Journal of Chromatography, 343 (1985) 9—20
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2659

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF FREE NOREPINEPHRINE, EPINEPHRINE, DOPAMINE, VANILLYLMANDELIC ACID AND HOMOVANILLIC ACID

ERIK GERLO* and RONALD MALFAIT

Dienst Klinische Scheikunde, Akademisch Ziekenhuis, Vrije Universiteit te Brussel, Laarbeeklaan 101, B-1090 Brussels (Belgium)

(First received January 25th, 1985; revised manuscript received March 26th, 1985)

SUMMARY

A procedure is described for the concurrent assay of free norepinephrine, epinephrine, dopamine, vanillylmandelic acid and homovanillic acid in physiological fluids using high-performance liquid chromatography with electrochemical detection. The column packing is an octadecyl-bonded silica. A single mobile phase containing 1-octanesulphonate is used for the assay of catecholamines and for the assay of the acidic metabolites.

An efficient sample preparation scheme is presented for the isolation of the catecholamines and their acidic metabolites from the same sample aliquot. Catecholamines are extracted by ion exchange on small columns and adsorption on alumina, using dihydroxybenzylamine as an internal standard. Vanillylmandelic acid and homovanillic acid are recovered from the combined loading and washing effluents of the ion-exchange column by a solvent extraction procedure. Recovery of catecholamines averages 67%. The limit of detection for individual catecholamines is ca. 30 pg. Recoveries of vanillylmandelic acid and homovanillic acid average 77% and 87%, respectively. The use of the same mobile phase for the concurrent assay of catecholamines and their acidic metabolites considerably increases the throughput of samples in the chromatographic system by eliminating the time-consuming column-equilibration periods.

INTRODUCTION

Several methods for the analysis of catecholamines using high-performance liquid chromatography (HPLC) have been described (see ref. 1 for a review of the literature). These methods usually involve an apolar stationary phase in combination with a mobile phase containing an alkylsulphonate as ion-pairing agent. The chromatographic assay of the major metabolites of catecholamines, vanillylmandelic acid (VMA) and/or homovanillic acid (HVA), has also been

0378-4347/85/$03.30

reported using similar reversed-phase columns (e.g., refs. 2–4). In most of these procedures no ion-pairing agent is added to the mobile phase.

We report a chromatographic method with electrochemical detection for the assay of catecholamines in urine or plasma and for the assay of urinary VMA and HVA. A single isocratic mobile phase is used for the different assays. An extraction scheme is also presented for the isolation of catecholamines, VMA and HVA from the same sample aliquot. This procedure offers several advantages over other chromatographic methods: VMA and HVA are determined simultaneously with good specificity, precision and accuracy, the throughput of samples is increased since no equilibration of the analytical column is required when changing from VMA/HVA assays to catecholamine assays, the column lifetime is extended by the use of a single mobile phase, and the chromatographic analyses can be automated as a result of increased baseline stability. The procedure has been applied to the analysis of catecholamines in plasma and urine and to the analysis of VMA and HVA in urine.

EXPERIMENTAL

Chemicals

Epinephrine (A), norepinephrine (NA), dopamine hydrochloride (DA), 3,4-dihydroxybenzylamine (DHBA), isoprenaline (IP), epinine (EPI), synephrine (SYN), metanephrine (MN), normetanephrine (NMN), dihydroxymandelic acid (DOMA), *p*-hydroxyphenylacetic acid (POPAC), *p*-hydroxymandelic acid (POMA), *m*-hydroxymandelic acid (MOMA), 3,4-dihydroxyphenylacetic acid (DOPAC), vanillylmandelic acid (VMA) and homovanillic acid (HVA) were obtained from Sigma (St. Louis, MO, U.S.A.); octopamine hydrochloride (OCT), 4-hydroxy-3-methoxyphenylglycol piperazine (MOPEG), 3,4-dihydroxyphenylglycol (DOPEG) and 2,5-dihydroxyphenylacetic acid (HGA) were from Janssen Chimica (Beerse, Belgium); 3,4-dihydroxybenzoic acid (PA) and 3,4-dihydroxyphenylethanol (DOPET) were from Serva (Heidelberg, F.R.G.). We received two metabolites of α-methyldopa as a gift from Merck Sharp and Dohme (Rahway, NY, U.S.A.): 1-methyl-2-(3,4-dihydroxyphenyl)ethylamine hydrochloride (α-MD1) and 1-methyl-2-(3-methoxy-4-hydroxyphenyl)ethylamine hydrochloride (α-MD2).

