ELSEVIER

Journal of Chromatography B, 769 (2002) 341–349

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Extraction and separation of urinary catecholamines as their diphenyl boronate complexes using C_{18} solid-phase extraction sorbent and high-performance liquid chromatography

Dinesh Talwar*, Cathie Williamson, Allison McLaughlin, Alan Gill, Denis St.J. O'Reilly

Department of Clinical Biochemistry, Macewen Building, Royal Infirmary, Glasgow G4 OSF, UK

Received 5 July 2001; received in revised form 16 November 2001; accepted 8 January 2002

Abstract

The clinical utility of a one-step extraction procedure based on the retention of a diphenyl boronate–catecholamine complex on a C_{18} solid-phase extraction sorbent was investigated for the measurement of urinary catecholamines. Although recoveries with the extraction procedure were optimal over a relatively broad pH range (7.5–9.5), analytical factors such as sample loading and elution flow-rates, wash step and elution conditions, the concentration of catecholamines in urine to be extracted and the type of C_{18} sorbent used for extraction were found to influence the efficiency of this procedure and would therefore need to be controlled for optimal recoveries. Under optimal conditions the recovery of noradrenaline, adrenaline and dopamine from spiked urine was high and reproducible (mean recoveries were >85% for all catecholamines). The effectiveness of sample clean-up step was demonstrated by reverse phase, ion pair high-performance liquid chromatography with electrochemical detection. The method described was found to be suitable for the routine measurement of catecholamines in urine in clinical biochemistry laboratories. It has a high sample extraction throughput (40/h) and has adequate precision (between batch C.V.<8%) and sensitivity (LOD<30 nmol/l; LOQ<65 nmol/l) for all the catecholamines measured. The method has acceptable accuracy, showing a mean bias of 6.6% for noradrenaline, 7.3% for adrenaline and 6.8% for dopamine from the mean value of laboratories (N=69) participating in an External Quality Assurance scheme for greater than 12 months.

Keywords: Catecholamines; Diphenyl boronate

1. Introduction

The importance of measuring urinary free catecholamines for the investigation of catecholamine secreting tumours is well recognised. Measurement of urinary catecholamines is usually by high-performance liquid chromatography (HPLC) with electrochemical detection, which requires an extensive sample purification step to remove potentially interfering compounds. This purification step has been performed mainly by using off line liquid–solid or liquid–liquid extraction procedures. Extraction with alumina requires complex washing procedures which introduces variability in recovery, is relatively time consuming and specificity tends to be poor [1–3]. Solid-phase extraction (SPE) procedures utilising sephadex G_{10} [4], weak or strong cation-exchange columns [2,3,5], C_{18} matrix [6,7] or boronate gels [2,3,8,9] tend to use extensive column washing

*Corresponding author. Tel.: +44-141-553-1703; fax: +44-141-211-5178.

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00022-3

procedures and often require two sequential extractions. In addition, optimal and reproducible recoveries with these procedures require careful adjustment of the sample pH [2,8,9]. Liquid–liquid extraction procedures are labour intensive and time consuming requiring extraction of catecholamines into an organic phase and then back extraction into an acid phase [10,11]. Several on-line extraction methods have been reported for procedural simplification and automation [12–14]. However, such methods require additional equipment such as column switching and automated sample processing systems, which are not readily available in clinical chemistry laboratories.

Grossi and co-workers [6] described a rapid, one-step liquid–solid procedure for the extraction of catecholamines from urine employing diphenyl boronic acid (DPBA) ethanolamine ester and a C_{18} SPE sorbent. Urinary catecholamines are only weakly retained by the C_{18} matrix; however, in alkaline media, DPBA-ethanolamine ester is dissociated into negatively charged diphenyl boronate and ethanolamine [14]. The diphenyl boronate forms a stable negatively charged complex (Fig. 1) with *cis*-hydroxyl groups of catecholamines [15], which is strongly retained on a C_{18} extraction sorbent when operating in alkali media [6]. This allows column washing with methanol–buffer solutions to remove interfering compounds without the loss of the catecholamines which are eluted by disrupting the complex under acid conditions. Recovery studies indicate that binding of urinary catecholamines to DPBA-ethanolamine ester at alkaline pH is nearly quantitative (>95%) [6]. However, the efficiency and robustness of this extraction procedure in terms of recovery and sample clean-up has not been investigated for analytical variables such as extraction pH, sample loading, elution flow-rates, wash step, elution conditions, stability of the diphenyl boronate–catecholamine complex during the extraction procedure, the capacity of the C_{18} sorbent to adsorb the diphenyl boronate–catecholamine complex from urine, type of C_{18} sorbent used for extraction, effect of sample matrix and potential interference by some structurally related compounds.

