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High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma[a]

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ABSTRACT

A procedure is described for the determination of α-methyldopa (MD) [L-3-(3,4-dihydroxyphenyl)-2-methylalanine], its metabolite and catecholamines in the urine and plasma of patients undergoing MD therapy, by high-performance liquid chromatography with dual working electrode coulometric detection.

An efficient sample preparation procedure is presented for the isolation of endogenous MD, its metabolite and catecholamines from plasma or urine. After deproteinization of a plasma sample with methanol containing 2% of 0.5 *M* perchloric acid and dilution of a urine sample (1:200), MD, dihydroxyphenylacetic acid (DOPAC), 3-O-methylmethyldopa (3-OMMD) and homovanillic acid (HVA) were separated with a Supelcosil LC-18 column. Catecholamines were extracted from the supernatant of deproteinized plasma or from urine by ion exchange on a Sephadex CM-25 column and subsequent adsorption on alumina. The use of the same mobile phase for the concurrent assay of MD, its metabolite and catecholamines increased considerably the efficiency of sample separation. Recoveries were close to 100% for MD, DOPAC, 3-OMMD and HVA and 70% for catecholamines.

The effects of various experimental parameters related to mobile phase composition on chromatographic performance are reported. The purity of the eluted compounds was tested by recording both the first detector response (oxidation current) and the second detector response (reduction current). The ratio of the detector responses yielded a chemical reversibility ratio for the detected compound. A number of applications such as monitoring data from patients under MD therapy are presented.

INTRODUCTION

α-Methyldopa (MD), L-3-(3,4-dihydroxyphenyl)-2-methylalanine, is a well known hypotensive agent, structurally related to the catecholamines and their precursors [1–5]. Recently, MD has been used to treat hypertension during pregnancy and no serious adverse effects on the foetus have been reported [6].

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MD has three major metabolic pathways [7]: sulphate conjugation catalysed by phenolsulphotransferase, O-methylation catalysed by catechol-O-methyltransferase and decarboxylation catalysed by aromatic L-amino acid decarboxylase. O-Methylation results in the formation of 3-O-methylmethyldopa (3-OMMD), while decarboxylation results in the formation of α-methyldopamine (α-MDA).

There is a wide range of individual variations in both MD metabolism and the dose of drug required to control blood pressure in hypertensive patients [8]. The individual differences in the activities of the above-mentioned enzymes might be one of the factors responsible for variations in MD metabolism [8]. Hence understanding the pharmacokinetics of MD is crucial to establishing the optimum therapeutic regimen for the drug. Also, the analysis of 24-h urine levels of MD and related compounds (3-OMMD, catecholamines and their metabolites) could be useful tools for studying how drug-metabolizing enzyme activities affect the wide range of individual variations in human MD metabolism [7]. Moreover, urine MD monitoring could be a sensitive and non-invasive means for clinical optimization of individual drug management.

Many methods have been developed for the determination of MD and its metabolites, such as fluorimetric [9], paper chromatographic [10], gas chromatographic [11,12] and high-performance liquid chromatographic (HPLC) methods with ultraviolet [13,14], fluorescence [15] and electrochemical detection [3,15–23]. Most of these methods have been applied to the evaluation of many interesting biogenic amines. There is general agreement that the complete profile is more informative in drug metabolism involving catecholamines. For this reason, we developed a procedure for detecting MD, 3-OMMD, norepinephrine (NE), epinephrine (E), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in biological fluids. MDA was not determined because the authentic standard was not commercially available.

The two major problems that arise in determining MD, its metabolites and catecholamines are first the very low levels of free catecholamines with respect to the amounts of MD and second the half-wave potential of 3-OMMD and HVA, which is greater than those of catecholamines and MD.

The purpose of this study was to overcome these difficulties by using a coulometric detector to solve the problems of high electrode potentials and by employing a sample preparation method that separates catecholamines from other interfering endogenous compounds. The method includes deproteinization of serum with cold methanol solution containing 2% of 0.5 *M* perchloric acid followed by centrifugation. An aliquot of the supernatant was used to measure MD and its metabolite. For the determination of the catecholamines, an aliquot of the supernatant was purified using a Sephadex CM-25 column (where MD is not retained) and alumina. The use of the same mobile phase for the concurrent assay of MD, its metabolite and catecholamines increased considerably the efficiency of sample separation in the chromatographic system.

