenilboric acid to block CA, which is retained by the cartridges. The complexation reaction, which needs a basic pH (8.5–9.0), is reported in Scheme 1(b). Without being previously complexed, the CAs cannot be eluted because they rapidly flow out of the cartridges during the loading step.The absolute recovery (extraction yield) percentage values, obtained using different kinds of cartridges, are reported in Table 2. The data were obtained by comparing the peak areas obtained by adding a known amount of the analytes to a blank plasma (that is ''reconstituted'' plasma, as reported in Experimental) sample and subjecting the mixture to the SPE procedure, with those peak areas obtained by injecting standard solutions having the same nominal concentrations. As can be seen, Oasis cartridges only gave recovery values near 100%, while the other cartridges gave much different recoveries, often far from 100%. Excluding the Oasis cartridges, the most consistent data were obtained from BondElut C_8 cartridges (100 mg).

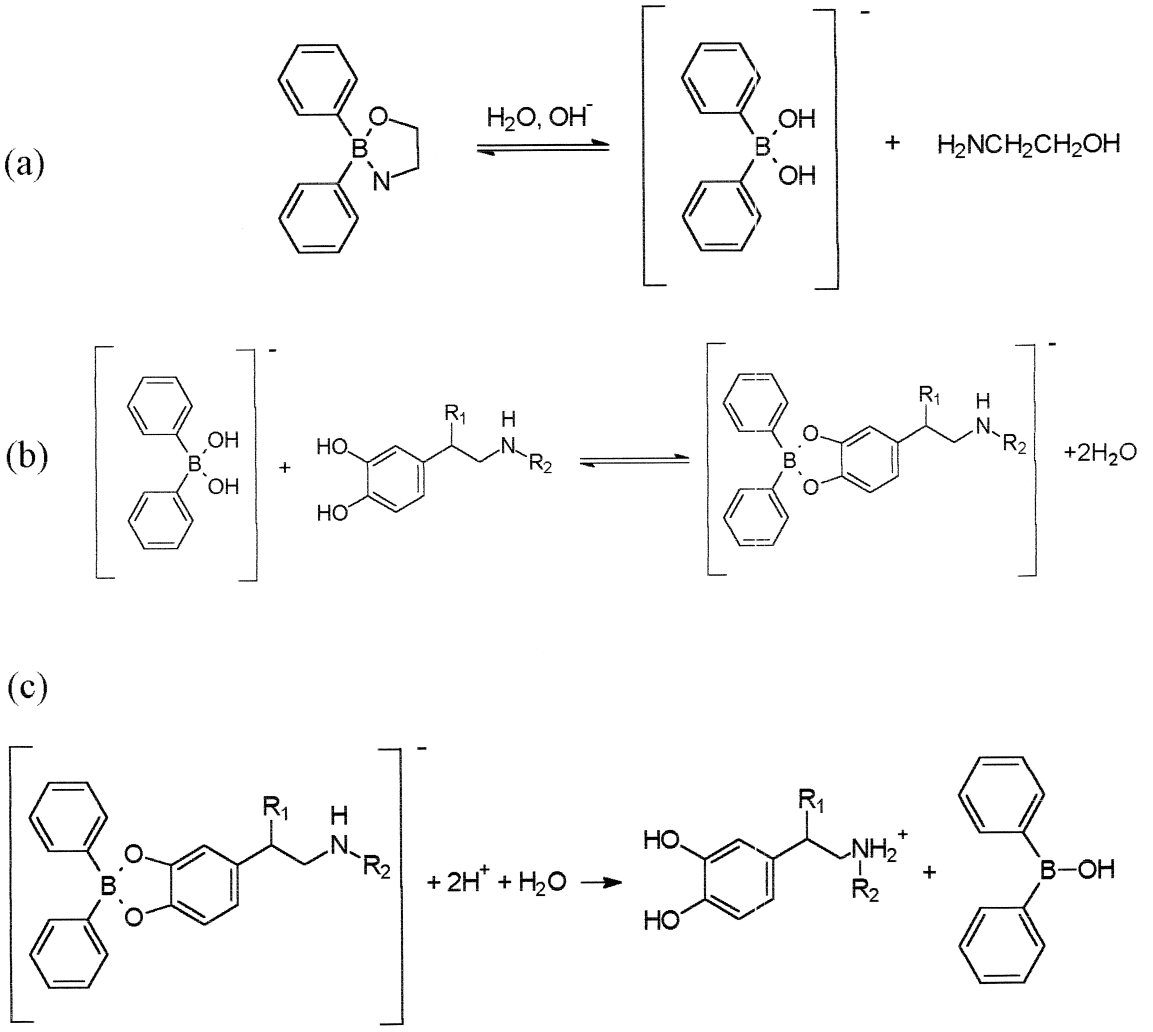
The cartridges were loaded with a mixture of plasma (500 μl), buffer A (500 μl) and I.S. solution (10 μl).