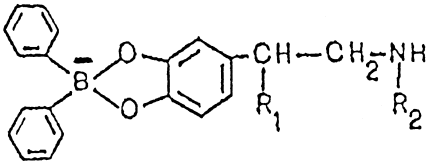

Fig. 1. Diphenyl boronate–catecholamine complex structure. (For noradrenaline: R1=OH, R2=H; for adrenaline: R_1=OH, R_2=CH_3; for dopamine: R_1=H, R_2=H). Adapted from Ref. [6].

The aim of this study was to investigate the above variables with a view to proposing an effective, reliable extraction method for the routine determination of free catecholamines in urine and to document the advantages and limitations of the proposed method. The effectiveness of the extraction procedure was demonstrated by reversed-phase HPLC with electrochemical detection. We report on the long term performance of this method in the UK External Quality Assurance scheme for the measurement of urinary catecholamines.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade. DPBA-ethanolamine ester was obtained from the Aldrich Chemical Company (Poole, UK). Methanol, acetonitrile, sodium dihydrogen phosphate, sodium dodecyl sulphate (SDS), EDTA disodium salt, ammonium chloride, concentrated ammonia and acetic and phosphoric acids were obtained from BDH Chemicals (Poole, UK).

The catecholamine standards (noradrenaline, adrenaline and dopamine); the internal standard (I.S.), dihydroxybenzylamine (DHBA); catecholamine metabolites [normetadrenaline, metadrenaline, 4-hydroxy-3-methoxymandelic acid (VMA), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG)] were all obtained from the Sigma Chemical Company (Poole, UK).

Urine catecholamine calibrator and spiked controls (levels 1 and 2) were obtained from Biorad (Hemel Hempstead, UK). These are lyophylised materials based on human urine. They were reconstituted in 0.05 mol/l HCl according to the manufacturers instructions, aliquoted and stored at −20 °C.

2.2. Instrumentation

The HPLC system consisted of a Waters solvent delivery system, a Waters autosampler and a Coulometric electrochemical detector (ESA model 5100A). The HPLC column used was C_{18} Luna, 250×4.6 mm I.D. (5 μm particle size, 100 Å pore diameter), obtained from Phenomenex (Macclesfield, UK). The column effluent was monitored using the ESA detector with a high sensitivity analytical cell (ESA 5011). The detector settings were as follows: det. 1, 0.1 V; det 2, 0.35 V; gain 10×10. Hydrodynamic voltamograms demonstrated that using these settings gave optimal detector response for catecholamines with minimal response from other potentially interfering compounds and a low background signal (0.002 μΩ). Above 0.35 V, there was a sharp increase in signal-to-noise ratio (S/N) using the mobile phase described. Voltamograms were obtained using DHBA since its oxidation potential is higher than that of catecholamines.

Urinary catecholamines were extracted manually using the Vac Elut system and monofunctional C_{18} SPE cartridges (Isolute MF C_{18} 100 mg/10 ml XL column reservoir), both obtained from Jones Chromatography (Glamorgan, UK).

2.3. Procedures

2.3.1. Preparation of mobile phase

The isocratic mobile phase used for ion pair, reversed-phase HPLC was a modification of that used by Grossi et al. [6]. It consisted of dihydrogen phosphate buffer (50 mmol/l; sodium salt), 200 ml/l acetonitrile, 100 ml/l methanol, 250 mg/l EDTA and 500 mg/l sodium dodecyl sulphate (SDS) as the counter ion. The pH of the mobile phase was adjusted to 2.9 with 6 mol/l orthophosphoric acid, filtered through a 0.45-μm nylon filter and pumped through the HPLC column (protected with a 10-mm guard column) at a flow-rate of 1.5 ml/min.

2.3.2. Preparation of standards

Stock standards (1 mmol/l) of noradrenaline, adrenaline, dopamine and the internal standard (DHBA) were prepared in 0.1 mol/l HCl and stored at 4 °C. These were further diluted in 0.05 mol/l HCl to provide working standards in the concentration range of 50–10 000 nmol/l for catecholamines and 5000 nmol/l for the internal standard.