EXPERIMENTAL

Materials

NE, E, DA, DOPAC, HVA and N-methyldopamine (NMDA, internal standard) were purchased from Sigma (St. Louis, MO, U.S.A.) and MD and 3-OMMD

were gifts from Merck, Sharp & Dohme (Darmstadt, Germany). Sodium 1-octanesulphonate (OSA) and ethylene glycol-O,O′-bis-(2-aminoethyl-N,N,N′,N′-tetraacetic acid (EGTA) were purchased from Fluka (Buchs, Switzerland). Methanol (HPLC grade) and all other analytical-reagent grade chemicals were obtained from Carlo Erba (Milan, Italy). A Sephadex CM-25 column was obtained from Pharmacia (Uppsala, Sweden) and acid alumina AG-4 from Bio-Rad Labs. (Richmond, CA, U.S.A.). Water was treated with a Milli-Q system (Millipore, Milford, MA, U.S.A.).

HPLC apparatus

The HPLC system consisted of a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.) and a Model 7125 injector (equipped with a 100-μl loop). The column used was a reversed-phase Supelcosil LC-18 (15 cm × 4.6 mm I.D.), particle size 5 μm (Supelco, Bellefonte, PA, U.S.A.). A Model 5100 A Coulochem detector (ESA, Bedford, MA, U.S.A.) was equipped with a Model 5011 A analytical cell. The potentials were set at +0.40 V for the first electrode and −0.30 V for the second electrode. The gain control used for signal amplification resulting in peak height and area changes was maintained at 2000 for the first electrode and 3500 for the second. Chromatograms were analysed with a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan) monitoring both detector signals. The mobile phase was 13 m*M* sodium acetate containing 0.5 m*M* OSA (ion-pairing reagent), 0.5 m*M* disodium EDTA and 14% methanol (pH 3.10). The compounds were eluted isocratically at room temperature at a flow-rate of 1.0 ml/min.

Preparation of plasma sample

Blood samples from patients receiving MD were drawn by venipuncture and collected in tubes containing 50 μl of a solution of EGTA (60 mg/ml) and reduced glutathione (90 mg/ml) and immediately centrifuged at 4°C for 5 min at 2000 *g*. The supernatants were stored at −80°C for assay later. A sample was allowed to thaw at room temperature and an aliquot of plasma (1 ml) was deproteinized by the addition of three volumes of ice-cold methanol containing 2% of 0.5 *M* perchloric acid and centrifuged at 4°C for 3 min at 4000 *g*. The supernatant (0.2 ml) was collected, spiked with 0.1 ml of NMDA (80 ng/ml) and evaporated to dryness under vacuum. The residue was dissolved in 0.2 ml of mobile phase. The resulting solution (5–20 μl) was injected into the HPLC system for the determination of MD, DOPAC, 3-OMMD and HVA. The isolation of catecholamines was carried out in 1 ml of the supernatant spiked with 40 μl of NMDA (80 ng/ml) as an internal standard [24].

A 3-ml volume of 0.1 *M* phosphate buffer (pH 7) was added to the solution and the mixture was applied to a Sephadex CM-25 column (2 cm × 0.5 cm I.D.). The column was conditioned with 5 ml of 0.1 *M* hydrochloric acid and 10 ml of distilled water and buffered with 10 ml of 0.1 *M* phosphate (pH 7). The compounds retained in the column were washed with 5 ml of distilled water, then catecholamines were eluted with 5 ml of 1.5 *M* perchloric acid into conical tubes with caps. A 2-ml volume of 1.5 *M* Tris buffer (pH 9.3) containing 0.06 *M* EDTA and 20 mg of acid-washed alumina [25] were added to the solution. The tube was vortex mixed for 2 min, the supernatant was removed by vacuum aspiration and the alumina was washed three times with 1 ml of water. Each wash was followed by centrifugation. The catecholamines were extracted with 100 μl of 0.1 *M* acetic acid by vortex mixing for 2 min,

allowed to settle and then centrifuged at 4°C for 2 min at 3000 *g*. The resulting solution (20–50 μl) was injected into the HPLC system.

