Journal of Chromatography, 228 (1982) 143—154
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1138

DETERMINATION OF CATECHOLAMINES IN RAT HEART TISSUE AND PLASMA SAMPLES BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

BRITT-MARIE ERIKSSON* and BENGT-ARNE PERSSON

Analytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)

(First received August 10th, 1981; revised manuscript received October 1st, 1981)

SUMMARY

Liquid chromatography with electrochemical detection is used for the determination of adrenaline, noradrenaline and dopamine in rat heart tissue, and the method has also been applied to the determination of basic levels of these compounds in blood plasma. The catecholamines are isolated from the biological sample by adsorption onto alumina and are then desorbed by elution with perchloric acid. The stability of the compounds during the different stages in the work-up process has been studied. A greatly simplified procedure for the preparation of alumina is presented. Both ion-pair reversed-phase and ion-exchange liquid chromatography have been used for the separation of the catecholamines. For plasma samples the method has been validated against radioenzymatic assay and the choice of method is discussed.

INTRODUCTION

The release of noradrenaline in ischemic myocardium is assumed to be correlated with the development of serious ventricular arrhythmias [1]. Ischemia-induced changes of catecholamines in rat myocardium have been studied with the presented analytical methodology [2].

In the last few years, analytical methods for catecholamines have improved in both sensitivity and selectivity. Liquid chromatographic methods, using either cation-exchange or reversed-phase chromatography with electrochemical detection, have been used for the determination of catecholamines in brain tissue [3—5] as well as in plasma samples [6—12], and a review on this subject has been presented [13]. Improved fluorometric assays have also been described [14]. However, the most sensitive methods are still the radioenzymatic ones [15—17] which require only 100 μl of sample.

Since in many physiological samples the adrenaline and dopamine concentrations may be a hundredfold less than that of noradrenaline, a more effective

0378-4347/82/0000—0000/$02.75

separation system than the one used in the radioenzymatic method is required. Therefore, we have investigated the use of ion-pair reversed-phase and ion-exchange liquid chromatographic separation systems. The effects of stabilizing agents, alumina adsorption and desorption, handling of the samples in the work-up procedure and choice of chromatographic system have also been examined. The described analytical methodology was applied to the analysis of catecholamines in samples of rat heart tissue and plasma.

EXPERIMENTAL

Apparatus

The liquid chromatograph was composed of a moderate-pressure pump Constametric II G (Riviera Beach, FL, U.S.A.) with extra pulse dampeners, an injection valve Rheodyne 7125 (Berkeley, CA, U.S.A.) with a 100-μl loop, a stainless-steel separation column (150 × 4.5 mm I.D.) and an electrochemical detector LC 4 [Bioanalytical Systems (BAS), West Lafayette, IN, U.S.A.]. The detector was operated at +0.6 to 0.8 V with an Ag/AgCl reference electrode (Model BAS RE 1) and a thin-layer cell (Model BAS TL 4A) consisting of a paraffin-oil-based carbon paste (CPO) working electrode. Homogenization was performed with a Braun Potter S homogenizer (Melsungen, G.F.R.) equipped with pestles and 5-ml ground-glass tubes. The refrigerated centrifuges used were a Du Pont Sorvall RC 2 (Newtown, CT, U.S.A.) and a Beckman TJ 6 centrifuge (Palo Alto, CA, U.S.A.). A Julabo Paramix II (Seelbach, G.F.R.) and a rotary mixer for 56 tubes (Breda Sci., Breda, The Netherlands) were used to mix the tubes.

Chemicals

Adrenaline (A) and noradrenaline (NA) (hydrogen tartrate form) were obtained from Société des Usines Chimiques, Paris, France), and Österreichische Stickstoffwerke AG, Linz, Austria, respectively. Dopamine (DA) hydrochloride, normetanephrine (NMN) hydrochloride, reduced glutathione (GSH), the sodium salt of thioglycolic acid, and ethyleneglycol-bis-(β-aminoethyl ether) N′,N′-tetraacetic acid (EGTA) were from Sigma (St. Louis, MO, U.S.A.); dihydroxybenzylamine (DHBA) hydrobromide was purchased from EGA-Chemie (Steinheim, G.F.R.). α-Methyldopamine (MDA) hydrochloride was obtained from Merck Sharp and Dohme (Rahway, NJ, U.S.A.) and tris(hydroxymethyl)aminomethane (Tris), analytical grade, was of Fluka quality (Buchs, Switzerland). Alumina, Woelm neutral, was from Woelm Pharma (Eschwege, G.F.R.) and 3,5-dimethylcyclohexyl sulfate (DMCHS) was supplied by the Department of Organic Chemistry, AB Hässle, Mölndal, Sweden. Disodium EDTA, sodium bisulfite, ascorbic acid, methanol, sodium hydroxide, acids and buffer substances were all of analytical grade from E. Merck (Darmstadt, G.F.R.).