2.3.3. Sample collection

The 24-h urine samples were collected in plastic containers containing 10 ml of 6 mol/l HCl, so that the final pH of the samples was between 1 and 3. Acidified urine samples were stored at 4 °C until analysis.

2.3.4. Sample preparation and extraction

To each glass tube (16×100 mm) 1 ml of urine calibrator, control or sample; 100 μl of the internal standard (DHBA, 5 μmol/l in 0.05 mol/l HCl) and 2 ml of buffer containing a complexing agent (0.2% DPBA-ethanolamine ester and 5 g/l EDTA in 2 mol/l NH_4Cl–NH_4OH buffer, pH 8.5) was added. After mixing, the pH of the complexed sample preparation was checked with a pH probe or narrow range pH paper. At this stage most of the complexed urine samples had a pH value of between 7.5 and 8.5. If the pH was less than 7.5, it was adjusted with concentrated ammonia to be between 7.5 and 9.5.

For the extraction, the SPE columns were placed on a Vac Elut extraction system, activated and equilibrated with 2 ml of methanol followed by 2 ml of wash buffer (0.2 mol/l NH_4Cl–NH_4OH, pH 8.5). The complexed samples (1.5 ml) were applied to the equilibrated SPE columns which were then washed with 2 ml of wash buffer followed by 2 ml of 20% methanol in wash buffer (pH 8.5) to remove interferences and finally dried for 30 s at full pressure (20 inches Hg). The catecholamines were eluted with 1.5 ml of acetic acid (1 mol/l), an aliquot transferred to glass autosampler vials and 40-μl injected via an autosampler. The flow-rate of each of the above steps was <0.5 ml/min (flow-time=4–5 min).

Using the Vac Elut system, 40 urine samples could be extracted within 1 h for overnight analysis by HPLC.

2.3.5. Quantitation

Quantitation was done by the method of internal standardisation using a single level calibration. Catecholamine concentration in urine (nmol/l) was calculated by multiplying the peak height ratio of the catecholamine–internal standard in the sample and calibrator chromatogram with the concentration of

the catecholamine in the urine calibrator. The concentrations of noradrenaline, adrenaline and dopamine in the urine calibrator were 1159, 578 and 1978 nmol/l, respectively. The catecholamine peaks were identified by retention times relative to the internal standard

2.3.6. Quality control

Commercial quality control (QC) material (Biorad urine levels 1 and 2) was used for the internal QC. The accuracy and performance of the extraction and separation procedures was monitored by taking part in External Quality Assurance scheme for the measurement of urine catecholamines (UKNEQAS, Wolfson EQA laboratory, Birmingham, UK).

2.3.7. Recovery studies

Studies on recovery and the effect of various analytical factors on extraction efficiency were performed using aliquots of an acidified 24-h urine sample obtained from a healthy adult. The mean concentrations of catecholamines in this urine sample when extracted and analysed were noradrenaline, 103 nmol/l; adrenaline, 56 nmol/l; dopamine, 180 nmol/l ($N=6$). Four 50-ml aliquots of this urine were spiked to increase the concentration of noradrenaline, adrenaline and dopamine by 200, 2000, 4000 and 6000 nmol/l. Each aliquot was then extracted six times and analysed as described above for samples to obtain the mean recoveries. Recovery was calculated by comparing the net chromatographic peak heights obtained by subtraction of peak heights after and before standard addition with those obtained after injecting the appropriate aqueous standards directly onto the HPLC column.

2.3.8. Assessment of analytical variables on extraction efficiency

Preliminary work had shown that recovery of catecholamines from urine increased depending on the DPBA concentration in the complexing buffer and reached a plateau at concentrations over 0.15% (w/v). A DPBA concentration of 0.2% was therefore selected as the optimal concentration for the extraction procedure.

The efficiency of the extraction method with respect to optimal recovery and sample clean-up was examined by investigating the following analytical variables: pH of the complexed sample, sample loading and elution flow-rates, methanol wash step and acetic acid elution step. Their effect on the recovery of catecholamines from urine was studied by spiking an aliquot of the acidified healthy adult urine sample with 1000 nmol/l of each of the catecholamine standards which was then extracted ($N=4$) and analysed as described above for samples to obtain mean recoveries. Recovery was calculated as described in recovery studies (Section 2.3.7).