Preparation of urine samples

Urine samples (24 h) were collected (2 h after administration) in plastic containers containing 10 ml of 6 *M* hydrochloric acid as a preservative, and a 1-ml aliquot was frozen at −80°C for assay later. A 50-ml volume of water was added to the thawed urine sample (1 ml) and 10 μl of the solution were injected into the HPLC system for the determination of MD, 3-OMMD, DOPAC and HVA. The same procedure as described above for the determination of plasma catecholamines was followed for 0.2 ml of urine sample. The values obtained represented the amount of unconjugated compounds present. In order to determine total levels, the sample was adjusted to pH 1, flushed with nitrogen and kept in a boiling water-bath for 20 min [26]. The hydrolysed sample was then processed in the same manner as the non-hydrolysed sample.

RESULTS AND DISCUSSION

Separation

Several approaches can be used to improve the separation in the reversed-phase ion-pairing technique to obtain a balance between ion-pairing reagent, organic solvent and pH.

The pH of the mobile phase is perhaps the best means of separating the various substances as it can modify the charge of the functional groups. The finding that on increasing the pH the retention times of the carboxylic acids and amino acids decreased can be explained by the fact that ion-pair formation increases as the protonation of carboxylic groups increases [27]. The retention times of HVA, MD and 3-OMMD were the most affected by the modest pH range of 2.60–3.10, whereas the retention times of amines were not influenced.

Fig. 1A shows the effect of pH on the retention of the compounds in the acidic range. For MD, DOPAC, 3-OMMD and HVA (solid lines) good separation was obtained at the pH of the mobile phase (3.1), and these conditions also provide a good compromise between resolution and duration of chromatography. Throughout the test, the concentrations of sodium acetate and methanol were maintained at 13 m*M* and 14%, respectively.

An increase in the methanol content of the mobile phase caused a significant decrease in the k' values of all compounds in the standard mixture (Fig. 1B), the strongest effect being on HVA. The pH and sodium acetate concentration were 3.1 and 13 m*M*, respectively.

Finally, keeping a constant pH of 3.1 and a 14% methanol concentration, the effect of ionic strength on the retention was investigated. Fig. 1C shows that an increase in the molar concentration of sodium acetate caused a significant decrease in the capacity factors of all catecholamines and an increase in retention for DOPAC, MD, 3-OMMD and HVA. The increase in the capacity factors with increasing salt concentration implies that the retention is due to hydrophobic interaction [28].

A representative chromatogram illustrating the resolution of a standard mixture of MD, 3-OMMD, DOPAC and HVA, and also NE, E, DA and NMDA, is