Preparation of alumina

The method used for the purification of alumina for catecholamine analysis is generally that of Anton and Sayre [18]. This procedure is very time-consuming as it involves heating 4 times in hydrochloric acid and rinsing 25—50

times with water to remove excess acid. We simplified the procedure in the following manner: 100 g of alumina were added to a beaker containing 500 ml of perchloric acid (2 mol/l). The mixture was heated at 95°C for 45 min with continuous and rapid stirring with a propeller stirrer, after which the supernatant fluid was discarded along with the fines. The precipitate was treated once more with a fresh 500-ml portion of perchloric acid (2 mol/l) at 95°C for 15 min; the supernatant with the fines was discarded and the precipitate transferred to a glass column (500 × 25 mm). Water was run through the column until the eluate reached a pH of 3.5. Finally the alumina was transferred to a beaker, heated in a warming cupboard at 120°C for 1 h and at 200°C for 2 h, after which it was stored in a desiccator and kept dry. The recoveries of catecholamines purified by the two methods differed by less than 1%.

Sample preparation

The heart tissue was quickly frozen on dry ice and stored at −70°C. Before the homogenization, the frozen tissue was cut into small pieces, put into a tissue grinder and weighed. One millilitre of perchloric acid (0.1 mol/l) containing EDTA (2.7 mmol/l) and the internal standard DHBA (0.2 μmol/l) was added per 0.1 g of tissue and the homogenization was performed in an ice-bath. The homogenate was then centrifuged in a refrigerated centrifuge at 4°C for 10 min at 25,000 *g*. The supernatant was transferred to a new tube and frozen if not being analysed the same day. The blood samples were collected into chilled tubes containing a small volume (20 μl/ml blood) of a solution (pH 6.0—7.0) of an anticoagulant, EGTA (0.2 mol/l), and an antioxidant, GSH (0.2 mol/l). After centrifugation (1000 *g*, 5 min) at 4°C the plasma phase was separated and stored at −70°C until analysis.

Alumina adsorption

One millilitre of tissue homogenate or 2 ml of plasma sample was transferred into a 4-ml centrifuge tube. Then, 50 μl of GSH (0.05 mol/l), 50 μl of EDTA (0.3 mol/l, pH 7) and 20 mg of alumina were added. While vortexing the tube, 1.0 ml of Tris buffer (1 mol/l, pH 8.6) was added to the tissue sample and 0.20 ml to the plasma sample; the tube was then placed in a rotary mixer and rotated for 10—30 min. Each series of analyses also included reference samples of 20 and 400 pmol of the catecholamines for the heart tissue analyses and 2 and 20 pmol for plasma samples. These reference samples were prepared by dilution of a stock solution of the catecholamines in 0.1 mol/l perchloric acid. All samples contained as internal standard DHBA, for the ion-pair reversed-phase system, and MDA for the ion-exchange system.

Alumina particles adhering to the walls were forced down to the bottom of the tube by shaking and the supernatant was discarded. The alumina was then washed three times by mixing for a few seconds with an EDTA solution (3 mmol/l, pH 7). After the final washing, the tube was centrifuged and any excess liquid was again discarded. The amines were eluted from the alumina by vortexing for 1 min with 150 μl of perchloric acid (0.2 mol/l). After centrifugation the tube was stored frozen in darkness and was thawed just before injection of 50 μl onto the chromatographic column. A volume of 100—150 μl can be injected when maximum sensitivity is required.

Chromatographic system

The separation columns were packed with LiChrosorb RP-18 (reversed-phase), 5 μm average particle size, from Merck, or with Nucleosil SA (strong cation-exchange), 5 or 10 μm average particle size, from Macherey-Nagel & Co. (Düren, G.F.R.). In the ion-exchange system, the mobile phase was an acetate—citrate buffer (pH 5.2) containing 10% methanol. The composition of the buffer was sodium acetate 100 mmol/l, sodium hydroxide 60 mmol/l and citric acid 40 mmol/l. The eluent for the ion-pair reversed-phase system was the same, except that it also contained DMCHS at a concentration of 3 mmol/l. The water used for the mobile phase was deionized and filtered through a Milli Q reagent-grade water system (Millipore, Bedford, MA, U.S.A.). Prior to use, the mobile phase was degassed by vacuum filtering through a 0.45-μm MF-Millipore filter. The flow-rate was 1 ml/min for both systems, ambient temperature being used. The separation column and the detector cell were housed in a Faraday cage to avoid electrical disturbances.

