Simultaneous analysis of human plasma catecholamines by high-performance liquid chromatography with a reversed-phase triacontylsilyl silica column

Maiko Machida [a], Ayumi Sakaguchi [a], Satoshi Kamada [a], Tetsuya Fujimoto [a], Shigeru Takechi [b], Shigeo Kakinoki [c], Akikazu Nomura [a],*

[a] Department of Pathophysiology, Hokkaido College of Pharmacy, 7-1 Katsuraoka, Otaru 047-0264, Hokkaido, Japan
[b] Department of Cardiovascular Medicine, Date Red Cross Hospital, Date, Hokkaido, Japan
[c] Otaru Kyokai Hospital, 1-6-15 Suminoe, Otaru, Hokkaido, Japan

Abstract

The clinical importance of simultaneous analysis of 3,4-dihydroxyphenylglycol with other human plasma catecholamines has been investigated to better understand the sympathetic nervous system. However, previous reports have had analytical difficulties with both resolution and extraction. The current study uses a reversed-phase triacontylsilyl silica (C30) column under the mobile phase condition without ion-pair reagents to separate catecholamines and their metabolites, with above 91% recoveries for intra-assay, above 85% for inter-assay, and less than 10% ($n = 5$) coefficient of variation. Lower detection limits (S/N = 4) and quantification limits (S/N = 6) were 40 and 100 pg/mL for norepinephrine, 3,4-dihydroxyphenylglycol, and 3,4-dihydroxyphenylalanine, 10 and 20 pg/mL for epinephrine, 10 and 40 pg/mL for dopamine. Linear ranges were from 40 to 5000 pg/mL for norepinephrine and 3,4-dihydroxyphenylalanine, from 100 to 5000 pg/mL for 3,4-dihydroxyphenylglycol, and from 10 to 2000 pg/mL for epinephrine and dopamine. The C30 column may prove clinically useful, as it provides a convenient and simultaneous method of evaluation of human plasma catecholamines.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Norepinephrine; 3,4-Dihydroxyphenylglycol; Triacontylsilyl silica (C30); Solid-phase extraction

1. Introduction

Catecholamines are well known for their role as central and peripheral neurotransmitters [1]. 3,4-Dihydroxyphenylglycol (DHPG) is formed from norepinephrine (NE) by pre-synaptic oxidative deamination catalyzed by monoamine oxidase [1–4]. Plasma DHPG is derived only from NE recaptured by sympathetic nerve endings after synaptic release [5–8]. Therefore, the measurement of both NE and DHPG plasma levels would offer accurate information about sympathetic activities, since these levels change under various physiological and pathological situations [2,5,9–11]. Various catecholamine analytical methods using high performance liquid chromatography (HPLC) with electrochemical detection have been reported [12–17]. However,

problems still exist with such analyses from potential interferences that may influence detection sensitivity. According to previous reports using a C18 (octadecyl groups) column, it is difficult to measure the levels of DHPG since the low k' value can lead to interferences and difficulties with subsequent analysis [2,10,11]. Therefore, these methods require various extraction procedures for cleaning the samples and require a mobile phase containing various ion pair reagents to improve the resolution of analytes [8–10,18–21]. However, these improvements have not been sufficient enough for simultaneous analysis of DHPG and catecholamines.

Various procedures for catecholamine extraction from plasma have been reported. However, the extraction methods using boric acid gel [22] and organic solvents [23,24] has not been applied in the analysis of DHPG. The other methods using alumina extraction of catecholamines as well as DHPG have been reported but these have lower DHPG recovery rates compared to the other analytes [9,10,19,20]. Various antioxidant agents are used to pro-

---

tect the degradation of DHPG throughout analysis [9,19,25,26]. Extraction with cation-exchange columns [27], boronate gels [21,27], C18 matrix [12,13,16,28–30], MFC18 [29] and PLRP-S or OASIS HLB [13,30] have provided good recoveries of NE and other catecholamines from plasma and urine. However, recoveries of other catecholamines and metabolites, such as DHPG and 3,4-dihydroxylalanine (DOPA) in plasma, have not been reported using this method.