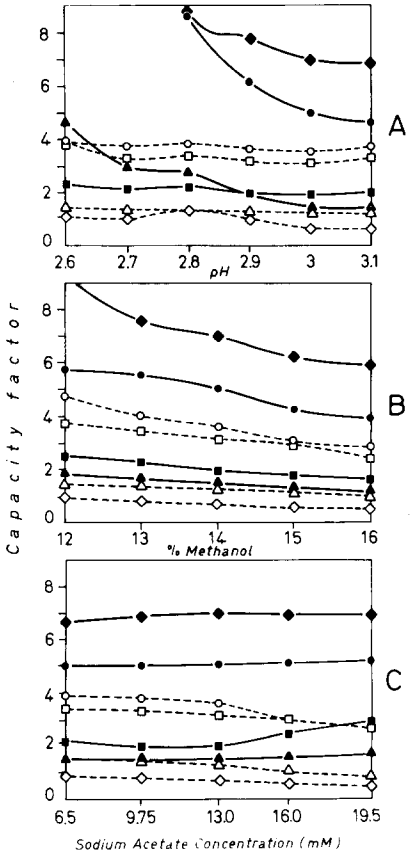

Fig. 1. Effect of (A) pH of mobile phase, (B) methanol content and (C) ionic strength on the capacity factors of (▲) MD, (●) 3-OMMD, (■) DOPAC, (◆) HVA, (△) E, (◇) NE, (□) DA and (○) NMDA. When analysing biological samples, the analytes are divided into two groups according to their different pretreatment procedures, *i.e.*, direct injection and treatment with Sephadex CM-25 and alumina (continuous and dotted lines, respectively). Column: Supelcosil LC-18, particle size 5 μm, 150 mm × 4.6 mm I.D. Mobile phase: the concentrations of OSA (0.5 m*M*) and disodium EDTA (0.5 m*M*) were always constant. The pH of the mobile phase (A) was varied by changing the ratio of sodium acetate to acetic acid, but maintaining the ionic strength (13 m*M*) and the methanol concentration (14%) constant. In (B), only the methanol concentration varied. The effect of ionic strength was investigated maintaining the pH at 3.1 and methanol concentration at 14% (C). The flow-rate was 1 ml/min.

shown in Fig. 2. When biological samples are processed, the eight compounds are divided into two groups according to their respective sample pretreatment (see Experimental) and analysed separately. This allows chromatograms to be obtained with more resolved peaks and the result could represent a substantial advantage as biological samples often include unknown substances (*i.e.*, endogenous compounds or drugs) which could give unexpected interfering peaks. Fig. 3 shows chromatograms from 24-h urine samples after administration of MD (Aldomet 250, MD 250 mg) (Merck Sharp & Dohme). Fig. 3A represents the detection of MD, DOPAC, 3-OMMD and HVA after direct injection of diluted urine. Aldomet is known to in-

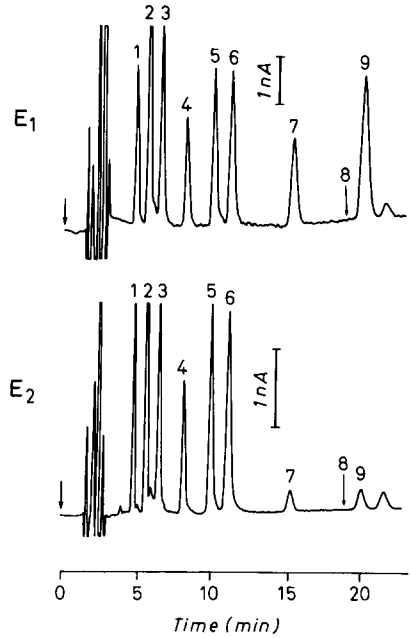

Fig. 2. Chromatograms of oxidation (E_1 level) and reduction (E_2 level) obtained from a mixture of standard NE (40 ng/ml), E (40 ng/ml), MD (80 ng/ml), DOPAC (40 ng/ml), DA (40 ng/ml), NMDA (80 ng/ml), 3-OMMD (80 ng/ml) and HVA (80 ng/ml). Peaks: 1 = NE; 2 = E; 3 = MD; 4 = DOPAC; 5 = DA; 6 = NMDA; 7 = 3-OMMD; 9 = HVA. The positions indicated by arrow No. 8 correspond to the retention time of the unknown peaks (No. 8) included in the urine chromatograms (Fig. 3), which perhaps could be ascribed to MDA. Conditions: column, Supelcosil LC-18, particle size 5 μm, 150 mm × 4.6 mm I.D.; flow-rate, 1 ml/min at ambient temperature; mobile phase, 13 m*M* sodium acetate containing 0.5 m*M* OSA, 0.5 m*M* disodium EDTA and 14% methanol (pH 3.1). Applied potential: E_1 = +0.40 V, E_2 = −0.35 V.

crease the level of MD in urine, *i.e.*, the size of MD peak is expected to be greater than those of other compounds. Therefore, the sample had to be further diluted 10-fold.