This mixture, containing plasma samples alone or plasma samples spiked with catecholamine standard solutions (2.5 ng ml^{-1} of each analyte), was prepared 5 min before loading, to allow for the formation of the catecholamine–diphenilborate complexes. The cartridges were sequentially washed with buffer A, then with buffer B–methanol (1:1) mixture and finally with water. It was considered useful to perform the first wash with buffer A, because it contains diphenylboric acid, which allows for the recovery of possible traces of uncomplexed CA. With regard to the elution, the employment of an acidic eluent is necessary in order to dissociate the CA–diphenilborate complex, as shown in Scheme 1(c). The acidic medium shifts the equilibrium to the right.

At first, acetic acid was chosen as the eluent, as reported by Grossi et al. [8].

Various acetic acid concentrations, from 0.08 to 0.8 mol l^{-1}, were tested. The extraction yields of the CA resulted better for the higher concentrations of acetic acid (about 90% for all CA) using Oasis cartridges. These cartridges gave the best overall results and were thus chosen for the subsequent assays. The resulting chromatograms however, showed the presence of plasma interfering peaks (Fig. 4). For this reason, other eluents were tested. The use of a mobile phase (pH=2.9), using an optimal volume of 500 μl, gave the best results in terms of both extraction yield and selectivity.

Under these conditions, plasma interference was eliminated (Fig. 5A) and the CAs were revealed as neat chromatographic peaks, like those of the stan-



Scheme 1.

Table 2
Absolute recovery[a] of plasma catecholamines using several kinds of cartridges

Analyte	Absolute recovery (%)					
	Isolute C_8		BondElut C_8		BondElut CH	Oasis HLB
	50 mg	100 mg	50 mg	100 mg	100 mg	30 mg
NE	137	120	70	75	46	95
E	65	73	55	68	50	92
I.S.	55	74	40	51	35	96
DA	120	140	52	67	40	98

[a] Each value is the mean of three independent assays.

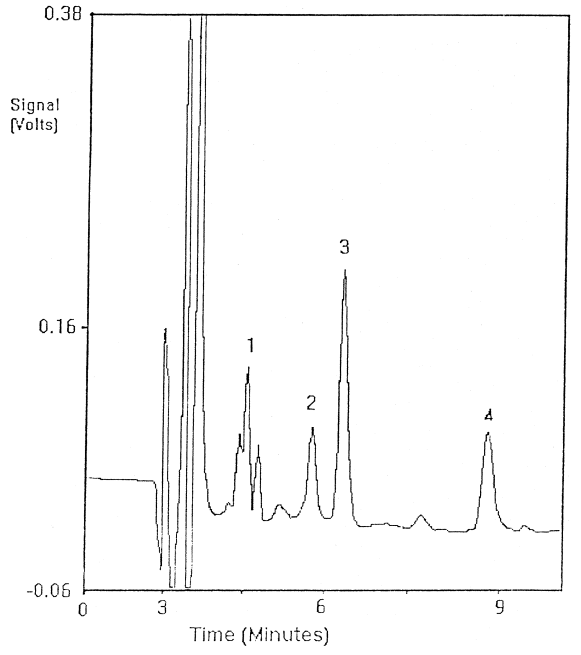


Fig. 4. Chromatogram of a "blank" (reconstituted) plasma extract, spiked with 2.5 ng ml^{-1} of NE, E, DA and DHBA, eluted from Oasis cartridges with 0.8 mol l^{-1} acetic acid. Peaks: 1=NE, 2=E, 3=DHBA, 4=DA.