2.3.9. Specificity studies

Several drugs and metabolites were tested for possible interference with the extraction and separation procedure by collecting and analysing acidified 24-h urine samples from patients who were taking paracetamol or antihypertensive drugs (Atenalol, Labetalol, Methyldopa, Captopril). These drugs were chosen because previous studies have shown that sample extraction methods using phenyl boronic acid or alumina are prone to interference from urine collected from patients on these medications [9,16–18]. Possible interference by catecholamine metabolites was checked by taking aqueous solutions of normetadrenaline, metadrenaline, VMA, HVA and MHPG (10 μmol/l in 0.05 mol/l HCl) through the extraction and separation procedures as described above for the samples.

3. Results

3.1. Chromatography

The chromatographic profiles corresponding to an extracted urine calibrator and urine sample are shown in Fig. 2a–c. The noradrenaline, adrenaline, the internal standard and dopamine peaks were easily separated with capacity ratios (k') of 5.2, 6.2, 7.5 and 9.3, respectively.

Several chromatographic parameters were examined, including the pH of the mobile phase and the concentrations of the ion pair, organic modifier and phosphate buffer in the mobile phase in order to optimise peak separation and analysis time for catecholamines. The concentration of the ion pair (SDS) and organic modifier in the mobile phase had a major effect on peak shape, separation and analysis

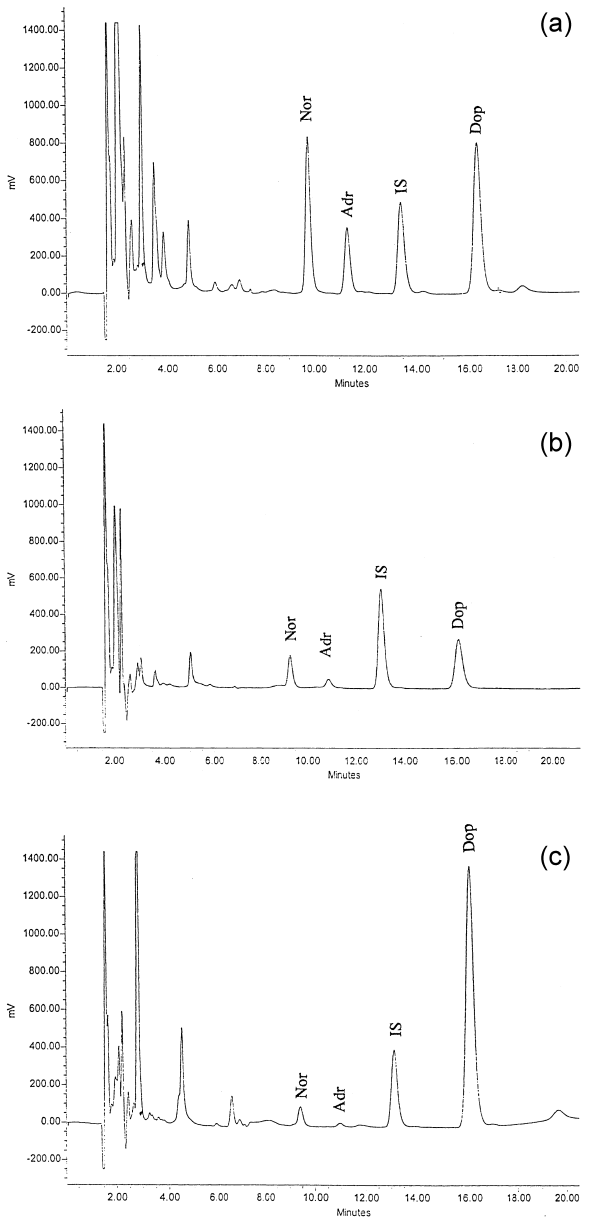

Fig. 2. Chromatographic profiles of urine extracts. (a) Urine based calibrator containing 1159 nmol/l noradrenaline, 578 nmol/l adrenaline and 1978 nmol/l dopamine; (b) normal urine containing 260 nmol/l noradrenaline, 80 nmol/l adrenaline and 660 nmol/l dopamine; (c) urine from a patient with ganglioneuroma. Urine was diluted five-fold prior to extraction. Concentration of noradrenaline, adrenaline and dopamine in the neat urine sample was 980, 218 and 17 530 nmol/l, respectively. Nor=Noradrenaline, Adr=adrenaline, I.S.=internal standard, Dop=dopamine.

time. A final concentration of 0.05% SDS, 20% acetonitrile and 10% methanol yielded catecholamine peaks that were well resolved with reasonable separation times. A combination of acetonitrile and methanol as the organic modifier was found to promote better resolution of catecholamine peaks than methanol on its own.

