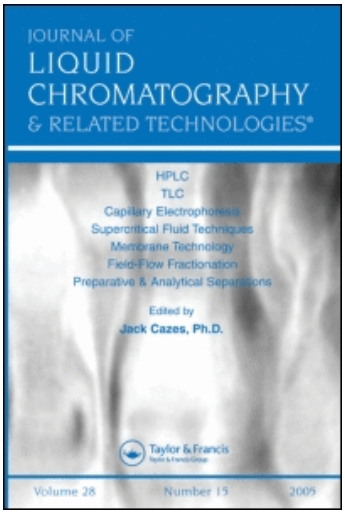

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Simultaneous Measurement of Plasma Catecholamine (Norepinephrine, Epinephrine, and Dopamine) and Free N—Methyl Dopamine (Epinine) Levels, by HPLC with Electrochemical Detection

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SIMULTANEOUS MEASUREMENT OF PLASMA CATECHOLAMINE (NOREPINEPHRINE, EPINEPHRINE, AND DOPAMINE) AND FREE N - METHYL DOPAMINE (EPININE) LEVELS, BY HPLC WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

Epinine is the active moiety of ibopamine, a cardiovascular prodrug used in congestive heart failure. This catecholic compound shows dopaminergic and adrenergic properties. Moreover the drug seems to affect plasma catecholamine levels in patients with heart failure. Here we present a method developed for the simultaneous determination of epinine and catecholamine plasma levels. Free epinine and catecholamines were extracted from human venous plasma via an alumina adsorption procedure. The extracts underwent an ion-pair reversed-phase HPLC separation with three-electrode coulometry. Quantitation was made by an internal standard method. Coefficients of variation were $< 9\ \%$. The validity was assessed as the peak

height - picograms correlation ($r > 0.997$). The detection limits were < 5 pg of each catechol after extraction. This method allows about 50 low cost determination to be done in a working day.

INTRODUCTION

N-methyl dopamine (otherwise called deoxyepinephrine or epinine) is the active moiety of ibopamine, an orally active drug, derivative of dopamine (DA), that shows cardiovascular effects (1). The presence of epinine has been demonstrated in rat renal tissue, and it was proposed to have some intrarenal effects (2). In vivo studies demonstrated significant decrease of plasma norepinephrine (NE) values, and increase of plasma epinephrine (Epi) concentrations in patients with congestive heart failure who received single or repeated doses of oral ibopamine (3,4).

In view of: i) the effects of epinine on plasma catecholamine (CA) levels, ii) the prognostic value of plasma NE concentration in patients with congestive heart failure, and iii) the possible intrarenal synthesis of epinine, we modified a previously reported method (5) of plasma CA analysis, attempting the

simultaneous determination of both plasma CA and plasma epinine values. Studies regarding disposition of epinine and its effects on plasma CA levels require sensitive methodologies for the simultaneous quantitation of epinine itself and of CA. This report details the analytical procedure developed to measure these compounds.

MATERIALS

Reagents

NE, Epi, DA, dihydroxybenzylamine (DHBA), epinine, alumina type WA-4, sodium metabisulfite, sodium phosphate, sodium acetate, sodium dodecilsulfate, Tris and microfilters were from Sigma Chemical Co. St.Louis, MO. Acetonitrile LiChrosolv, phosphoric, hydrochloric, and perchloric acids, and EDTA were Merck products (Darmstadt, FRG). Water was purified in a Milli-Q apparatus (15-18 Mohm, 0.22 um pore size, Millipore Corp. Bedford, MA). All solutions were filtered in a solvent clarification apparatus (0.22 um pore size, Millipore). pH was determined at room temperature.