The cation exchanger Bio-Rex 70 from Bio-Rad Labs. (Richmond, CA, U.S.A.) was regenerated after use by a batch method and equilibrated with 0.05 *M* phosphate buffer (pH 6.5).

Alumina oxide from Merck (Art. 1078, Darmstadt, F.R.G.) was used without further treatment.

Aqueous solutions were prepared in reagent-grade water obtained from a Milli-Q^{TM} 4-Bowl System (Millipore, Bedford, MA, U.S.A.).

Lyophilysed urine pools used in precision studies were from Hyland Diagnostics (Travenol Labs., Deerfield, IL, U.S.A.).

Reference solutions

All stock solutions were prepared in 0.01 *M* hydrochloric acid containing $Na_2S_2O_5$ (0.5 g/l) and Na_2EDTA (0.5 g/l).

Assay of catecholamines. Separate standard solutions (400 mg/l as the free base) were prepared in 100-ml amounts every two months. From these standard solutions a stock standard mixture (to contain, per litre, 4 mg of NA, A, DA and DHBA) and a stock solution of DHBA (4 mg/l) were made. Both stock solutions were aliquoted and stored at −20°C. The stock standard mixture was diluted weekly 100-fold in 0.5 *M* acetic acid containing $Na_2S_2O_5$ (0.5 g/l) and Na_2EDTA (0.5 g/l) for use as a calibration standard (40 μg/l). The DHBA stock solution was diluted weekly 10-fold in 0.01 *M* hydrochloric acid for use as an internal standard (400 μg/l) in the assay of urinary catecholamines. For the assay of plasma catecholamines a 100-fold dilution of the DHBA stock solution for use as an internal standard (40 μg/l) was prepared.

Assay of VMA and HVA. Stock solutions of POMA (0.5 g/l), VMA (1 g/l) and HVA (1 g/l) were prepared in 100-ml amounts every two months. A standard mixture prepared to contain 10 mg/l VMA and 10 mg/l HVA was aliquoted and stored at −20°C.

Sample collection

Urine specimens (24-h samples) were collected in polyethylene containers with 20 ml of 6 *M* hydrochloric acid and stored at 4°C. Blood samples (10 ml) were collected in chilled lithium-heparin tubes. Plasma was removed within 30 min after collection and stored at −20°C after addition of sodium metabisulphite (50 μl of a 50 g/l solution). All samples were analysed within one week after collection.

Chromatographic system

Apparatus. The liquid chromatograph comprised an Altex 100 A pump (Altex, Berkeley, CA, U.S.A.), a WISP 850 autosampler (Waters Assoc., Milford, MA, U.S.A.), a reversed-phase analytical column (Ultrasphere™ IP, 5 μm particle diameter, 25 or 15 cm × 0.46 mm I.D., from Altex) fitted with a 7.5 × 0.21 cm I.D. guard column (Chrompack, Middelburg, The Netherlands). The pellicular reversed-phase material of the guard column was changed every two months or after ca. 500 injections. The electrochemical detector consisted of a thin-layer LCC 231 K cell with glassy carbon electrodes and an E230 control module (Bruker, Karlsruhe, F.R.G.). The electrode potential was maintained at +0.7 V vs. the calomel reference electrode (+0.9 V for the assay of the acidic metabolites). The column and the detector were mounted in a Faraday cage. The signal generated by the detector was fed to a Model 4100 integrator (Spectra Physics, Santa Clara, CA, U.S.A.) operating in the peak height mode.

Mobile phase. Unless otherwise stated, the mobile phase was prepared by mixing 200 ml of HPLC-grade methanol (J.T. Baker, Deventer, The Netherlands) with 2000 ml of 0.04 *M* sodium dihydrogen phosphate containing 100 mg of octanesulphonate (OSA) (Eastman Kodak, Rochester, NY, U.S.A.) and 100 mg of Na_2EDTA. The phosphate solution was adjusted to pH 3.0 with concentrated phosphoric acid and filtered through a 0.45-μm membrane filter (Millipore). The mobile phase was continuously degassed with helium. The flow-rate was 0.9 ml/min.

Specimen handling and calculations.

To recover catecholamines and acidic metabolites from the same aliquot of urine, we developed a sample preparation scheme based on ion-exchange and alumina adsorption [5], combined with ethyl acetate extraction.