The concentration of the phosphate buffer in the mobile phase affected the intensity and reproducibility of the electrochemical signal and also column stability and performance. At concentrations of <10 mmol/l, the catecholamine peaks were broader and retention times longer. At concentrations of >100 mmol/l, the column was unstable. A phosphate concentration of 20–50 mmol/l avoided both problems yielding catecholamine peaks that were sharp and well resolved with reproducible retention times and signal response. The pH of the mobile phase had a significant effect on the k'-values of the catecholamines, increasing when the pH of the mobile phase was varied between 2 and 6. A pH of 2.9 was found to produce optimal retention times and resolution. Addition of EDTA to the mobile phase improved the stability of the ECD response by complexing with any metals present in the chromatographic system resulting in low base line noise.

Lunar reversed-phase analytical columns which are packed with a pH stable C_{18} bonded phase containing low metal contaminants yielded narrow symmetrical peaks, even for the late eluting dopamine peak which has a retention time of 16.5 min under the chromatographic conditions specified. Although Nucleosil and Apex C_{18} columns resolved catecholamines adequately using the mobile phase described, both columns produced broad tailing dopamine peaks.

3.2. Effect of analytical variables on extraction efficiency

3.2.1. pH of complexed sample

For high and uniform recoveries ($>85\%$), the optimal pH of the complexed sample was determined to be between 7.5 and 9.5. Below pH 7.0, mean catecholamine recovery from urine was poor (58% for noradrenaline, 55% for adrenaline and 61% for dopamine at pH 6.5; $N=4$).

3.2.2. Sample loading and elution flow-rates

The optimal loading and elution flow-rates were found to be less than 0.5 ml/min. Higher flow-rates resulted in poor and irreproducible catecholamine recoveries (ranging from 45 to 64% at approximately 1 ml/min.)

3.2.3. Column wash step

The 20% methanol in NH_4Cl–NH_4OH wash buffer allowed removal of interfering compounds while maintaining optimal recovery for all catecholamines (>85%). Increasing the methanol concentration in the wash buffer to 30% gave lower catecholamine recoveries (mean recovery=64% for noradrenaline, 61% for adrenaline and 70% for dopamine). Decreasing the methanol concentration to 10% did not produce effective sample clean up for HPLC analysis. Interfering peaks were observed between the noradrenaline and the internal standard region on the chromatogram.

3.2.4. Elution step

Elution with 1 mol/l acetic acid yielded optimal catecholamine recoveries. Mean catecholamine recoveries were lower when a more dilute acid (0.1 mol/l) was used for elution (noradrenaline 66%, adrenaline 63% and dopamine 55%).

From the results obtained with the above study, the following optimal extraction conditions could be selected: pH of complexed sample between 7.5 and 9.5; sample loading, wash step and elution flow-rates <0.5 ml/min; sample clean up with 20% methanolic buffer and elution with 1 mol/l acetic acid.

3.3. Analytical validation

3.3.1. Linearity and detection limits

The method was linear up to at least 2500 nmol/l for noradrenaline, adrenaline and dopamine. Linearity was assessed by taking aqueous solutions containing catecholamines through the entire procedure.

The limits of detection (LOD; $S/N=4$) were 14 nmol/l for noradrenaline, 22 nmol/l for adrenaline and 27 nmol/l for dopamine. The limits of quantitation (LOQ; $S/N=10$) were 32, 45 and 64 nmol/l, respectively. At these concentrations, the aqueous standard when taken through the extraction and separation procedures produced mean catecholamine values within 10% of the expected values ($N=6$) with 15% between batch variation (C.V.).