The purpose of this study was to develop a method for simultaneous analysis of clinically important catecholamines and metabolites, specifically NE, DHPG, DOPA, epinephrine, and dopamine.

2. Experimental

2.1. Chemicals

3,4-Dihydroxyphenylglycol (DHPG), epinephrine (Epi), norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), 3,4-dihydroxybenzylamine (DHBA), diphenylboric acid 2-aminoethyl ester (DPB), tetrabutylammonium bromide (TBA) and human serum albumin were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, citric acid, sodium metabisulfite, ethylene diamine tetra-acetic acid disodium salt (EDTA 2Na), potassium chloride (KCl), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Wako (Tokyo, Japan). All reagents used for the assays were of highest grade available. Ultra-pure water was obtained using the Milli-Q Millipore systems (Billerica, MA, USA).

2.2. Standard preparation

First stock standard solutions (1 g/L) of DHPG, Epi, NE, DOPA, DA and DHBA were prepared in an acid diluent (1 mmol/L HCl, 1 g/L sodium metabisulfite and 1.6 g/L NaCl) and stored at −80 °C. The assay standards were freshly obtained by diluting the stock standard solutions with ultra-pure water for each assay.

2.3. Human plasma samples

Human plasma samples were obtained from the hypertensive patients enrolled in this study after obtaining informed consent. The plasma was prepared from about 5 mL of whole blood, which was collected into a tube containing 0.01 mM of EDTA 2Na and spun at $1500 \times g$ in a refrigerated centrifuge at 4 °C for 10 min. Each samples was stored at −80 °C until analysis.

Artificial plasma samples containing 40 g/L human serum albumin in an aliquot (0.2 g/L KCl, 8 g/L NaCl, 0.2 g/L KH_2PO_4 and 1.2 g/L Na_2HPO_4) was used as control plasma, free from catecholamines.

2.4. Apparatus and column

The HPLC system used was an Eicom model PC-300 (Eicom, Kyoto, Japan) and a Rheodyne model 7725 injection (Rheo-

dyne, Cotati, CA, USA) with a loop of 100 μL. The electrochemical detector was an Eicom model ECD-300 (Eicom, Kyoto, Japan) with a glass carbon cell WE-GC (Eicom, Kyoto, Japan). It was operated in the d.c. mode at +550 mV versus an Ag/AgCl electrode. Chromatograms were recorded and analyzed by a Power Chrom System EPC-300 (Eicom, Kyoto, Japan). The column used was a Deverosil RPAQUEOUS-AR-5 (Nomura Chemical Co. Ltd., Aichi, Japan): column dimension was 250 mm × 4.6 mm. Packing material was a triacontylsilyl silica (C30). Particle diameter was 5 μm. Pore diameter is 14 nm. The temperature of the column was controlled using a thermoregulated bath, NTT 2200 (Tokyo Rikakikai Co. Ltd., Tokyo, Japan). Plasma samples were extracted using a Vac Elute system and solid-phase extraction (SPE) cartridges. The cartridges investigated for SPE were OASIS HLB (Sorbent material was Batch No. 044A, reservoir size was 30 mg/1 mL; Waters, Milford, MA, USA), Isolute MFC18 (Sorbent material is spherical porous mono functional silica C18, reservoir size is 30 mg/1 mL; Uniflex International Sorbent Technology, Chiba, Japan) and a C30 cartridge (Sorbent material is spherical porous mono functional silica C30; Nomura Chemical Co. Ltd., Aichi, Japan) packed in a vacant Extract-clean reservoir (reservoir size was 1.5 mL; Alltech Associated Inc. (Waukegan Road Deerfield, IL, USA) with 30 mg of Develosil spherical porous silica C30 (Nomura Chemical Co. Ltd., Aichi, Japan).