Fig. 3B shows catecholamines detected in the same urine sample as shown in Fig. 3A, which were obtained by analysing the extract from treatment with the Sephadex CM-25 column and alumina (see *Preparation of urine samples*). Interestingly, both (unknown) peaks No. 8 in Fig. 3A and B have the same retention time; the peak shown in Fig. 3B is out of the range and we confirmed its purity by the above reported "dilution and re-injection procedure". Further, we suspect that they could be due to MDA, one of the main MD metabolites in urine, but we have not been able to confirm this hypothesis as we were unable to obtain a pure standard. Our idea is based on the following: (a) the chemical structure of the unknown peak could be similar to that of other amines (NE, E, DA, NMDA), as it is also extracted by Sephadex CM-25 and alumina; (b) the k' value of the unknown peak could be compatible with MDA if it is compared with k' and the structures of DA and NMDA; (c) if we consider the different concentrations of the aliquots injected to determine catecholamines and MD, the area ratio of the unknown peak and the MD peak is not

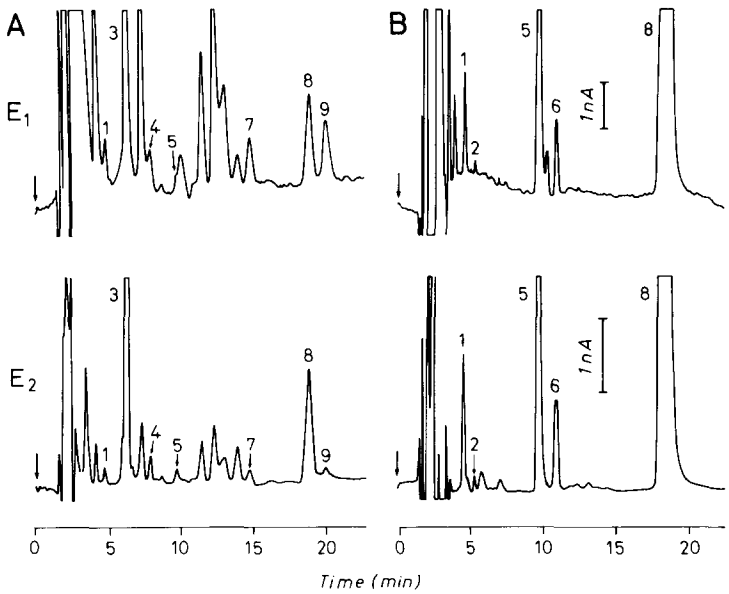

Fig. 3. Chromatograms of (A) 10 μl of 24-h urine (diluted to 1:200) from a hypertensive patient undergoing 250 mg per 24 h oral MD therapy and (B) 100 μl of acetic acid eluate from alumina using the same urine. Peaks: 1 = NE; 2 = E; 3 = MD; 4 = DOPAC; 5 = DA; 6 = NMDA; 7 = 3-OMMD; 8 = unknown; 9 = HVA. Peaks 1 and 4 reported in (A) cannot be ascribed to NE and DA because of their oxidation reduction peak-height ratios, which are different from those of standard compounds (some interfering substances are probably co-eluted). Peaks 8 are suspected to be due to MDA (see *Peak identification*). For chromatographic conditions, see Fig. 2.

very different from the concentration ratio between MDA and MD calculated from previous data [8].

Recovery and reproducibility

Deproteinization by adding trichloroacetic acid, perchloric acid, acetonitrile, methanol and methanol containing 2% of 0.5 or 1.0 *M* perchloric acid was examined. The use of methanol containing 2% of 0.5 *M* perchloric acid to prepare a protein-free sample from plasma gave highly reproducible recoveries of MD, its metabolite and HVA. The recovery was determined by comparing the peak heights of known amounts of standards added to pooled plasma from healthy subjects carried through the assay procedures with those resulting from the analysis of the same amount of a stock standard solution. Table I reports the data for plasma and urine recovery. Satisfactory recoveries were obtained with good relative standard deviations (R.S.D.). Table I also shows the linear regression analysis from calibration graphs for biological samples. The correlation coefficients for all these compounds were higher than 0.9909. Table II shows the between-assay and within-assay R.S.D.s for plasma and urine samples.