3.3.2. Recovery studies

Up to 4000 nmol/l, the mean recovery of each catecholamine was high and reproducible (Table 1). However, at 6000 nmol/l the SPE columns became overloaded and mean recoveries fell significantly for noradrenaline and adrenaline. Urine samples from patients with phaeochromocytoma with very high concentrations of catecholamines (total>12 000 nmol/l) would therefore have to be appropriately diluted for accurate quantitation of catecholamines. In our experience, high catecholamine recoveries were obtained only when MF C_{18} sorbent was used for extraction. Using end-capped C_{18} sorbent for extraction resulted in lower recoveries. This may be because the MF sorbent provides enhanced retention of the catecholamine–boronate complex as a result

Table 1
Absolute recovery of catecholamines added to aliquots of an acidified urine sample

Concentration of each catecholamine added (nmol/l)	Mean observed concentration ($N=6$)				Mean recovery (%) ($N=6$)			
	Nor	Adr	IS[a]	Dop	Nor	Adr	IS	Dop
0	103	56	465	180	–	–	93	–
200	286	230	450	363	91	87	90	91
2000	1983	1836	460	2040	94	89	92	95
4000	3663	3536	450	3900	89	87	90	93
6000	4723	4796	435	5580	77[b]	79[b]	87	90

Nor=Noradrenaline, Adr=adrenaline, Dop=dopamine.

[a] Concentration of DHBA, the internal standard, in each spiked sample was 500 nmol/l.

[b] Mean recovery significantly lower ($P<0.001$; Students *t*-test).

of additional polar interactions with the surface silanol groups.

Sample matrix appeared to have no effect on catecholamine recovery from urine. Analysis of urine spiked with 4000 nmol/l of each catecholamine from seven different patients yielded mean analytical recoveries in the range of 86–94% ($N=3$) for all catecholamines.

Although the mean recovery of exogenously added catecholamines was never 100% with the extraction procedure described, the use of an internal standard was the best way to correct for these differences in recovery (Table 1).

3.3.3. Precision studies

Precision data are shown in Table 2. The within batch imprecision for the measurement of catecholamines in urine was calculated by analysing Biorad QC levels 1 and 2 ten times on the same day. The between batch imprecision data were obtained from QC levels 1 and 2 analysed over a period of 6 months ($N=23$).

Table 2
Precision of catecholamine measurements in urine (nmol/l)

	Nor	Adr	Dop
QC1			
Intrabatch ($N=10$)			
Mean	219	70	603
SD	11.4	4.3	29
C.V. (%)	5.2	6.1	4.8
Intrabatch ($N=23$)			
Mean	217	66	588
SD	14	4.9	40
C.V. (%)	6.4	7.4	6.8
QC2			
Intrabatch ($N=10$)			
Mean	1620	418	2518
SD	78	25	128
C.V. (%)	4.8	5.7	5.1
Intrabatch ($N=23$)			
Mean	1577	406	2450
SD	96	33	176
C.V. (%)	6.1	8.0	7.2

Nor=Noradrenaline; Adr=adrenaline; Dop=dopamine.

3.3.4. Accuracy of measuring catecholamine concentrations in urine

The method showed a mean bias of 6.6% for noradrenaline, 7.3% for adrenaline and 6.8% for dopamine from the mean value of all laboratories ($N=69$) participating in the External Assurance scheme (UKNEQAS) for the measurement of catecholamines in urine by HPLC for longer than 12 months.

3.3.5. Stability studies

Unlike catecholamines which are unstable at alkaline pH, the diphenyl boronate–catecholamine complex (pH 8.5) was found to be stable for at least 2 h at room temperature which allows batch extraction of a large number of urine samples without loss of catecholamines.

Catecholamines eluted in acetic acid were stable in the autoinjector for at least 24 h at room temperature allowing a sample throughput of 40/day under the HPLC conditions described.

3.3.6. Specificity studies

Urine from patients taking labetalol or paracetamol produced an interfering peak whose k'-value was similar to that of adrenaline (Fig. 3a). Chromatograms from urine of patients receiving methyldopa showed two large peaks, one close to noradrenaline and the other with about twice the retention time of dopamine ($k'=18.0$). The first of these peaks, thought to be due to the drug metabolite α-methylnoradrenaline, has a k'-value of 3.1 which allows measurement of noradrenaline (Fig. 3b). However, if this peak is sufficiently large it can potentially swamp the noradrenaline peak preventing its quantitation. Attempts to solve this interference problem by varying the pH and the concentrations of the ion pair and organic modifier of the mobile phase proved unsuccessful.