dard solution (Fig. 5B). The values of the extraction yields (or absolute recovery) were: 95% for NE, 92% for E, 98% for DA and 96% for the I.S. (Table 2). These values are very satisfactory, indicating an improvement with respect to the literature data of 80 to 90% for other procedures usually performed using other SPE procedures [11] or activated alumina [3,19,20] or solvent extraction [22,23] for the analysis of catecholamines in human plasma.

3.4. Application to reconstituted plasma

Calibration curves were constructed by adding different amounts of CA to reconstituted plasma, subjecting the resulting mixture to the SPE procedure and analyzing the eluate by means of HPLC. Linearity was found in the two following concentration ranges: 40–1600 ng l^{-1} (using 5 nA as the full scale value) and 0.2–30 ng ml^{-1} (using 50 nA as the full scale value) for each analyte. The limit of quantitation (LOQ) was 40 ng l^{-1} for all three analytes, while the limit of detection (LOD) was 12 ng l^{-1}. Both parameters were calculated according to USP XXIII guidelines [29]. These values are better than others reported in the literature [3,5].

The precision of the method (extraction and chromatography) was evaluated by injecting samples of reconstituted plasma spiked with CA (5 ng ml^{-1}), all prepared in the same manner, six times into the HPLC system. RSD values were: 3.2% for NE, 3.4% for E and 3.1% for DA (intra-day precision or repeatability); 4.3% for NE, 3.8% for E and 3.9% for DA (intermediate precision or inter-day precision).

3.5. Application to human plasma samples

Having verified the analytical parameters needed to validate the method, samples of plasma of young volunteers under emotional stress, were analyzed.

It was found that NE, E and DA were detected at retention times of 3.5, 5.2 and 8.8 min, respectively, without any interfering peaks (Fig. 6A). The peak at retention time of 6.1 min is due to I.S. The method is thus suitable for the dosage of the CAs by interpolation on the calibration curve.

The levels of CAs obtained in the plasma sample (Fig. 6A) were: 2.4 ng ml^{-1} for NE, 0.5 ng ml^{-1} for E and 1.8 ng ml^{-1} for DA.

The identification of the analytes is effected by addition of CA standard solutions. As Fig. 6B shows, the CA peaks increase significantly.

The accuracy of the method was verified by recovery studies, by adding known amounts of CA standard solutions (containing also metabisulfite and I.S.) to a known amount of human plasma and subjecting the mixture to the above procedure. The recovery was good: 96% for NE, 93% for E and 97% for DA, for additions of 2 ng ml^{-1} of each analyte.

The accurate assay of catecholamines in response to experimental psychological stress in young adults could be useful to investigate the possible changes associated with aggressiveness [30,31] and anxiety.

4. Conclusion

The proposed HPLC method with coulometric detection, which employs a careful SPE pretreatment of plasma samples, seems to be suitable for the analysis of CAs in the blood of adolescents subjected