Quantitative evaluation

The median value of the ratios between the peak heights of the catecholamine (CA) and the internal standard (I.S.) for the standard samples was used to calculate the concentration in the unknown samples according to the following formula

$$\text{pmol/g tissue} = \frac{\text{pmol (standard)} \times \frac{\text{CA}}{\text{I.S.}}\text{ (sample)}}{\frac{\text{CA}}{\text{I.S.}}\text{ (standard)} \times \text{g tissue per ml perchloric acid solution}}$$

To obtain pmol/ml plasma, g tissue per ml perchloric acid solution in the formula is changed to ml plasma.

RESULTS AND DISCUSSION

Work-up procedure

We studied several parameters that may influence the adsorption and elution procedures and also the decomposition of the catecholamines during storage. The ionic strength of the sample did not seem to have any influence, since the same recoveries of catecholamines were obtained for a sodium chloride solution of 1 mol/l as for pure water. Generally, 1 ml of tissue homogenate or 2 ml of plasma sample is used for the analysis. If higher sensitivity is required, a sample volume of at least 4 ml can be adsorbed onto 20 mg of alumina without any decrease in the recovery. The adsorption of the catecholamines onto alumina was found to be linear from 0.1 to 400 pmol and the linear range is probably even wider. As the catecholamines are easily oxidized at alkaline pH, stabilizing agents are added prior to the Tris buffer. In our studies, the stabilizing agents did not show any significant effect, provided that the work-up procedure was performed in accordance with the described method. However, under inappropriate conditions, the type and concentration of stabilizing agents were found

to be of utmost importance, as can be seen in Table I, where results from samples that were left to stand for 40 min before mixing are shown. In our method, we use a combination of the antioxidant glutathione and the anticoagulant and complexing agent EDTA. Ascorbic acid and thioglycolic acid are also good stabilizing agents but the former has the drawback of causing an extensive front peak in the chromatogram and the latter is an unstable corrosive substance with a strong unpleasant odour. Sodium bisulfite appeared to be the least effective antioxidant, the same observation being made for metabolites of propranolol [19]. It is noticeable that, under non-optimal conditions, the internal standard did not compensate for the degradation, especially in the case of dopamine.

TABLE I

EFFECT OF DIFFERENT STABILIZING AGENTS ON THE RECOVERY OF SAMPLES ALLOWED TO STAND FOR 40 min BEFORE MIXING

Stabilizing Agent	Concentration (mol/l)	Recovery (%)			
		NA	A	DA	DHBA
—	—	24	24	14	52
EDTA	$5.2 \cdot 10^{-3}$	80	79	73	85
	$5.2 \cdot 10^{-2}$	82	79	76	86
Sodium bisulfite	$5.0 \cdot 10^{-4}$	35	38	18	37
	$5.0 \cdot 10^{-3}$	46	49	26	47
Glutathione	$0.9 \cdot 10^{-3}$	59	60	46	60
	$4.7 \cdot 10^{-3}$	68	69	73	71
	$0.9 \cdot 10^{-2}$	70	72	76	71
Thioglycolic acid	$5.1 \cdot 10^{-3}$	73	75	76	75
	$5.1 \cdot 10^{-2}$	78	80	82	83
Ascorbic acid*	$5.0 \cdot 10^{-4}$				
	$5.0 \cdot 10^{-3}$				
EDTA + glutathione	$5.2 \cdot 10^{-3}$	78	78	77	81
	$0.9 \cdot 10^{-3}$				
EDTA + sodium bisulfite	$5.2 \cdot 10^{-3}$	77	78	76	80
	$5.0 \cdot 10^{-4}$				

*The recoveries could only be roughly estimated to 85—100% due to interference from the ascorbic acid in the chromatograms.