Peak identification

The peaks of MD, its metabolite and catecholamines were identified by a combination of methods. The peak identification was initially performed on the basis of

TABLE I

RECOVERY AND REGRESSION LINES OF MD, ITS METABOLITE AND CATECHOLAMINES IN NORMAL PLASMA AND URINE SAMPLES

The values were calculated by analysing plasma and urine samples spiked with standards at three different concentrations ($n = 5$). The recoveries of MD, DOPAC, 3-OMMD, HVA and NMDA(I) were obtained after the deproteinization and evaporation steps; the recoveries of NE, DA, E and NMDA(II) were obtained after the weak cation-exchange extraction procedure and subsequent adsorption on alumina. The internal standard NMDA was used in both experiments. x = added amount of MD, DOPAC, 3-OMMD, NMDA(I) (expressed as ng/ml) and NE, E, DA and NMDA(II) (expressed as pg/ml); y = amount found.

Compound	Plasma				Urine			
	Recovery (mean ± S.D.)	R.S.D. (%)	Concentration range	r	Recovery (mean ± S.D.)	R.S.D. (%)	Concentration range	r
MD	95.6 ± 5.0	5.2	0.8–3.2	0.9996	99 ± 4	4.3	0.4–4.0	0.9991
DOPAC	89.9 ± 4.3	4.8	0.4–1.6	0.9908	102 ± 3	4.9	0.2–3.2	0.9954
3-OMMD	91.8 ± 4.8	4.8	0.8–3.2	0.9999	104 ± 5	4.8	0.4–4.0	0.9996
HVA	102.3 ± 4.4	4.3	0.8–3.2	0.9997	98 ± 2	3.5	0.4–4.0	0.9972
NMDA(I)[a]	95.1 ± 3.4	4.1	0.8–3.2	0.9939	99 ± 4	3.2	0.4–4.0	0.9989
NE	74.5 ± 4.5	5.1	150 –1200	0.9919	73 ± 5	4.6	300 –3000	0.9939
E	68.9 ± 3.3	4.9	50 –800	0.9997	70 ± 4	4.8	50 –800	0.9992
DA	69.1 ± 4.2	6.1	50 –800	0.9968	68 ± 3	4.4	150 –2400	0.9967
NMDA(II)[b]	66.1 ± 5.2	5.7	400 –1600	0.9990	69 ± 5	4.3	400 –1600	0.9998

[a] NMDA internal standard used for determination of MD, DOPAC, 3-OMMD and HVA.
[b] NMDA internal standard used for determination of NE, E and DA.

TABLE II

REPRODUCIBILITY

Between-assay and within-assay R.S.D.s. Plasma and urine samples were spiked with known amounts of MD, its metabolite and catecholamines. MD, DOPAC, 3-OMMD, HVA and NMDA(I) are expressed as ng/ml and NE, E, DA and NMDA(II) as pg/ml.

Compound	Concentration	Plasma		Urine	
		Within-assay R.S.D. (%) ($n=6$)	Between-assay R.S.D. (%) ($n=20$)	Within-assay R.S.D. (%) ($n=5$)	Between-assay R.S.D. (%) ($n=10$)
MD	1.6	5.1	6.3	4.7	4.9
DOPAC	2.1	4.4	5.2	5.2	5.1
3-OMMD	1.6	3.2	4.1	4.3	4.5
HVA	3.8	5.4	5.7	3.9	4.2
NMDA(I)[a]	1.6	5.1	6.2	3.2	3.9
NE	300	4.5	5.3	4.1	4.2
E	50	4.8	5.5	4.4	4.6
DA	50	5.9	6.8	5.4	5.6
NMDA(II)[b]	400	5.2	6.3	3.9	4.1

[a] NMDA internal standard used for the determination of MD, DOPAC, 3-OMMD and HVA (deproteinization and evaporation steps).