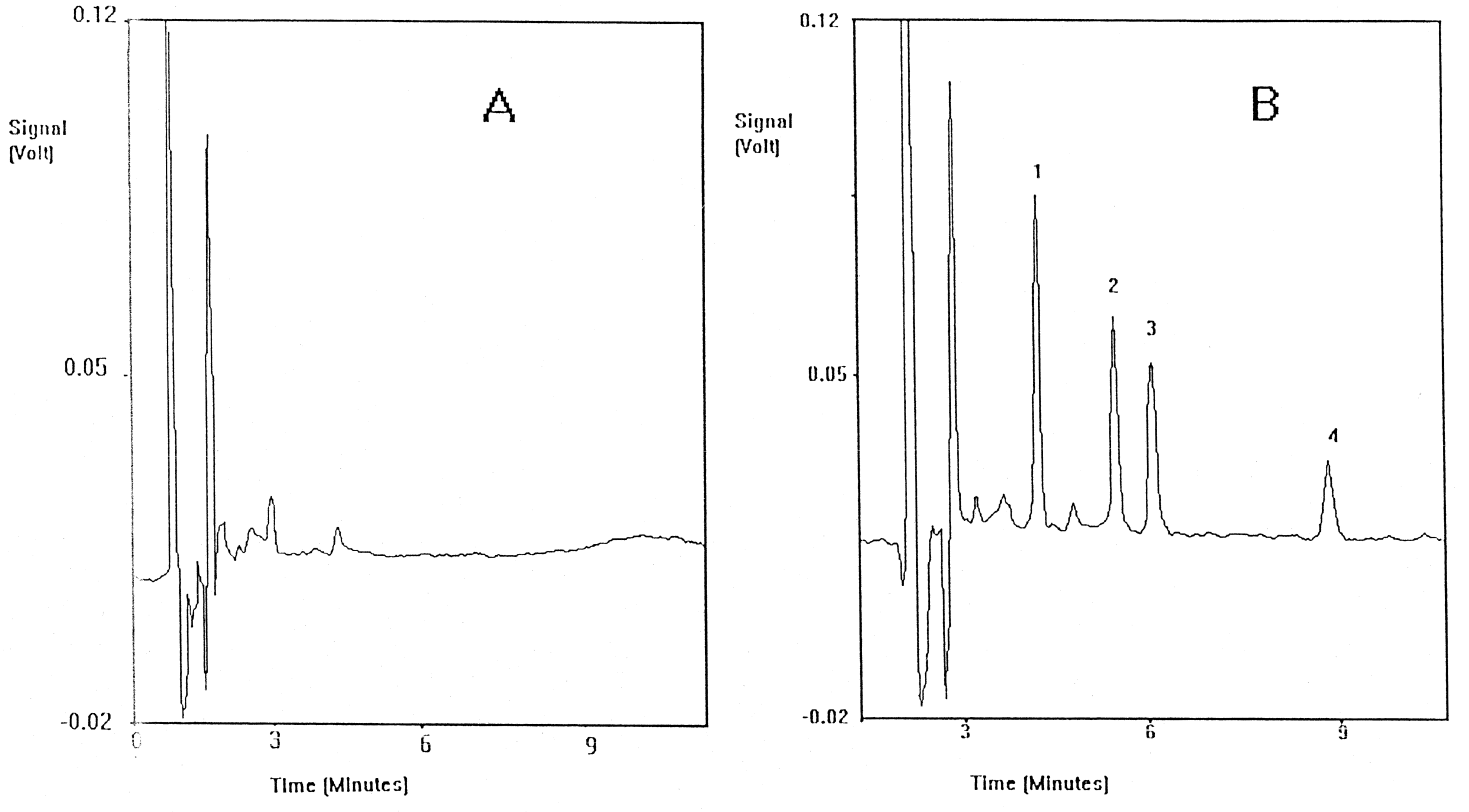


Fig. 5. Chromatographic profiles from a ''blank'' (reconstituted) plasma extract. (A) ''Blank'' plasma unspiked, (B) ''blank'' plasma spiked with 2.5 ng ml^{-1} of NE, E, DA and DHBA. Peaks: 1=NE, 2=E, 3=DHBA, 4=DA.