2.5. Chromatographic conditions

The mobile phase consisted of 2.0% (v/v) acetonitrile and 98.0% (v/v) of an aqueous solution (10.5 g/L citric acid and 20 mg/L EDTA 2Na) adjusted with 1 mol/L NaOH to pH 2.8. The mobile phase was pumped at a flow rate of 0.3 mL/min, the temperature of the column was kept at 34 °C, and 20 μL samples were then injected.

The capacity ratio (k'-value) of analyte was determined by its elution time and hold-up time (t0) was 9.2 min.

2.6. Procedure for solid-phase extraction

0.5 ng of internal standard, 10 μL of 1.9 mg/mL sodium metabisulfite, and 0.5 mL of DPB buffer were added to a 500 μL aliquot plasma sample. The DPB buffer consisted of agents involved in the formation of a catecholamine complex (0.01%, w/v DPB, 1.2%, w/v TBA, 1% methanol, and 5 g/L EDTA 2Na in 2 mol/L NH_4Cl–NH_4OH buffer, pH 8.5). The SPE cartridges were placed on a Vac Elute extraction system for extraction. They were activated and equilibrated by passing through 1 mL of methanol two times and then 1 mL of water followed by 500 μL of DPB buffer. The mixed aliquots were applied to the equilibrated SPE cartridges, which were washed with 1 mL of washing buffer (20%, v/v methanol, 0.4%, w/v TBA and 5 g/L EDTA 2Na in 0.2 mol/L NH_4Cl–NH_4OH buffer, pH 8.5), then with 1 mL of water, and finally dried for 30 s. The catecholamines trapped by the cartridges were eluted with 500 μL of mobile phase. The eluted samples were then injected into the HPLC.

2.7. Recovery studies

Known amounts of catecholamines and 0.5 ng of DHBA as an internal standard were added to 500 μL of artificial plasma, and the resulting mixture was extracted with a C30 cartridge. The absolute recovery was estimated as the ratio between the amount of analyte from extraction and the corresponding standard solution. The repeatability of assay intra-variances was evaluated from five replicate analyses on the same day, while assay inter-variances were evaluated from 5 consecutive days.

2.8. Plasma calibration curves

Various amounts of catecholamine (as a standard solution) in 500 μL of artificial plasma were extracted. Each elution was injected into the HPLC system. Quantification was done using the method of internal standardization. Calibration curves were evaluated by plotting the catecholamine peak height values against the respective concentrations of analyte standards.

3. Results

3.1. Analytical potential

In order to determine the appropriate potential for the electrochemical detection of analytes, a hydrodynamic voltammogram was investigated. A good response presented at above 550 mV and the maximum signal of each analyte reached a plateau at 650 mV. We applied 550 mV throughout this study, since the background current was almost 0 nA at this potential.

3.2. Mobile phase conditions

Some chromatographic parameters, including the pH of the mobile phase, the ion pair reagent, and organic modifier were investigated. The pH was varied from 2.2 to 3.9. At the lowest pH value, analyte k' values approached each other but were not enough to separate the interfering peaks, specifically the solvent front peak and large negative peaks. As the pH of the mobile phase increased, only the k' value of DOPA became smaller. The best resolution was obtained at pH 2.8; DOPA interfered with DA at pH 2.5, with DHPG at pH 3.0, with DHBA at pH 3.2, and with Epi at pH 3.9 (Fig. 1).

The mobile phase concentration of the ion pair reagent (SOS) was varied from 0 to 0.1%, and that of acetonitrile was varied from 1 to 5%. The retention time of DA was too prolonged to detect it in reasonable times at SOS concentrations above 0.01%. Therefore, the ion pair reagent was not used for this analysis. Two percent acetonitrile without SOS was found to produce the best analyte resolution.