Assay of urinary catecholamines. To a 1-ml aliquot of acidified urine contained in a 10-ml plastic tube add 100 μl of $Na_2S_2O_5$ (50 g/l), 100 μl of the internal standard solution (400 μg/l DHBA) and 5 ml of 0.05 *M* phosphate buffer (pH 6.8). If VMA and/or HVA are to be assayed add also 100 μl of the POMA standard stock solution (0.5 g/l). Adjust the pH to 6.5 ± 0.1 by addition of 0.6 *M* sodium hydroxide. Pour the samples onto small polypropylene columns (Bio-Rad Labs.) filled with ca. 2 ml of cation exchanger. Rinse the columns with 5 ml of 0.05 *M* phosphate buffer (pH 6.5). Collect the combined effluents from the loading and rinsing step in a graduated 20-ml glass test tube and save for the assay of the acidic metabolites (see below). Rinse the columns next with 1.5 ml of 0.7 *M* sulphuric acid. Do not collect the effluent of the acid rinsing. Elute the catecholamines with 5 ml of 2 *M* ammonium sulphate. Collect the eluate in 15-ml conical plastic tubes containing 50 μl of $Na_2S_2O_5$ (50 g/l) and 500 μl of Na_2EDTA (50 g/l). Add 50 mg of alumina to the eluate and then 500 μl of 3 *M* Tris buffer (pH 8.6). The tubes are vortex-mixed and placed on a rotary mixer for 15 min. Remove the liquid by aspiration and wash the alumina twice with 10 ml of water. After the second wash, centrifuge the tubes briefly and remove as much water as possible. Elute the catecholamines from the alumina with 500 μl of 0.5 *M* acetic acid containing $Na_2S_2O_5$ (0.5 g/l) and EDTA (0.5 g/l). After brief vortex-mixing, allow the tubes to stand for 15 min. Remove the supernatant after brief centrifugation. Inject 50 μl.

Catecholamines were quantified using the internal standard method. Peak identification was based on retention time relative to DHBA. Sample concentrations were quantified by comparing peak height ratios (relative to DHBA) obtained for samples to peak height ratios obtained for the not extracted calibration standard (NA, A, DA and DHBA each 40 μg/l).

$$\text{Concentration of NA in sample } (\mu g/l) = \frac{\text{peak height ratio NA/DHBA for sample}}{\text{peak height ratio NA/DHBA for standard}} \times \frac{[IS]}{SA} \quad (1)$$

[IS] is the concentration of the internal standard solution (400 μg/l) and SA is the ratio of the sample volume to the volume of the internal standard solution (1:0.1 = 10).

Assay of plasma catecholamines. Mix 2—4 ml of plasma with 100 μl of $Na_2S_2O_5$ (50 g/l), 100 μl of internal standard solution (40 μg/l DHBA) and 5 ml of 0.05 *M* phosphate buffer (pH 6.0). The extraction procedure is the same as the one described above for urine samples, except for the elution of catecholamines from the alumina where 200 μl instead of 500 μl of acidic solution are used. Inject 50 or 100 μl of the alumina eluate. Concentrations were calculated from eqn. 1 with [IS] = 40 μg/l and SA = 40 (assuming a sample volume of 4 ml).

Assay of urinary VMA and HVA. Acidify the combined loading and rinsing effluents from the cation-exchange column (see above) with 200 μl of 6 *M* hydrochloric acid and adjust the volume to 15 ml with Milli-Q water. After

mixing, transfer 500 μl to a 10-ml glass tube containing ca. 400 mg of sodium chloride. The acidic metabolites are extracted by shaking for 10 min with 2 ml of ethyl acetate. After brief centrifugation, shake 1 ml of the organic layer with 1 ml of 0.1 *M* dipotassium hydrogen phosphate (pH 8.3) for 10 min. Separate the phases again and acidify 0.5 ml of the aqueous phase with 10 μl of concentrated perchloric acid. Inject 10 μl.

Peak identification was based on retention time relative to POMA. Sample concentrations were quantified by comparing peak heights obtained for samples to peak heights obtained for the extracted standard mixture (external standard method).

$$\text{Concentration of VMA in sample (mg/l)} = \frac{\text{peak height for sample}}{\text{peak height for standard}} \times \text{standard concentration} \quad (2)$$

RESULTS AND DISCUSSION

Mobile phase composition

Fig. 1 shows the influence of the OSA concentration in the mobile phase on the retention of catecholamines and acidic metabolites. The retention of amines increased with increasing OSA concentration, whereas that of the acidic compounds decreased slightly. A concentration of 50 mg/l offered excellent resolution of both classes of compounds. Fig. 2 illustrates the effect of ionic strength on retention and peak height of DHBA. The endogenous catecholamines behaved similarly. The retention time of catecholamines gradually decreased when the phosphate concentration of the mobile phase was increased from 0.01 *M* to 0.05 *M*, and remained constant at higher concentrations. The peak height was maximal at a phosphate concentration of 0.04 *M*.