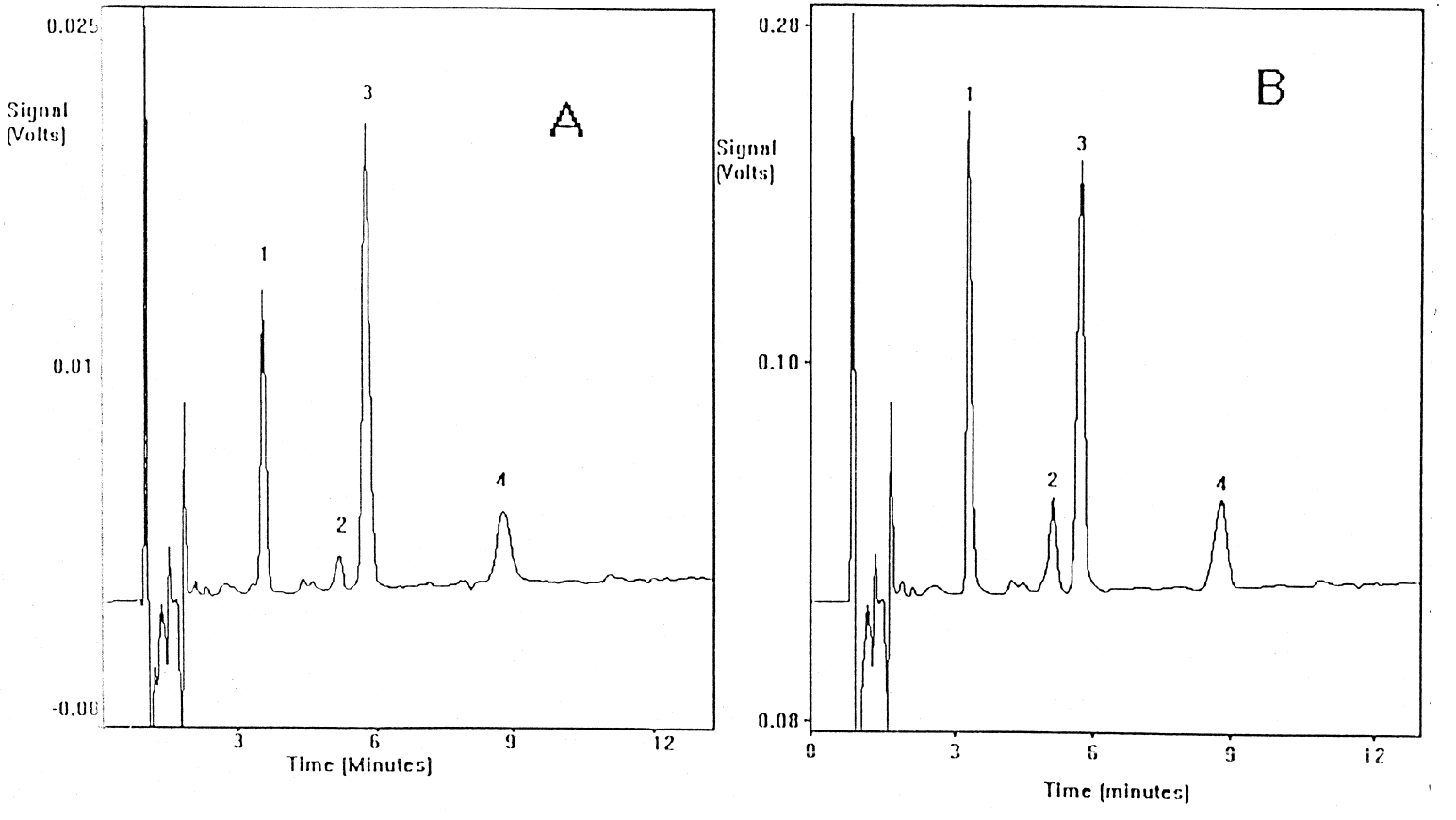


Fig. 6. Typical chromatograms of a human plasma sample: (A) spiked with 5 ng ml^{-1} of DHBA; (B) spiked with 5 ng ml^{-1} of NE and DHBA, and with 2.5 ng ml^{-1} of E and DA. Peaks: 1=NE, 2=E, 3=DHBA, 4=DA.

to emotional and physical stress. The method has various advantages: high precision and accuracy, good selectivity and feasibility and, above all, high sensitivity due both to the electrochemical detection and to the SPE procedure.