[b] NMDA internal standard used for the determination of NE, E and DA (cation exchange and alumina extraction).

the chromatographic retention time and by simultaneous injection of a standard. Second, the ratios of the first detector response (E_1, oxidation current) *versus* the second (E_2, reduction current) were calculated and compared with those obtained with plasma or urine samples. The peak-height ratios of reference compounds and those obtained with plasma or urine samples are reported in Table III.

TABLE III

REVERSIBILITY RATIOS

The values represent the ratios of the detector responses (oxidation current/reduction current) of MD, 3-OMMD, DOPAC, HVA, NMDA and catecholamines. The results are the means ± S.D. of ten experiments. Under the conditions of detector sensitivity, the gain was set at 2000 for the first electrode and 3500 for the second. The plasma levels of E and DA were not determined.

Compound	Standard	Plasma	Urine
MD	1.05 ± 0.06	0.96 ± 0.08	0.98 ± 0.06
3-OMMD	3.37 ± 0.06	3.34 ± 0.03	3.40 ± 0.02
DOPAC	0.73 ± 0.01	0.69 ± 0.71	0.71 ± 0.03
HVA	8.24 ± 0.03	8.28 ± 0.06	8.23 ± 0.05
NE	0.74 ± 0.02	0.71 ± 0.05	0.72 ± 0.04
E	0.83 ± 0.04	–	0.80 ± 0.05
DA	0.73 ± 0.02	–	0.76 ± 0.03
NMDA	0.76 ± 0.01	0.78 ± 0.03	0.79 ± 0.04

The comparison of peak-height ratios allowed some false peak identifications to be avoided. As an example of this, the two chromatograms shown in Fig. 3A should be carefully observed. If peaks are simply identified by comparing their retention times with those of a standard mixture (Fig. 2), peaks 1 and 4 seem to be NE and DA, respectively. On closer examination of the two chromatograms, we observed that the ratios of peak heights (oxidation/reduction) for reference compounds was significantly different from those obtained with urine. As the chromatograms were obtained by direct injection of diluted urine, there are probably some interfering substances which are co-eluted with NE and DA. Our conclusion is that peaks 1 and 4 in Fig. 3A are not homogeneous and they cannot be ascribed to NE and DA. On the other hand, if samples are treated by the appropriate catecholamine extraction (Sephadex CM-25 and alumina) the peaks are clearly identified (Fig. 3B). The selection of the detector potentials is important in obtaining an effective resolution.

Fig. 4 shows the hydrodynamic voltammograms of the standard solution. The substances can be separated according to their half-wave potentials ($E_{1/2}$) into three classes: DA, NMDA, NE, E and DOPAC with the lowest $E_{1/2}$, MD with a higher $E_{1/2}$ value than catecholamines, and finally 3-OMMD and HVA with the highest $E_{1/2}$ values. An operating oxidation potential of +0.40 V was chosen for the determination detection of all the compounds tested, including MD and HVA. The reduction half-wave potential is less indicative and the value chosen of −0.30 V is sufficient for the complete reduction of all compounds.

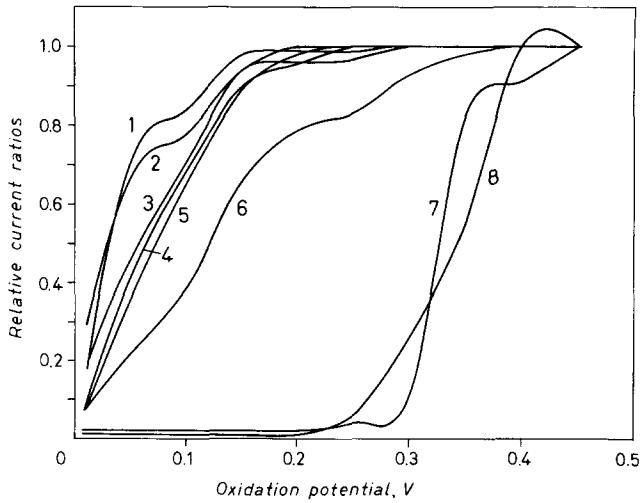

Fig. 4. Hydrodynamic voltammograms for standard substances obtained under the conditions described in Fig. 2. The response (current) at several potentials was recorded and the ratios of the current at any given potential to that of the average response at the plateau level were plotted as a function of oxidation potential. Each point represents the mean of two determinations. An oxidation potential of +0.40 V was chosen for the quantitative oxidation of all compounds. 1 = DA; 2 = NMDA; 3 = DOPAC; 4 = NE; 5 = E; 6 = MD; 7 = HVA; 8 = 3-OMMD.