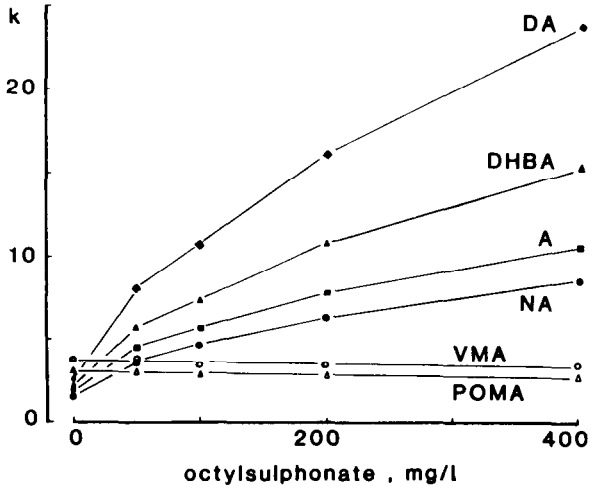

Fig. 1. Effect of octylsulphonate (OSA) concentration on the capacity factor (k') of norepinephrine (NA), epinephrine (A), dopamine (DA), dihydroxybenzylamine (DHBA), vanillylmandelic acid (VMA) and *p*-hydroxymandelic acid (POMA). Column, Ultrasphere IP 25 × 0.46 cm I.D. (particle size 5 μm); mobile phase, 0.04 *M* sodium dihydrogen phosphate containing 50 mg/l EDTA and various concentrations of OSA (pH 3.0), 15% methanol; flow-rate, 1 ml/min.

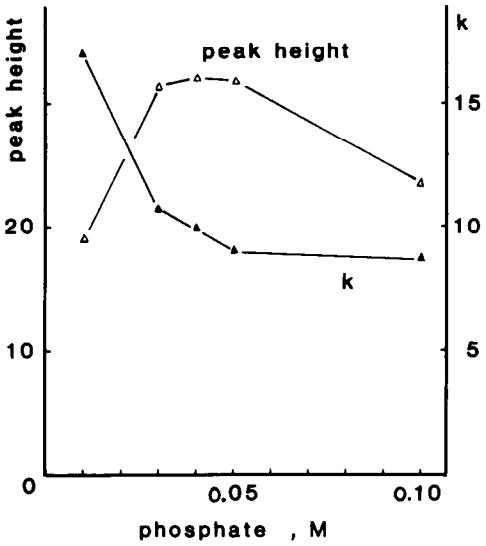

Fig. 2. Effect of phosphate concentration on the capacity factor k' (▲) and the peak height (△) of DHBA (4 ng). Column, Ultrasphere IP 25 × 0.46 cm I.D. (particle size 5 μm); mobile phase, various concentrations of sodium dihydrogen phosphate with 50 mg/l EDTA and 50 mg/l OSA (pH 3.0), 6% methanol; flow-rate 1 ml/min.

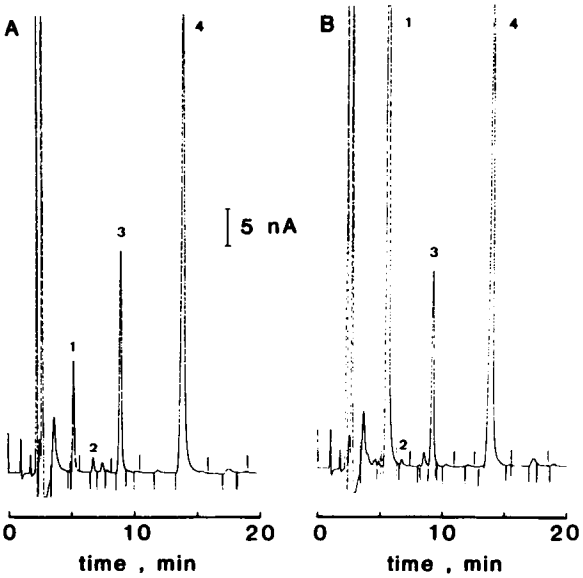

Fig. 3. Chromatograms of catecholamine extracts of 1-ml urine samples from a patient with essential hypertension (A) and from a patient with a histologically proven pheochromocytoma (B). Column, Ultrasphere IP 25 × 0.46 cm I.D. (particle size 5 μm); mobile phase, 0.04 *M* sodium dihydrogen phosphate with 50 mg/l EDTA and 50 mg/l OSA (pH 3.0), 6% methanol; flow-rate, 0.9 ml/min. The injected volume is 50 μl, corresponding to 100 μl of extracted urine. Catecholamine concentrations in μg/l for A and B, respectively, are: norepinephrine (peak 1), 19 and 590; epinephrine (peak 2), 3.5 and 1.7; dopamine (peak 4), 142 and 137. Peak 3 is the internal standard dihydroxybenzylamine (40 ng added to a 1-ml sample).