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References

[1] I.J. Kopin, Pharmacol. Rev. 37 (1985) 333.
[2] W.H. Kaye, H.E. Gwirtsman, D.T. George, M.H. Ebert, D.C. Jimerson, T.P. Tomai, G.P. Chrousos, P.W. Gold, J. Clin. Endocrinol. Metab. 64 (1987) 203.
[3] D.H. Fischer, L.M. Fischer, M. Broudy, J. Liq. Chromatogr. 18 (1995) 3311.
[4] R.M. Villanueva Camanas, J.M. Sanchis Mallols, J.R. Torres Lapasiò, G. Ramis-Ramos, Analyst 120 (1995) 1767.
[5] J.M. Sanchis Mallols, J.R. Torres Lapasiò, R.M. Vilanueva Camanas, G. Ramis-Ramos, Chromatographia 39 (1994) 591.
[6] J.M. Sanchis Mallols, R.M. Villanueva Camanas, G. Ramis-Ramos, Chromatographia 39 (1994) 365.
[7] F. Monaldi, D. Cavani, S. Seghedoni, G. Chiossi, L. Roli, A.T. Sacchetti, G.F. Baraghini, Chromatographia 24 (1987) 407.
[8] G. Grossi, A. Bargossi, A. Lippi, R. Battistoni, Chromatographia 24 (1987) 842.
[9] M. Candito, F. Bree, A.M. Krstulovic, Biomed. Chromatogr. 10 (1996) 40.
[10] P. Kubalec, E. Brandsteterova, J. Leothay, J. Cizmàrik, Pharmazie 49 (1994) 897.
[11] B.M. Eriksson, M. Wikström, J. Chromatogr. 593 (1992) 185.
[12] M. Hay, P. Mormede, J. Chromatogr. B 703 (1997) 15.
[13] M.J. Ribotta, N. Rodriguez, J.M. Marioli, J. Liq. Chromatogr. 21 (1998) 1003.
[14] H.B. He, C.M. Stein, B. Christman, A.J.J. Wood, J. Chromatogr. B 701 (1997) 115.
[15] E. Hollenbach, C. Schulz, H. Lehnert, Life Sci. 63 (1998) 737.
[16] J.E. Wolff, H.J. Bauch, A. Roessner, J. Boos, V. Gressner-Brocks, J. Ritter, H. Jurgens, Med. Pediatr. Oncol. 21 (1993) 460.
[17] C. Lucarelli, P. Betto, G. Ricciarello, M. Giambenedetti, F. Sciarra, C. Tosti Croce, P.L. Mottironi, Chromatographia 24 (1987) 423.
[18] E. Brandsteterova, K. Krajnak, I. Skacani, Pharmazie 50 (1995) 825.
[19] C.R. Benedict, J. Chromatogr. 385 (1987) 369.
[20] M. Candito, A.M. Krstulovic, V. Sbirazzuoli, P. Chambon, J. Chromatogr. 526 (1990) 194.
[21] Y. Imai, S. Ito, K. Maruta, K. Fujita, Clin. Chem. 34 (1988) 528.
[22] I. Meineke, E. Stuwe, E.M. Henne, G. Rusteberg, E. Brendel, C. De Mey, J. Chromatogr. 493 (1989) 287.
[23] F. Smedes, J.C. Kraak, H. Poppe, J. Chromatogr. 231 (1982) 25.
[24] G. Grossi, A.M. Bargossi, G. Sprovieri, Chromatographia 36 (1993) 110.
[25] M. Beschi, M. Castellano, E. Agabiti-Rosei, D. Rizzoni, P. Rossini, G. Muiesan, Chromatographia 24 (1987) 455.
[26] F. Blandini, G.V. Melzi d'Eril, G. Sances, C. Lucarelli, C. Herborg, E. Martignoni, Farmaco 46 (1991) 1217.
[27] A. Pappa-Louisi, F. Zougrou, Chromatographia 44 (1997) 348.
[28] W.A. Bartlett, J. Chromatogr. 493 (1989) 1.
[29] United States Pharmacopeial Convention, Rockville, MD, United States Pharmacopeia, XXIII, 1995, p. 1983.
[30] G. Gerra, A. Zaimovic, B. Chittolini, G. Giucastro, F. Folli, D. Maestri, A. Tessoni, P. Avanzini, R. Caccavari, S. Bernasconi, F. Brambilla, Life Sci. 62 (1998) 617.
[31] G. Gerra, A. Zaimovic, P. Avanzini, B. Chittolini, G. Giucastro, R. Caccavari, M. Palladino, D. Maestri, C. Monica, R. Delsignore, F. Brambilla, Psychiatry Res. 66 (1997) 33.