3.3. Temperature of the column

The column temperature greatly influenced the resolution obstacle. An unknown peak interfered with DOPA and DHPG
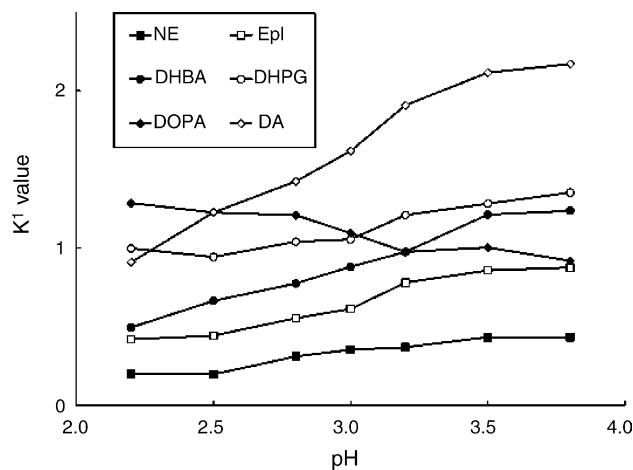

Fig. 1. Effects of mobile phase pH on the k' values of analytes under 2% (v/v) acetonitrile conditions. NE: norepinephrine; Epi: epinephrine; DHBA: 3,4-dihydroxybenzylamine used as an internal standard; DHPG: 3,4-dihydroxyphenyl glycol; DOPA: 3,4-dihydroxyphenylalanine and DA: dopamine.

at temperatures above 40 °C and with DA at temperatures below 30 °C. This unknown peak detected between DOPA and DA, did not interfere with the analytes at 34 °C. Good separation between this unknown peak and the analytes were achieved at this temperature.

3.4. Chromatography

Chromatograms obtained from standard and plasma samples are shown in Fig. 2a–c. The mobile phase consisted of 1.05% (w/v) citric acid adjusted with NaOH to pH 2.8 and 2.0% (v/v) acetonitril. The column temperature was set at 34 °C. NE, Epi, DHBA, DHPG, DOPA and DA peaks were separated with capacity ratios (k'-value) of 0.3, 0.5, 0.8, 1.2, 1.3 and 1.4, respectively. Fig. 2c illustrates a chromatogram from patient plasma with good peak separation.

3.5. Concentrations of complexing reagent

The absolute recoveries of analytes were influenced by the concentration of DPB for the SPE procedure (Fig. 3). Below the concentration of 0.01% DPB, all analyte recoveries decreased compared to those at 0.01% DPB. At concentrations above 0.01%, only that of NE decreased compared to those at 0.01% DPB, while that of other analytes occurred at almost the same level. Greater than 90% recoveries was obtained for all of the analytes at 0.01% DPB.

3.6. Cartridges for SPE

The extraction efficiency for three types of SPE cartridges was evaluated based on the absolute recovery of each analyte (Table 1). Silica-based C30, MFC18 and polymer-based OASIS HLB were used for the SPE procedure using 0.01% DPB solution. C30 cartridge presented with the highest recovery rates for

Table 1
Extraction efficiency based on cartridges for SPE ($n = 6$)

	Actual value (pg/mL)	Recovery (%)								
		C30			MFC18			OASIS		
		Mean	S.D.	CV	Mean	S.D.	CV	Mean	S.D.	CV
NE	900	87	5	6	66	6	9	63	3	5
Epi	300	90	9	10	85	5	6	90	4	4
DHBA	500	94	5	6	81	7	8	85	2	3
DHPG	1600	98	8	8	98	7	7	68	3	5
DOPA	900	96	7	8	92	3	4	27	3	9
DA	100	87	7	7	89	7	9	99	8	8