The effects of the volume and concentration of the acid used in the elution procedure were also studied; 50—400 μl of the perchloric acid at 0.1, 0.2 and 0.4 mol/l were tested. For maximum recovery at least 100 μl of 0.1 mol/l acid or 50 μl of 0.2 or 0.4 mol/l have to be used. Depending on the quantity of acid used in the elution procedure, the storage method of the samples before injection may be important. As can be seen in Table II, both the temperature and the presence of alumina were critical when 50 μl of acid (0.1 mol/l) were used. When samples were stored without alumina present, the recovery was maintained during the studied period both in the refrigerator (4°C) and freezer (−20°C). Storage of the samples in contact with alumina for 2 days at 4°C resulted in a decomposition of 95% compared to 28% in the case of storage

TABLE II

DECREASE IN RECOVERY IN RELATIVE PER CENT DEPENDING ON THE VOLUME OF ELUENT ACID (0.1 mol/l) AND THE STORAGE CONDITIONS

Storage conditions	Elution volume 50 μl		Elution volume 300 μl
	2 days	5 days	5 days
−20°C with alumina	28	50	0
−20°C without alumina		0	0
+ 4°C with alumina	95	95	0
+ 4°C without alumina		0	0

at −20°C. After 5 days, the corresponding figures were 95% and 50%. On the other hand, with 300 μl of acid (0.1 mol/l) the concentration was unaffected at both 4°C and −20°C. Reduced recovery when storing the eluent on alumina overnight at 4°C was also found by Wenk and Greenland [20].

It was also important to keep the tissue and plasma samples in darkness before injection as samples kept in daylight showed a growing peak close to the dopamine peak when chromatographed on the reversed-phase system. Similar observations were reported by Watson [11].

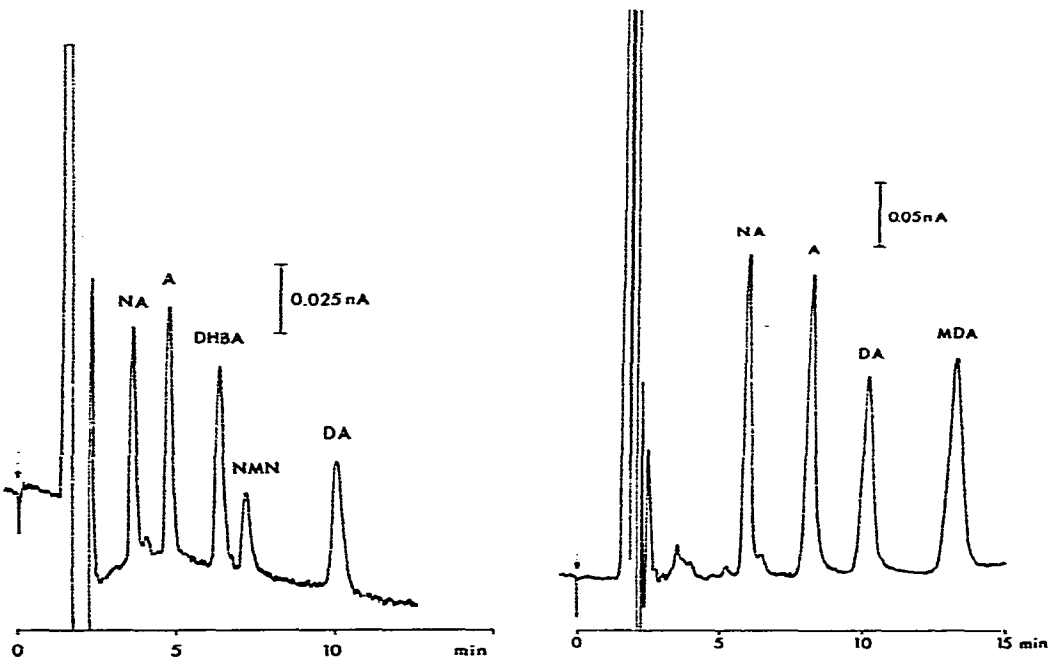

Fig. 1. Separation of noradrenaline (NA), adrenaline (A), dihydroxybenzylamine (DHBA) and dopamine (DA), 0.25 pmol of each, and 0.16 pmol of normetanephrine (NMN) in a reference solution. Stationary phase: LiChrosorb RP-18, 5 μm. Mobile phase: acetate—citrate buffer (pH 5.2) containing DMCHS (3 mmol/l) and 10% methanol. Potential: + 0.7 V.

Fig. 2. Separation of noradrenaline (NA), adrenaline (A) and dopamine (DA), 1.5 pmol of each, and 2.0 pmol of α-methyldopamine (MDA) in a reference solution. Stationary phase: Nucleosil 5 SA. Mobile phase: acetate—citrate buffer (pH 5.2) containing 10% methanol. Potential: + 0.7 V.