Urine from patients on atenalol or captopril showed no interference. No interference was observed with the catecholamine metabolites normetadrenaline, metadrenaline, MHPG, VMA and HVA. When a urine specimen distributed by UKNEQAS [spiked with normetadrenaline (6 μmol/l), metadrenaline (3 μmol/l), VMA (37 μmol/l), HVA (21 μmol/l) and 5HIAA (80 μmol/l)] was extracted and analysed by the method described, no interference was observed with catecholamine measurements.

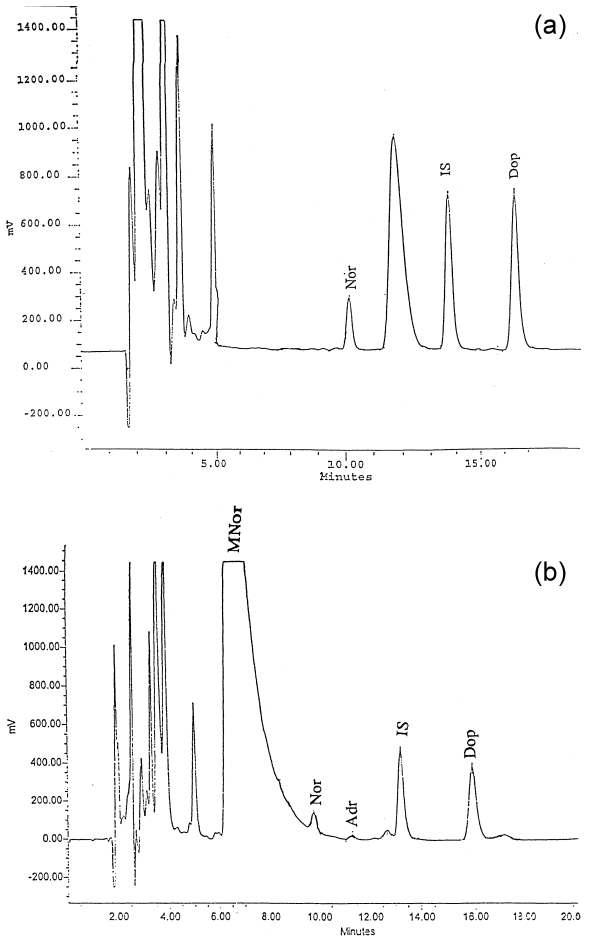

Fig. 3. (A) Chromatographic profile of urine extract from a patient on labetalol, 800 mg/day. The concentrations of noradrenaline and dopamine in this sample were 264 and 1210 nmol/l, respectively. Adrenaline could not be measured due to drug interference. (B) Chromatographic profile of urine extract from a patient on methyldopa, 750 mg/day. The concentrations of noradrenaline, adrenaline and dopamine in the sample were 160, 33 and 1060 nmol/l, respectively. Nor=Noradrenaline, MNor=α-methylnoradrenaline, Adr=adrenaline, I.S.=internal standard, Dop=dopamine.

4. Discussion

In this study we have examined the effect of various analytical variables on the efficiency of the one-step extraction procedure described by Grossi et al. [6] for the extraction of catecholamines from urine. The extraction efficiency in terms of recovery and effective sample clean-up was found to be influenced by analytical factors such as the pH of the complexed sample, its flow-rate through the SPE sorbent, concentration of methanol in the wash buffer, concentration of acetic acid used for elution, concentration of catecholamines in urine and type of C_{18} SPE sorbent used.

The diphenyl boronate extraction procedure described here has several advantages over extraction methods utilising the alumina, cation-exchange or boronate sorbents that are commonly used for urinary catecholamines: (i) Unlike these latter procedures which often require time consuming two sequential purification steps and careful adjustment of sample pH for effective sample clean up and optimal recoveries [2,8,9], the extraction method described is rapid requiring only a single purification step to yield sample preparations which show low residual interference when analysed by HPLC. In addition, since catecholamines recoveries were found to be optimal over a relatively broad alkaline pH range, it obviates the need for careful sample pH adjustment making the extraction procedure more robust. (ii) In contrast to alumina [2,3], extraction of urine catecholamines as their diphenyl boronate complexes using monofunctional (MF) C_{18} SPE sorbent shows high uniform recoveries. The SPE sorbent also has a high adsorption capacity to adsorb catecholamines from urine. The capacity of the sorbent was sufficient to adsorp catecholamines in concentrations up to at least 20 times the total concentration found in urine of normal individuals. (iii) With the extraction procedure described, stability and recovery studies showed that once the complexing agent was added to urine samples, there was no significant loss of catecholamines at alkaline pH. This is presumably due to the rapid covalent bond formation between the hydroxyl groups on the boronate ion and *cis*-diol groups on the catechol molecules at alkaline pH [15] which confers a stabilising effect on catecholamines during extraction. This is in contrast with some commonly used extraction procedures where the pH of urine samples has to be adjusted immediately before extraction to minimise oxidative loss of catecholamines during their extraction in neutral or alkaline conditions [1,4,9,13].