Extraction efficiencies were evaluated as absolute analyte recoveries. The particles for extraction cartridge were triacontylsilyl silica for C30, mono functional octadecylsilyl silica for MFC18, and polymer for OASIS. NE: norepinephrine; Epi: epinephrine; DHBA: 3,4-dihydroxy benzylamine as internal standard; DHPG: 3,4-dihydroxyphenyl glycol; DOPA: 3,4-dihydroxyphenylalanine; DA: dopamine; SD: standard deviation and CV: coefficient variation. The amount of each analyte was added to the reconstituted plasma. Recoveries are presented as mean values, S.D. and CV.

all the analytes among these three types of cartridges, where the mean recoveries by C30 were above 87% and coefficients of variation were less than 10%. Therefore, we selected to use C30 cartridges throughout our analysis.
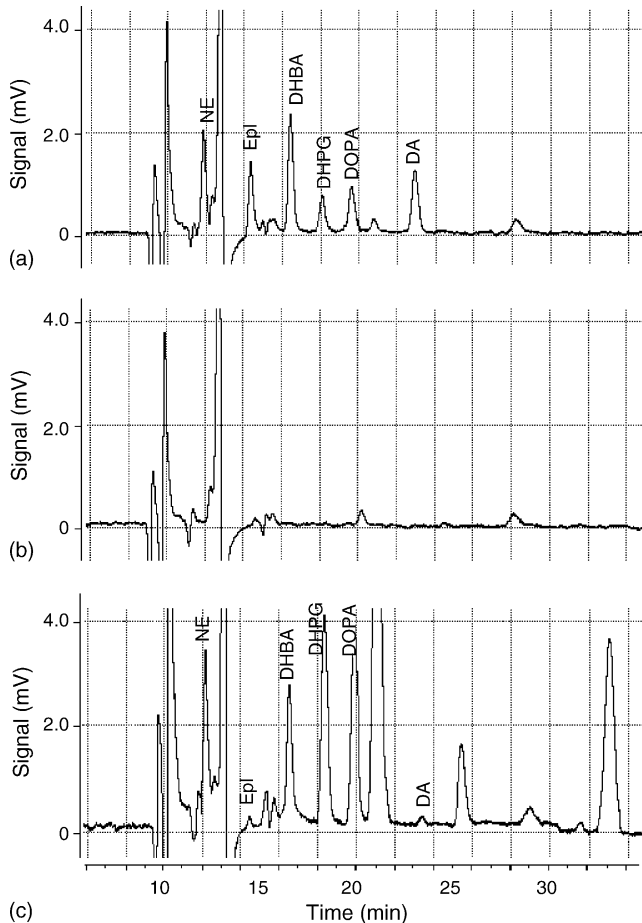

Fig. 2. Chromatograms. (a) Artificial plasma added with identical doses of each standard solution (500 pg/mL); (b) blank artificial plasma; (c) a sample obtained from plasma of a patient containing 600 pg/mL NE, 43 pg/mL Epi, 1770 pg/mL DHPG and 1340 pg/mL DOPA. DA was detected but was not quantified (below 40 pg/mL, above 10 pg/mL). The added dose of DHBA as internal standard was 1000 pg/mL for (a) and (c). NE: norepinephrine; Epi: epinephrine; DHBA: 3,4-dihydroxybenzylamine; DHPG: 3,4-dihydroxyphenyl glycol; DOPA: 3,4-dihydroxyphenylalanine and DA: dopamine.

3.7. Recovery studies

Assay procedure precision was evaluated from absolute recoveries (Table 2). At the three different concentrations of each analyte, including DHBA as an internal standard, mean absolute recoveries were above 91% for intra-assay, above 85% for inter-assay, and the coefficients of validations were less than 10%.

3.8. Linearity, detection limits, and precision

Analyte linearity was obtained in the concentration ranges from 40 to 5000 pg/mL for NE and DOPA, from 100 to 5000 pg/mL for DHPG, and from 10 to 2000 pg/mL for Epi and DA. These levels are sufficient for clinical use when plasma catecholamines were measured. Linear regression equations and the correlation coefficients for the analytes are presented in Table 3.