Chromatographic systems

Ion-pair reversed-phase and ion-exchange chromatography are used in the analytical procedure with the mobile phases of acetate buffer solutions of pH 5.2 and methanol as the organic modifier. In the reversed-phase system, dimethylcyclohexyl sulfate was found to be suitable as ion-pairing agent for the catecholamines. Chromatograms of test solutions obtained by the two separation systems are shown in Figs. 1 and 2, respectively. A lower pH of the mobile phase improved the chromatograms slightly but also lowered the detector response, as can be seen in Fig. 3.

The presence of 10% of methanol in the mobile phase decreased the detector response by about 10% but significantly improved the chromatographic performance of the ion exchanger. In the absence of methanol, the retention times increased and a long period was needed for conditioning the columns.

For the analysis of tissue homogenates, the ion-pair reversed-phase system was found to be more efficient than ion exchange (Fig. 4). However, for plasma samples use of the ion-exchange system is preferable since plasma samples from some subjects showed a peak interfering with adrenaline when chromatographed on the ion-pair reversed-phase system. This is exemplified in Figs. 5 and 6, where a plasma sample was chromatographed on the ion-exchange and the ion-pair reversed-phase system, respectively.

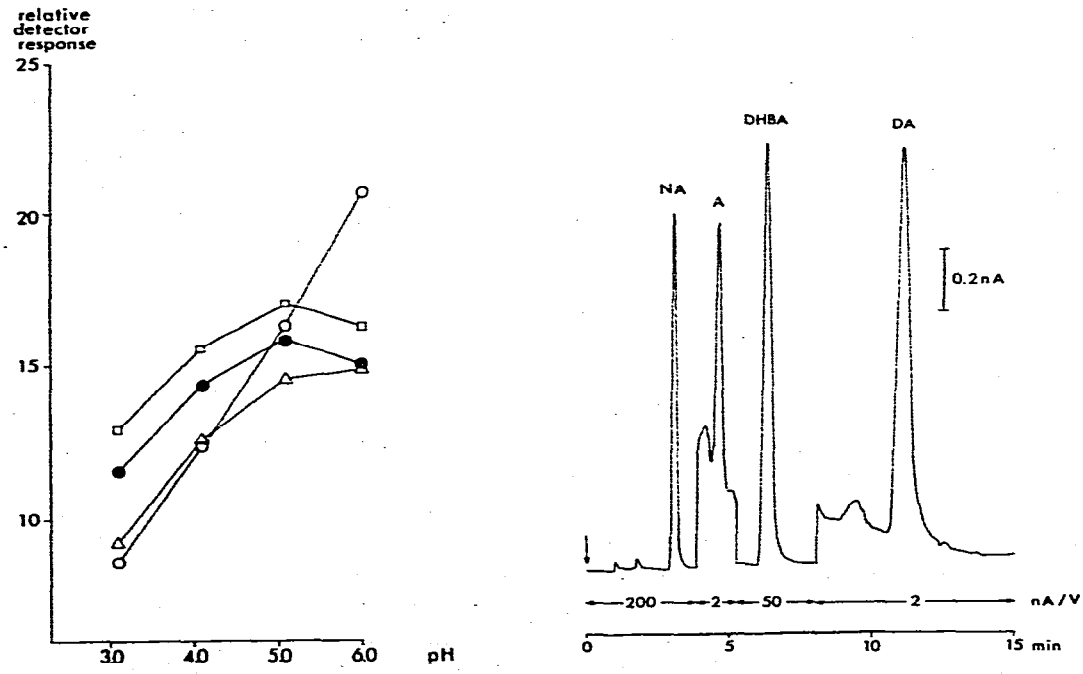

Fig. 3. Detector response versus pH of the aqueous mobile phase consisting of citrate buffer (μ=0.1) and methanol (90:10). The catecholamine mixture consisted of 50 pmol each of adrenaline (○), noradrenaline (△), dopamine (□) and α-methyldopamine (●).

Fig. 4. Chromatogram of catecholamines from 0.1 g of rat heart tissue containing 608 pmol of noradrenaline (NA), 5.2 pmol of adrenaline (A) and 11.6 pmol of dopamine (DA). Internal standard was dihydroxybenzylamine (DHBA) 250 pmol. The chromatographic conditions were the same as in Fig. 1.