TABLE IV

TIME COURSE OF CONCENTRATION OF MD PLASMA FOLLOWING AN ORAL DOSE OF 500 mg ALDOMET (MD 500 mg)

Each value represents the means ± standard errors of three determinations. The values are expressed as ng/ml.

Time after administration (h)	Subject No.				
	1	2	3	4	5
1	110 ± 13	642 ± 70	205 ± 20	104 ± 13	1027 ± 121
2	1820 ± 68	1072 ± 118	745 ± 121	580 ± 53	1842 ± 118
3	2576 ± 141	1480 ± 136	1152 ± 115	826 ± 46	2327 ± 265
4	1127 ± 103	1152 ± 116	908 ± 132	912 ± 68	1746 ± 70
5	942 ± 138	815 ± 75	648 ± 72	627 ± 51	1322 ± 145
6	525 ± 59	584 ± 64	552 ± 79	389 ± 53	827 ± 182
Mean	1183 ± 86	958 ± 52	702 ± 49	573 ± 53	1515 ± 149

Clinical applications and conclusion

The method described has been used extensively for the quantitative analysis of plasma and urine samples. Table IV gives the plasma MD concentrations for five patients with essential hypertension, dosed orally with 500 mg of MD. Maximum plasma concentrations occurred 2–3 h after administration.

Table V reports the 24-h urine excretion of MD, its metabolite and catecholamines (free and conjugate) from five hypertensive patients receiving 250 mg of MD orally. Urinary excretions of MD and 3-OMMD from twelve healthy subjects are shown in Table VI. None of the subjects were hypertensive and none of them was taking other medication. Each subject ingested 250 mg of MD and 24-h urine samples were collected.

TABLE V

URINARY CONCENTRATIONS OF FREE AND CONJUGATED MD, ITS METABOLITE AND CATECHOLAMINES IN FIVE PATIENTS RECEIVING MD ORALLY (250 mg)

24-h urine samples; urine collection began 2 h after drug administration. The results reported are the mean ± standard errors for five determinations (each value is the mean of three experiments). MD, 3-OMMD and HVA are expressed as μg/ml and NE, E and DA as ng/ml. The urine levels of HVA and E conjugated were not determined.

Compound	Free	Range	Conjugated	Range
MD	33.5 ± 6.9	25.5–40.6	19.5 ± 7.1	13.1–28.9
3-OMMD	4.9 ± 2.1	2.7–6.8	2.7 ± 0.8	1.7–3.7
DOPAC	0.9 ± 0.3	0.5–1.2	0.6 ± 0.2	0.4–0.8
HVA	3.8 ± 1.2	2.4–5.0	–	–
NE	37.2 ± 20.2	22–68	26 ± 6	19–34
E	13.8 ± 8.0	6–24	–	–
DA	376.1 ± 231.0	226–712	106 ± 24	81–136

TABLE VI

24-HOUR FREE MD AND 3-OMMD IN URINE FROM TWELVE HEALTHY PATIENTS RECEIVING 250 mg OF MD ORALLY

Urine collection began 2 h after drug administration. The results reported are the means of twelve values (each value is the mean of three experiments).

Compound	Mean ± S.E.M.[a] (μ/ml)	Range (μg/ml)
MD	17.70 ± 4.0	11.51–32.15
3-OMMD	3.80 ± 1.56	0.56–11.42

[a] Standard error of the mean.

The present procedure was found to be sufficiently reliable and simple to use for the clinical optimization of therapeutic regimes. Studies of the peak-height ratio reduced the risks of false identification of peaks. The method should be suitable for the study of how drug metabolic enzyme activities influence the wide individual variations in MD metabolism in man.

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