Previous studies have shown that maximum detector response for amines is obtained at a solvent pH between 5 and 6 [6]. We used a pH of 3.0 since it enabled better separation. The same was observed by others [7—9]. Typical chromatograms of catecholamine extracts are shown in Figs. 3 and 4. The purity of the chromatographic peaks was verified by the complete superimposition of the hydrodynamic voltammograms of catecholamine reference solutions and urinary extracts (Fig. 5).

With a mobile phase pH of 3.0, POMA and VMA were detected as clearly resolved peaks, eluting close to the solvent front, whereas HVA, which was the last peak to be detected upon injection of urinary ethyl acetate extracts, had a retention time of ca. 30 min (15-cm column; Fig. 6). With a mobile phase of higher pH, the retention of HVA could be decreased, but VMA was then no longer separated from the solvent front and could not be quantified accurately.

Detection

The detection limits of the various compounds under standard assay conditions are listed in Table I, together with the corresponding concentrations for plasma and/or urine samples. The dynamic range of the assay was somewhat limited for dopamine: the upper limit (1250 μg/l) may be exceeded in urine samples from children. However, the linear range could be expanded considerably by injecting a smaller volume of the final extract or by decreasing the sensitivity setting of the electrochemical detector. Plasma dopamine concentrations are usually too low to be detected.

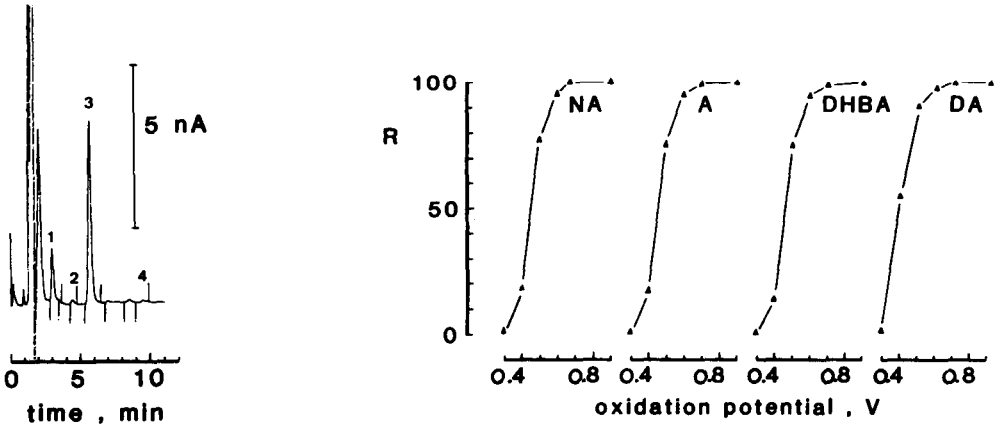

Fig. 4. Chromatogram of an extract of a 4-ml plasma sample. Chromatographic conditions are the same as in Fig. 3 except column length (15 cm). The injected volume is 50 μl, corresponding to 1 ml of extracted plasma. Catecholamine concentrations in μg/l are: norepinephrine (peak 1), 0.30; epinephrine (peak 2), 0.030; dopamine (peak 4), 0.016. Peak 3 is the internal standard dihydroxybenzylamine (4 ng added to a 4-ml sample).

Fig. 5. Hydrodynamic voltammograms for norepinephrine (NA), epinephrine (A), dihydroxybenzylamine (DHBA) and dopamine (DA). The detector response at a particular potential expressed as percentage of the maximal response (R) is plotted as a function of the oxidation potential (V). Identical voltammograms are obtained for peaks from standard solutions and for peaks from urinary extracts. Chromatographic conditions as in Fig. 3.