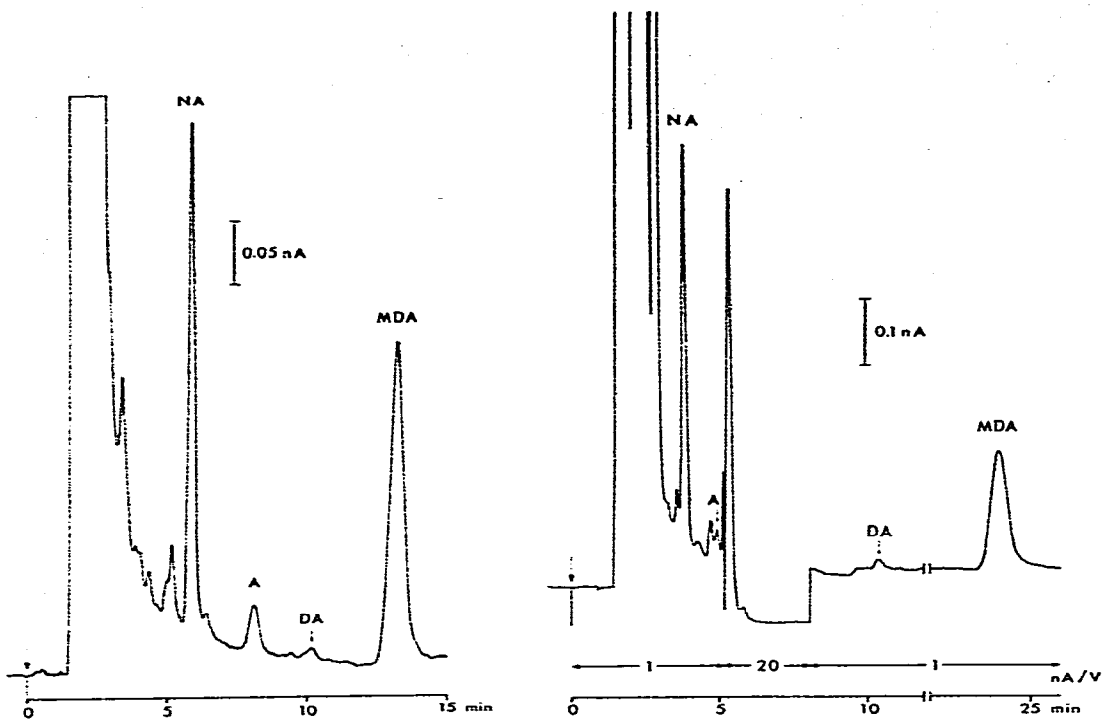

Fig. 5. Chromatogram from 2.0 ml of human plasma containing 1.95 nmol/l noradrenaline (NA) and 0.20 nmol/l adrenaline (A). Internal standard was α-methyldopamine (MDA) 2.5 nmol/l. A 75-μl sample was injected. The chromatographic conditions were the same as in Fig. 2.

Fig. 6. Chromatogram of 60 μl of the same sample as in Fig. 5, injected on the reversed-phase system. The chromatographic conditions were the same as in Fig. 1.

Detection

From the hydrodynamic voltammograms (Fig. 7) a potential of + 0.60 V was chosen for the detection of catecholamines. A higher voltage may increase the extent of interfering compounds in the chromatograms.

When checking the reference electrode after several months against a Radiometer AgCl electrode, the potential measured was + 0.20 V. This means that, by that time, the working potential was rather + 0.80 V with respect to the Radiometer AgCl electrode. The reference electrode rapidly reassumed a low potential when soaked in 3 mol/l sodium chloride solution. Separate measurements showed that the drift of the reference electrode was about $5 \cdot 10^{-4}$ V per day.

The linearity range of different detector cells was also tested. The TL 4A thin-layer cell, with the auxiliary electrode across from the working electrode, showed linearity even with injection up to 2 nmol of the compounds, while the TL 3 cell was linear up to about 250 pmol.

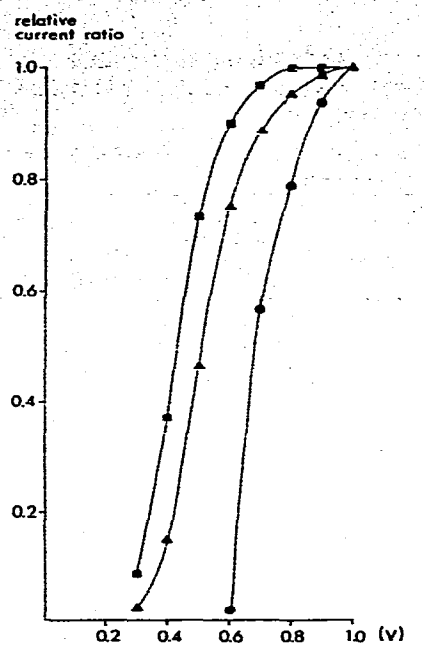

Fig. 7. Hydrodynamic voltammograms for the oxidation of adrenaline (A), noradrenaline (NA), dopamine (DA), dihydroxybenzylamine (DHBA) and normetanephrine (NMN) on a CPO electrode. (■), DA; (▲) A+NA+DHBA; (●), NMN.