Lower detection limits ($S/N = 4$) were 40 pg/mL for NE, DHPG and DOPA, and 10 pg/mL for both Epi and DA, while
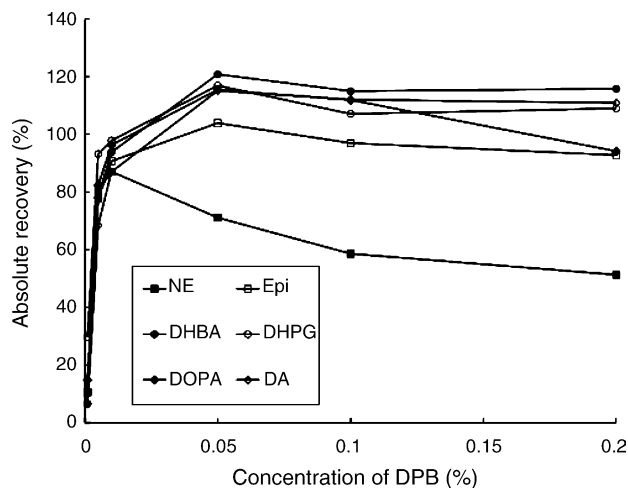

Fig. 3. The effect of DPB concentration on absolute recovery of analyte. The various amounts of DPB in the DPB buffer were used for solid-phase extraction. DPB: diphenylboric acid 2-aminoethyl ester; NE: norepinephrine; Epi: epinephrine; DHBA: 3,4-dihydroxybenzylamine; DHPG: 3,4-dihydroxyphenyl glycol; DOPA: 3,4-dihydroxyphenylalanine and DA: dopamine.

Table 2
Precision of catecholamine absolute recovery

	Actual value (pg/mL)	Intra-assay recovery (%)				Inter-assay recovery (%)	
		Mean	S.D.	CV	Mean	S.D.	CV
NE	5000	96	4	4	91	7	7
	900	91	5	5	85	8	9
	100	94	7	8	92	10	10
Epi	1000	92	1	1	87	10	9
	300	102	9	9	93	11	9
	20	92	10	10	107	10	10
DHPG	5000	93	6	6	87	10	10
	500	129	5	4	105	11	10
	100	94	8	9	102	10	10
DOPA	5000	91	5	5	99	5	5
	900	102	6	6	92	10	10
	100	98	10	10	86	10	10
DA	2000	102	3	4	97	10	10
	600	102	3	4	89	9	10
	40	95	10	10	105	10	10

Precision was evaluated as absolute analyte recovery. Intra-assay data was based on five replicate analyses and inter-assay data was based on 5 consecutive days. S.D.: standard deviation and CV: coefficient variation.

the lower quantification limits (S/N = 6) were 100 pg/mL for NE, DHPG and DOPA, 20 pg/mL for Epi, and 40 pg/mL for DA. Each of the precision data observed from inter- and intra-assay variation was less than 10% at the concentration of lower detection limits (Table 2).

4. Discussion

Our triacontylsilyl silica column (C30), sufficiently end-capped by the trimethylsilyl silica [31], provided good resolution and made it possible for simultaneous analyte measurements such as norepinephrine (NE), epinephrine (Epi), 3,4-dihydroxyphenylglycol (DHPG), 3,4-dihydroxyphenylalanine (DOPA) and dopamine (DA). This column has little polar groups and a small pore size (14 nm) on the surface [31]. The analysis with such a column reportedly maintains good repeatability, even under 100% aqueous mobile phase conditions and under a wide range of temperatures from room temperature to approximately 60 °C [31,32]. These column characteristics might enable us to use our mobile phase, which contained little organic modifier. Improved resolution might also be the result