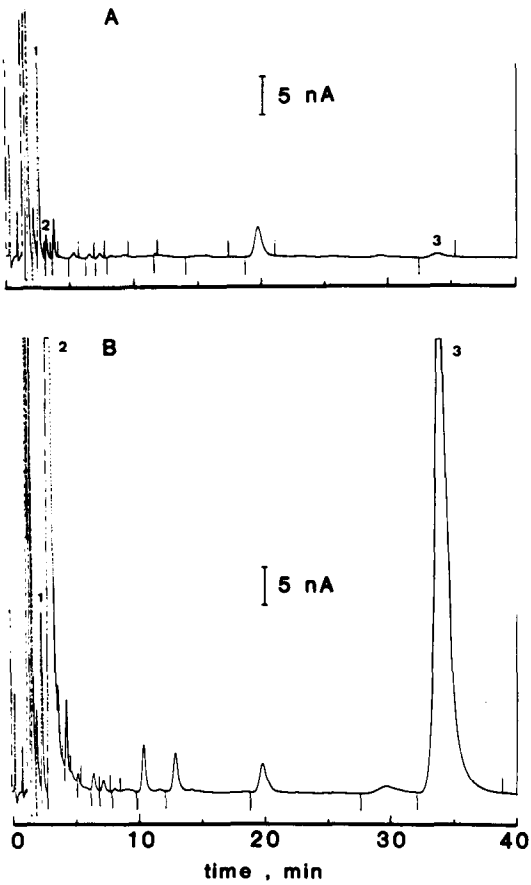

Fig. 6. Chromatograms of acidic extracts of urine samples from a healthy child (A) and from a child with neuroblastoma (B). Chromatographic conditions as in Fig. 3 except column length (15 cm). The injected volume is 10 μl, corresponding to 0.16 μl of extracted urine. Concentrations in mg/l for A and B, respectively, are: vanillylmandelic acid (peak 2), 1.8 and 500; homovanillic acid (peak 3), 1.9 and 287. Peak 1 is the reference peak of *p*-hydroxymandelic acid (50 μg added to a 1-ml sample).

Recovery of catecholamines

The overall absolute analytical recovery was studied by extracting standard mixtures containing from 2 ng to 4 μg of NA, A and DA (Table II, A). The recovery values for the individual amines were similar over the concentration range tested and averaged 67%. Absolute recovery was calculated assuming that the volume of the alumina eluate equals the volume of acid added and was thus not corrected for the dilution caused by water remaining on the alumina after the final wash (ca. 100 μl). As in the assay of plasma samples the alumina was eluted with only 200 μl instead of 500 μl of acid, the dilution effect was more pronounced in the assay of plasma samples than in the assay of urinary samples. This explains the lower apparent recovery values of DHBA in the extraction of plasma samples. However, recoveries of NA, A and DA, relative to that of DHBA were not affected by the sample matrix, justifying the use of DHBA as an internal standard.

Inter-specimen variation of extraction efficiency was studied by spiking individual urine and plasma samples. These analyses were performed over a period of several months. The results (Table II, B2 and C2) illustrate the consistency of recovery values for different samples and justify the proposed calibration procedure using an unextracted standard solution.

TABLE I

DETECTION LIMITS OF NOREPINEPHRINE (NA), EPINEPHRINE (A), DOPAMINE (DA), VANILLYLMANDELIC ACID (VMA) AND HOMOVANILLIC ACID (HVA)

Compound	Lower limit* (pg)	Upper limit** (ng)	Measuring range***	
			Urine §	Plasma §§
NA	20	50	0.3— 750 μg/l	0.015—38 μg/l
A	27	65	0.4—1000 μg/l	0.020—50 μg/l
DA	32	80	0.5—1250 μg/l	0.025—63 μg/l
VMA	22	55	0.1— 220 mg/l	
HVA	140	360	0.5— 950 mg/l	

*The amount for which a peak height of 0.2 nA is obtained, corresponding to a signal-to-noise ratio of 4.
**The amount for which a peak height of 500 nA is obtained, corresponding to the maximum current output of the electrochemical detector at a sensitivity setting of 50 nA/V.
***Taking into account the losses during sample clean-up.
§Sample volume = 1 ml; injection volume = 50 μl (1/10 of the final extract).
§§Sample volume = 4 ml; injection volume = 100 μl (1/2 of the final extract).

TABLE II

RECOVERY OF NOREPINEPHRINE (NA), EPINEPHRINE (A), DOPAMINE (A), AND DIHYDROXYBENZYLAMINE (DHBA)

Mean values ± standard deviation.