Recovery

The absolute recovery of the analytical procedure was determined by comparing peak heights of the catecholamines in the samples with peak heights of injections of reference solutions. Correction for dilution by the washing solution remaining in the alumina was performed by measuring peak heights of normetanephrine present in the eluent as a volume marker. Normetanephrine was chosen as it separated well from the catecholamines and did not adsorb onto the alumina. The dilution was found to be about 10%. Absolute yields and relative standard deviations are shown in Table III. As can be seen, the use of an internal standard lowers the relative standard deviation significantly, and obviously compensates for minor variations in the analytical procedure, i.e. in the alumina adsorption, eluent volumes and detection response.

Comparison of methods of analysis

For the last few years, we have routinely been using the radioenzymatic method described by Peuler and Johnson [16] for plasma catecholamines. This method, slightly modified [21], has proved to be quite satisfactory and, so far, thousands of plasma samples have been assayed.

The liquid chromatographic and radioenzymatic methods were validated against each other in order to separate the features of each method. When analysing plasma samples there was a good agreement between the two methods (Table IV) and the sensitivity was of about the same magnitude, i.e.

TABLE III

ABSOLUTE RECOVERY AND RELATIVE STANDARD DEVIATION FOR CATECHOLAMINES IN REFERENCE SOLUTIONS, RAT HEART AND PLASMA SAMPLES ASSAYED ACCORDING TO THE ANALYTICAL PROCEDURE

The rat heart sample (0.1 g) contained 400 pmol of NA, 8 pmol of A and 9 pmol of DA; the plasma sample contained 1.53 pmol of NA and 0.25 pmol of A per ml.

Substance	1 pmol/sample (n = 9)		100 pmol/sample (n = 7)		Rat heart (n = 10)	Plasma (n = 8)
	Recovery	S.D. (%)	Recovery	S.D. (%)	S.D. (%)	S.D. (%)
NA	90	5.6	83	3.7	2.3	2.7
NA/DHBA		4.0		0.5	1.0	2.0
A	85	5.4	83	3.7	4.1	8.6
A/DHBA		2.7		0.4	2.7	7.3
DA	92	6.5	82	3.6	7.6	
DA/DHBA		2.6		0.8	6.5	
DHBA	88	6.3	85	3.8	2.1	
NMN	94	7.8	90	2.5		
MDA						3.9

TABLE IV

DETERMINATION OF NORADRENALINE AND ADRENALINE IN PLASMA SAMPLES BY THE RADIOENZYMATIC (^{3}H) AND THE ELECTROCHEMICAL (EC) METHODS

The values are expressed as nmol/l ± relative standard deviation (± S.D. %)

Sample	Noradrenaline			Adrenaline		
	^{3}H	EC	± S.D. (%)	^{3}H	EC	± S.D. (%)
1	1.27	1.13	8.2	0.1	—	
2	1.34	1.40	3.1	0.83	0.96	10.3
3	2.07	2.11	1.4	4.36	4.91	8.4
4	1.05	1.03	1.4	1.40	1.51	5.3
5	1.17	1.19	1.2	0.41	0.45	6.6
6	2.11	2.13	0.7	0.23	0.22	3.1
7	2.55	2.77	5.8	0.16	0.17	4.3
8	1.95	2.11	5.6	0.14	0.16	9.4
9	1.71	2.17	17	0.12	0.12	0

0.1 nmol/l plasma. The radioenzymatic method has the advantage of requiring only 100 µl of plasma, as compared to 2 ml for the liquid chromatographic method, which is of particular value for samples from small animals. There are, however, occasions when the radioenzymatic method is less suitable, for instance when one of the catecholamines occurs in a much higher concentration than the others. This is exemplified in Table V, where dopamine had been administered intravenously. In this case, the resolution of thin-layer chromatography was insufficient and the adrenaline values were falsely elevated due to contamination from the very high dopamine levels. This was confirmed when the plasma samples were assayed by ion-exchange liquid chromatography. Some of the samples were also chromatographed on the ion-pair reversed-phase system as well.