Table 3
Parameters of catecholamine calibration curves

Analyte	Linear regression equation[a]	r^2
NE	$y = 0.944x + 0.041$	0.997
Epi	$y = 0.609x + 0.005$	1.00
DHPG	$y = 1.16x + 0.048$	0.996
DOPA	$y = 0.956x + 0.077$	1.00
DA	$y = 0.989x + 0.005$	0.998

[a] In the linear regression equations, y is the expressed as the peak-height ratio of each analyte to internal standard and x is the expressed as the amount of each analyte (ng/mL). r^2 is the correlation coefficient. NE: norepinephrine; Epi: epinephrine; DHPG: 3,4-dihydroxyphenylglycol; DOPA: 3,4-dihydroxyphenylalanine and DA: dopamine.

of the triacontyl-groups, which has about a 1.7-fold longer chain in the surface of the particles than the previous column. Such a long chain might also supply enhanced secondary polar interaction between polar analytes and its surface. This interaction by the C30 could cause more retention of polar analytes than one by octadecylsilyl silica (C18). Among the analytes investigated, DHPG consisted of the polar material without amino groups, whose charged potential against the surface in the mobile phase is different from that of the other analytes. Its peak was not sufficient enough to separate the interfering peaks, such as the leading solvent, when the C18 column is used. The C18 column required various ion pair reagents and some organic modifiers in the mobile phase to improve analyte resolution [18–21]. However, the C30 column made it possible to measure the level of DHPG since its chain provided DHPG for enough retention to the surface. Our methods, uses this C30 column and does not require the ion pair reagent for analysis of these substrates, meaning that we can make prompt measurements. The previous method involving the C18 column also proves difficult, in that the separation of DOPA from the interference peaks was a challenge. We observed that the DOPA k' value using the C30 column was influenced more by temperature as well as by mobile phase pH than the C18 column. Thus, we focused on these different characteristics in order to obtain good separation.

The good analyte extraction efficiencies were provided from the solid-phase extraction (SPE) with a C30 particle packed cartridge, when 0.01% diphenyl boronic acid (DPB) was used. Since DPB forms a complex with *cis*-hydroxyl groups of catecholamines, the selective extraction of analytes possessing catechol groups was caused by adequate retention of such complexes to solid particles in the cartridges [33]. These retention times, caused by intermolecular forces, change depending on the concentration of DPB and the particles. The recovery of NE was greatly improved when the concentration of DPB was decreased to 0.01%, compared with previously reports of 0.1 or

0.2% [12,16,29,30]. This improvement might be partly due to C30 particle characteristics. The difference in the DPB concentration indicates that higher ionic strength caused by excess DPB might disturb the smooth formation of a DPB-NE complex. This complex, as well as diphenylboronic acid formed from DPB, might be negatively charged and stabilized in alkali media. In fact, it may be subsequently hydrolyzed in acidic media and hydrolyzed even more in a lower concentration of DPB. This explains why we could improve the recovery of NE through the respective extraction procedures, using 0.01% DPB under controlled pH conditions.

The C30 particle improved extraction resolution and efficiency. Our method is convenient and provides simultaneous analysis of human catecholamines, such as NE, Epi, DHPG, DOPA and DA. Thus, it can be used for clinical examination.