However, there are some limitations with the proposed extraction method. Since urine catechol-

amine recoveries with this extraction procedure fall sharply below pH 7, it is necessary prior to extraction to check, and if required to, adjust the pH of every sample to between 7.5 and 9.5 after addition of the complexing buffer. The extraction is therefore pH dependent for optimal recoveries. Also, like many other extraction methods previously described for urinary catecholamines [9,16–18], the method suffers from interference by some structurally related drug metabolites, e.g., metabolites of paracetamol and labetalol and potentially methyldopa. It is therefore important to have a full drug history with each request for urine catecholamine analysis in order to avoid misinterpretation of the chromatograms. Urine from patients receiving these medications should be collected 48 h after stopping their medication for catecholamine analysis.

The chromatographic separation and detection procedures were optimised with respect to all major variables to achieve the desired k'-value, sensitivity and analysis time. Coulometric detection provided adequate sensitivity for the reliable measurement of urinary catecholamines in the physiological range.

The method described here for the extraction of catecholamines from urine and their subsequent measurement by HPLC has acceptable precision and adequate sensitivity for use in the routine measurement of catecholamines in urine. It is an excellent alternative to other published methods, its main advantages being simplicity, robustness, effective sample clean-up, efficient chromatographic separation and relatively high sample extraction capacity. The method has been in routine use for over 3 years during which time it has been shown to provide an accurate measurement of urinary catecholamines as assessed by an External Quality Assurance Scheme.

References

[1] D.F. Davidson, J. Fitzpatrick, Ann. Clin. Biochem. 22 (1985) 297.
[2] A.H. Wu, T.G. Gornet, Clin. Chem. 31 (1985) 298.
[3] E. Gerlo, R. Malfait, J. Chromatogr. 343 (1985) 9.
[4] B.H. Westerink, F.J. Bosker, J.F. O'Hanlon, Clin. Chem. 28 (1982) 1745.
[5] P. Volin, J. Chromatogr. 655 (1994) 121.
[6] G. Grossi, A. Bargossi, A. Lippi, R. Battistoni, Chromatographia 24 (1987) 842.
[7] F. Mashige, Y. Matsushima, C. Miyata, R. Yamada, H. Kanazawa, I. Sakuma, N. Takai, N. Shinozuka, A. Ohkubo, K. Nakahara, Biomed. Chromatogr. 9 (1995) 221.
[8] C.R. Benedict, J. Chromatogr. 385 (1987) 369.
[9] R.T. Peaston, J. Chromatogr. 424 (1988) 263.
[10] H. Tsuchiya, T. Hayashi, J. Pharmacol. Methods 23 (1990) 21.
[11] E. Hollenbach, C. Shulz, H. Lehnert, Life Sci. 63 (1998) 737.
[12] G. Grossi, A.M. Bargossi, C. Lucarelli, R. Paradisi, C. Sprovieri, G. Sprovieri, J. Chromatogr. 541 (1991) 273.
[13] T. Soga, Y. Inoue, J. Chromatogr. 620 (1993) 175.
[14] A. Pastoris, L. Cerutti, R. Sacco, L. De Vecchi, A. Sbaffi, J. Chromatogr. 664 (1995) 287.
[15] L. Hansson, M. Glad, C. Hansson, J. Chromatogr. 265 (1983) 37.
[16] T.A. Jones, E.R. Walwick, Clin. Chem. 27 (1981) 1951.
[17] A.J. Speek, J. Odink, J. Schrijver, W.H. Schreurs, Clin. Chim. Acta 128 (1983) 103.
[18] D.F. Davidson, Ann. Clin. Biochem. 25 (1988) 583.