TABLE V

NORADRENALINE, ADRENALINE AND DOPAMINE DETERMINED BY THE RADIOENZYMATIC (^{3}H) METHOD AND BY ION-EXCHANGE (IE) AND ION-PAIR REVERSED-PHASE (RP) CHROMATOGRAPHY IN PLASMA SAMPLES AFTER DOPAMINE INFUSION

The values are expressed as nmol/l ± relative standard deviation (± S.D. %).

Sample	Noradrenaline				Adrenaline				Dopamine			
	^{3}H	IE	RP	± S.D. (%)	^{3}H	IE	RP	± S.D. (%)	^{3}H	IE	RP	± S.D. (%)
1	2.27	1.97	2.05	7.4	0.18	0.15	0.19	12	3.89	2.95	3.23	14.4
2	2.26	2.56		8.8	0.26*	0.13		47	92.4	94.9		1.9
3	2.49	2.80	2.66	5.9	0.96*	0.25	0.18	93	315	318		0.7
4	4.30	4.21	4.02	3.4	1.52*	<0.1	0.14	118	477	537	538	6.8
5	4.62	4.86		3.6	1.03*	0.17		101	564	573		1.1
6	4.68	4.49		2.9	2.60*	0.22		119	463	459		0.6
7	3.33	3.13		4.4	1.15*	<0.1			218	224		1.9
8	2.95	2.84	2.76	3.3	1.66*	<0.1	0.11	124	171	177	175	1.8
9	2.92	2.83		2.2	1.04*	0.14		108	111	117		3.7
10	2.65	2.93	2.85	5.1	1.87*	<0.1	0.10	127	116	133	132	7.5

*Falsely high values due to contamination from the high dopamine concentrations.

CONCLUSION

The recovery of catecholamines from biological samples and the accuracy of the assay using alumina adsorption and liquid chromatography are affected by different factors in the work-up procedure such as nature of stabilizing agents, elution media and mode of storage of the samples before injection. The assay by liquid chromatography and electrochemical detection has been validated against the radioenzymatic method, showing good agreement even at low plasma levels. The two methods are complementary and the choice is governed by the nature of the analytical object.

REFERENCES

1 Å. Hjalmarsson, Cardiology, 65 (1980) 226.
2 S. Holmgren, T. Abrahamsson, O. Almgren and B.-M. Eriksson, Cardiovasc. Res., in press.
3 L.J. Felice, J.D. Felice and P.T. Kissinger, J. Neurochem., 31 (1978) 1461.
4 J. Wagner, M. Palfreyman and M. Zraika, J. Chromatogr., 164 (1979) 41.
5 Y. Maruyama, T. Oshima and E. Nakajima, Life Sci., 26 (1980) 1115.
6 H. Hallman, L.-O. Farnebo, B. Hamberger and G. Jonsson, Life Sci., 23 (1978) 1049.
7 P. Hjemdahl, M. Daleskog and T. Kahan, Life Sci., 25 (1979) 131.
8 S. Allenmark, L. Hedman and A. Söderberg, Microchem. J., 25 (1980) 567.
9 D.S. Goldstein, G. Feuerstein, J.L. Izzo, I.J. Kopin and H.R. Keiser, Life Sci., 28 (1981) 467.
10 I.N. Mefford, M.M. Ward, L. Miles, B. Taylor, M.A. Chesney, D.L. Keegan and J.D. Barchas, Life Sci., 28 (1981) 477.
11 E. Watson, Life Sci., 28 (1981) 493.
12 G.C. Davis, P.T. Kissinger and R.E. Shoup, Anal. Chem., 53 (1981) 156.
13 P.T. Kissinger, C.S. Bruntlett and R.E. Shoup, Life Sci., 28 (1981) 455.
14 Y. Yui, Y. Itokawa and C. Kawai, Anal. Biochem., 108 (1980) 11.
15 M. Da Prada and G. Zürcher, Life Sci., 19 (1976) 1161.
16 J.D. Peuler and G.A. Johnson, Life Sci., 21 (1977) 625.
17 L. Bauce, J.A. Thornhill, K.E. Cooper and W.L. Veale, Life Sci., 27 (1980) 1921.
18 A.H. Anton and D.F. Sayre, J. Pharmacol. Exp. Ther., 138 (1962) 360.
19 M. Lo and S. Riegelman, J. Chromatogr., 183 (1980) 213.
20 G. Wenk and R. Greenland, J. Chromatogr., 183 (1980) 261.
21 B.-M. Eriksson, Clin. Chem., 27 (1981) 341.