References

[1] I.J. Kopin, Pharmacol. Rev. 37 (4) (1985) 333.
[2] J. Ludwig, T. Gerhardt, T. Halbrugge, J. Walter, K.H. Graefe, Eur. J. Clin. Pharmacol. 35 (1988) 261.
[3] G. Eisenhofer, M.D. Esler, I.T. Meredith, A. Dart, R.O. Cannon, A.A. Quyyumi, G. Lambert, J. Chin, G.L. Jennings, D.S. Goldstein, Circulation 85 (1992) 1775.
[4] K.-H. Graefe, M. Henseling, Gen. Pharmacol. 14 (1) (1983) 27.
[5] J.L. Izzo, D. Greulich, Life Sci. 33 (1983) 483.
[6] J.L. Izzo, D.A. Thompson, D. Horwitz, Life Sci. 37 (1985) 1033.
[7] D.S. Robinson, G.A. Johnson, A. Nies, J. Corcella, T.B. Cooper, D. Albright, D. Howard, J. Clin. Psychopharmacol. 3 (1983) 282.
[8] G. Jackman, J. Snell, H. Skews, A. Bobik, Life Sci. 31 (1982) 923.
[9] L.G. Howes, S. Miller, J.L. Reid, J. Chromatogr. 338 (1985) 401.
[10] G. Eisenhofer, D.S. Goldstein, R. Stull, H.R. Keiser, T. Sunderland, D.L. Murphy, I.J. Kopin, Clin. Chem. 32 (1986) 2030.
[11] T. Halbrugge, T. Gerhardt, J. Ludwig, E. Heidbreder, K.-H. Graefe, Life Sci. 43 (1988) 19.
[12] D.H. Fischer, L.M. Fischer, M. Broudy, Biomed. Chromatogr. 10 (1996) 161.
[13] M.A. Raggi, C. Sabbioni, G. Casamente, G. Gerra, N. Calonghi, L. Masotti, J. Chromatogr. B 730 (1999) 201.
[14] J.M. Sanchis Mallols, J.R. Torres Lapaiso, R.M. Villanueva Camanas, G. Ramis-Ramos, Chromatographia 39 (1994) 591.
[15] J.M. Sanchis Mallols, R.M. Villanueva Camanas, G. Ramis-Ramos, Choromatographia 39 (1994) 365.
[16] G. Grossi, A. Bargossi, A. Lippi, R. Battistoni, Chromatographia 24 (1987) 842.
[17] M. Candito, F. Bree, A.M. Krsrulovic, Biomed. Chromatogr. 10 (1996) 40.
[18] S. Xie, R.F. Suckow, T.B. Cooper, J. Chromatogr. B 677 (1996) 37.
[19] B.-M. Eriksson, B.-A. Persson, J. Chromatogr. 228 (1982) 143.
[20] C. Holmes, G. Eisenhofer, D.S. Goldstein, J. Chromatogr. B 653 (1994) 131.
[21] R.T. Peaston, J. Chromatogr. 424 (1988) 263.
[22] Y. Imai, S. Ito, K. Maruta, K. Fujita, Clin. Chem. 34 (1988) 528.
[23] I. Meineke, E. Stuwe, E.M. Henne, G. Rusteberg, E. Brendel, C. De Mey, J. Chromatogr. 493 (1989) 287.
[24] F. Smedes, J.C. Kraak, H. Poppe, J. Chromatogr. 231 (1982) 25.
[25] D.C. Jimerson, S.P. Markey, J.A. Oliver, I.J. Kopin, Biomed. Mass Spectrom. 8 (1981) 256.
[26] B.-M. Eriksson, B.-A. Person, J. Chromatogr. 386 (1987) 1.
[27] A.H. Wu, T.G. Gornet, Clin. Chem. 31 (1985) 298.
[28] F. Mashige, Y. Matsushima, C. Miyata, R. Yamada, H. Kanazawa, I. Sakuma, N. Takai, N. Shinosuka, A. Ohkubo, K. Nakahara, Biomed. Chromatogr. 9 (1995) 221.
[29] D. Talwar, C. Williamson, A. McLaughlin, A. Gill, D.St.J. O'Reilly, J. Chormatogr. B 769 (2002) 341.
[30] A. Pastoris, L. Cerutti, R. Sacco, L. De Vecchi, A. Sbaffi, J. Chromatogr. B 664 (1995) 287.
[31] N. Nagae, T. Enami, S. Doshi, LCGC North Am. 20 (10) (2002) 964.
[32] S. Doshi, T. Enami, N. Nagae, LCGC Eur. 16 (7) (2003) 2.
[33] L. Hansson, M. Glad, C. Hansson, J. Chromatogr. 265 (1983) 37.