Sample matrix	Added concentration (μg/l)			DHBA recovery* (%)	Recovery relative to DHBA (%)		
	NA	A	DA		NA	A	DA
A. 0.01 *M* Hydrochloric acid (1 ml)							
1	2	2	2	60	104	112	100
2	20	20	20	64	98	102	94
3	200	200	200	69	97	99	102
4	2000	2000	2000	72	97	98	101
5	4000	4000	4000	70	99	101	101
B. Urine (1 ml)							
1 $n = 7$**	15—240	3—48	100—1600	67 ± 5	101	87	100
2 $n = 20$***	80	20	40	67 ± 5	100 ± 7	97 ± 6	99 ± 7
3 $n = 250$***	—	—	—	65 ± 7	—	—	—
C. Plasma (4 ml)							
1 $n = 7$**	0.05—1	0.05—1	0.05—1	52 ± 2	97	100	102
2 $n = 32$***	0.5	0.125	—	50 ± 6	96 ± 5	101 ± 10	—
$n = 7$***	0.5	0.5	0.5	55 ± 6	94 ± 5	99 ± 3	95 ± 8
3 $n = 105$***	—	—	—	53 ± 7	—	—	—

*DHBA (40 ng) was added to aqueous and urine samples, 4 ng to plasma samples.
**Different aliquots of a single specimen spiked with different amounts and analysed the same day; relative values calculated as 100 times the slope of a plot of measured versus added concentration.
***Different specimens analysed during a six-month period.

Recovery of VMA and HVA

Recovery values are listed in Table III. The recovery values for spiked urine samples (Table III, B) were similar to those for aqueous solutions. The calibration of the assay using a standard solution taken through the entire procedure is therefore justified.

The use of POMA as an internal standard in the assay of VMA has been previously suggested [2]. However, POMA is not an ideal standard as it can be excreted in considerable amounts after the ingestion of fruit juice [10]. We added it to the urine samples to obtain a high reference peak that allowed peak identification based on relative retention times, and to detect erroneously low recoveries.

Precision

Within-run variation was determined by extracting six aliquots of one sample. For 1-ml aliquots of a urine sample coefficients of variation were 2%, 12%, 2%, 5% and 8% for NA, A, DA, VMA and HVA, respectively (the concentrations were NA = 17 μg/l, A 1.7 μg/l, DA = 84 μg/l, VMA = 4.2 mg/l and HAV = 4.4 mg/l). For 4-ml aliquots of a spiked plasma pool the coefficients of variation were 2%, 2% and 1% for NA, A and DA, respectively (respective concentrations of 0.88 μg/l, 0.70 μg/l and 0.60 μg/l).

Total variation was determined by analysing two lyophilysed urine controls during a six-month period (n = 30). Mean values (and coefficients of variation) were: 252 μg/l (3.5%) and 12 μg/l (3.6%) for NA, 2.4 μg/l (6.6%) and 5.4 μg/l (7.1%) for A, 149 μg/l (6.5%) and 68 μg/l (4.4%) for DA, 2.5 mg/l (4.1%) and 26 mg/l (6.7%) for VMA and 2.9 mg/l (11%) and 2.6 mg/l (10%) for HVA.

TABLE III

RECOVERY OF VANILLYLMANDELIC ACID (VMA), HOMOVANILLIC ACID (HVA) AND *p*-HYDROXYMANDELIC ACID (POMA)

Mean values ± standard deviation.

Sample matrix	Added concentration (mg/l)		Absolute recovery (%)		
	VMA	HVA	POMA*	VMA	HVA
A. 0.01 *M* Hydrochloric acid (1 ml)					
1	2	2	88	68	93
2	20	20	92	74	94
3	200	200	85	76	93
4 n = 70	10	10	86 ± 5	82 ± 5	85 ± 6
B. Urine (1 ml)					
1 n = 7**	1—50	1—50	89 ± 3	77	88
2 n = 20***	10	10	90 ± 5	77 ± 7	87 ± 9

*POMA (50 μg) was added to all samples.

**Different aliquots of a single specimen spiked with different amounts and analysed the same day; recovery values derived from the slope of a plot of peak height vs. added concentration.

***Different specimens analysed during a two-month period.

Interferences

Table IV lists relative retention times of catecholamines, metabolites and related compounds. For the assay of catecholamines a single chromatographic run was normally completed in less than 12 min (15-cm column, Fig. 4). For samples from patients treated with α-methyldopa or isoproterenol, the analysis time was increased to 35 min to avoid interference from late-eluting compounds. Extracts of samples from patients treated with labetalol showed an additional chromatographic peak coeluting with DHBA. Catecholamine concentrations in these samples were determined using isoproterenol instead of DHBA as an internal standard. No interference was noted in the VMA/HVA assay.

TABLE IV

RETENTION TIMES OF CATECHOLAMINE METABOLITES, ANALOGUES AND DRUGS RELATIVE TO THE RETENTION TIME OF DIHYDROXYBENZYLAMINE (DHBA) AND *p*-HYDROXYMANDELIC ACID (POMA)

Compound*	Retention time relative to**		Compound*	Retention time relative to**	
	DHBA	POMA		DHBA	POMA
Basic			*Neutral*		
NA	0.61	*1.33*	DOPEG	*0.42*	0.91
A	0.78	*1.70*	MOPEG	*0.75*	1.62
OCT	*0.80*	*1.74*	DOPET	*1.31*	2.84
DHBA	1	*2.17*			
SYN	*1.12*	*2.43*	*Acidic*		
NMN	*1.14*	*2.48*	DOMA	*0.35*	0.76
DA	1.53	*3.33*	POMA	*0.46*	1
MN	*1.68*	*3.65*	VMA	*0.56*	1.21
EPI	1.82	*3.96*	MOMA	*0.64*	1.40
IP	2.61	*5.67*	HGA	*0.97*	2.11
α-MD 1	3.37	*7.32*	PA	*1.33*	2.89
α-MD 2	Not detected		DOPAC	*1.66*	3.61
			POPAC	*3.24*	7.05
			HVA	*5.24*	11.4

*Abbreviations are defined under Experimental.

**Numbers in italics mean that the compound is not recovered after extraction.

Correlation studies

The chromatographic procedure for the assay of urinary catecholamines was compared with a trihydroxyindole fluorometric procedure [11]. The correlation of the results was poor and suggested interferences in the fluorometric procedure. The regression equations were (n = 25; fluorescence data as the independent variable): for NA: y (μg/l) = 17 + 0.66x, r = 0.77; for A: y (μg/l) = 2.5 + 0.70x, r = 0.67.

The results for the assay of urinary VMA correlated well with those obtained using the colorimetric method of Pisano [12]. The regression equation was (n = 55; colorimetric data as the independent variable): y (mg/l) = 0.1 + 0.88x, r = 0.93.

Urinary excretion values

Urine specimens (24-h samples) were collected from 448 adults, most of whom were hypertensive (258 women and 190 men, ages 16 to 83 years). The range of excretion values derived by deleting the last 2.5% of values from each end of the distributions were: for NA, 10—87 μg per 24 h; for A, 1.2—18 μg per 24 h; for DA, 60—460 μg per 24 h; for VMA, 1.4—6.5 mg per 24 h; for HVA 1.4—8.8 mg per 24 h. These values agree with the normal adult values determined by Moyer et al. [13] and Soldin and Hill [3].

CONCLUSIONS

The described HPLC method allows the detection of ca. 30 pg of individual catecholamines. We recommend a thorough sample clean-up procedure in order to increase the specificity of the analysis and to safeguard the efficiency of the chromatographic system. We think that the additional time spent in sample preparation is outweighed by the improved chromatograms. The use of the same mobile phase for the concurrent assay of catecholamines and their acidic metabolites eliminates the time-consuming column equilibration periods, extends the lifetime of the analytical column and favours the stability of the electrochemical detector. As a result of increased baseline stability, the analysis can be automated, and samples can be injected 24 h a day. Over 5000 extracts can be analysed on a single column without appreciable loss of resolution.

ACKNOWLEDGEMENTS

We are grateful to Dr. Claude Sevens for assistance in the preparation of the manuscript. We thank Miss Gerda Winckelmans for skillful technical assistance and Miss Annie De Nil for excellent secretarial work.

REFERENCES

1 A. Krstulovic, J. Chromatogr., 229 (1982) 1.
2 I. Molnar, C. Horvath and P. Jatlow, Chromatographia, 11 (1978) 260.
3 S.J. Soldin and J.G. Hill, Clin. Chem., 27 (1981) 503.
4 P. Moleman and J.J.M. Borstrok, Clin. Chem., 29 (1983) 878.
5 G.C. Davis, P.T. Kissinger and R.E. Shoup, Anal. Chem., 53 (1981) 156.
6 T.P. Moyer and N.S. Jiang, J. Chromatogr., 153 (1978) 365.
7 R.L. Michaud, M.J. Bannon and R.H. Roth, J. Chromatogr., 225 (1981) 335.
8 J.J. Warsh, A. Chiu and D.D. Godse, J. Chromatogr., 228 (1982) 131.
9 B.M. Eriksson and B.A. Persson, J. Chromatogr., 228 (1982) 143.
10 J.R. Crowley, M.W. Couch, C.M. Williams, R.M. Threatte and M.J. Fregly, Clin. Chim. Acta, 109 (1981) 125.
11 A.H. Anton and D.F. Sayre, J. Pharmacol. Exp. Ther., 138 (1962) 360.
12 J.J. Pisano, R.J. Crout and D. Abraham, Clin. Chim. Acta, 7 (1962) 285.
13 T.P. Moyer, N.S. Jiang, G.M. Tyce and S.G. Sheps, Clin. Chem., 25 (1979) 256.