Liquid Chromatography

Instrumentation: HPLC apparatus consisted of: Model 510 constant flow pump (Waters, Milford, MA), Model 7125 valve fitted with a 50 uL sample loop (Rheodyne, Cotati, CA), Model 5100A electrochemical detector (ESA, Bedford, MA) consisting in a series of three electrodes (Model 5011 and 5021 cells) working in ox-red mode. The analytical column was a stainless steel Supelcosil LC-18-DB 7.5 cm x 4.6 mm id prepacked with 3 um ODS (Supelco Inc. Bellefonte, PA), protected by a precolumn Supelguard LC-18-DB 2 cm x 4.6 mm id, also from Supelco. Mobile phase consisted of an 85:15 mixture of 50 mmol/L sodium phosphate, 50 mmol/L sodium acetate, and acetonitrile and also contained 0.6 mmol/L of sodium dodecilsulfate and 0.5 mmol/L of EDTA. The final pH was adjusted to 3.10 with 85% phosphoric acid. The column was equilibrated with the mobile phase at least 6 hours before use.

The elution profiles were integrated by an LCD CI-10B (Milton Roy, Riviera Beach, FL) and displayed on a Sekonics plotter.

METHODS

Sample collection and handling

Venous blood was drawn via a 21 gauge needle into chilled tubes containing EDTA and sodium metabisulfite, and centrifuged immediately at +4°C for 10 minutes. The plasma was separated and stored in liquid nitrogen until assayed, within 1 week.

Extraction

For assay of free CA and free epinine, 2 mL freshly thawed plasma were added to 22-25 mg of activated alumina in a 12 mL plastic tube. To this were added 1 mL of filtered Tris hydrochloride buffer (1.5 mol/L, pH 8.70, containing 0.5 mmol/L of EDTA and 0.4 mmol/L sodium metabisulfite per liter) and 500 pg of DHBA. The sample was vortexed for 10 minutes. The supernatant was discarded and the alumina was washed three times with 3 mL portions of 50-fold dilutions of Tris hydrochloride buffer in chilled water. The alumina slurry was transferred by disposable pipette in microfilters and centrifuged. CA and epinine were desorbed with 0.1 mL of filtered 0.1 mol/L perchloric

acid. The mixture was vortexed for at least 10 seconds. Finally the supernatant was separated by centrifuge.

Analysis

The plasma extracts were analyzed by injecting 50 uL aliquots into the column. The effluent was monitored at the following potentials: +300 mV (first electrode), +60 mV (second, screen electrode), and -300 mV (third, quantifying electrode). Full-scale sensitivity was 20 nA. Pump flow was 1.1 ml/minute.

RESULTS

The response of the detector was linear from the detection limits up to 20 ng/mL ($r > 0.997$) for CA and epinine. The detection limits (signal to noise ratio = 3) were 2 pg per 50 uL of extracts of standard for NE and epinine and 4 pg for Epi and DA. All within-run CV were $< 9\%$ (Range 20 ng / mL to 10 pg / mL). The between-run CV was $< 7\%$ respect to DHBA during five consecutive weeks (total assay n = 28). The chromatographic separations were completed in 11 minutes. Each

TABLE 1.

Response Factors of Norepinephrine, Epinephrine, Dopamine, and Epinine Respect to DHBA (Int. Standard).

Amounts	Norepinephrine	Epinephrine	Dopamine	Epinine
0 pg	0.00000	0.00000	0.00000	0.00000
25 pg	0.00151	0.00122	0.00066	0.00063
50 pg	0.00154	0.00121	0.00063	0.00061
100 pg	0.00150	0.00118	0.00070	0.00065
200 pg	0.00158	0.00120	0.00064	0.00066
500 pg	0.00152	0.00119	0.00067	0.00060
1000 pg	0.00156	0.00124	0.00066	0.00062
2000 pg	0.00148	0.00128	0.00076	0.00065
Mean value	0.00153	0.00122	0.00066	0.00063
SD as %	2.29%	2.79%	3.48%	3.49%

of the peaks was resolved to the baseline. Average analytical recovery was: NE 86%, Epi 95%, DA 76%, epinine 82%; it was calculated as the recovery of standards diluted in plasma respect to standards directly injected (peak height for plasma + standard) - (peak height for plasma) / (peak height for standard directly injected), total n = 30 (Range 2 ng / mL to 25 pg / mL). Table 1 shows the response factors for CA and epinine. Figure 1A shows chromatograms of stan-

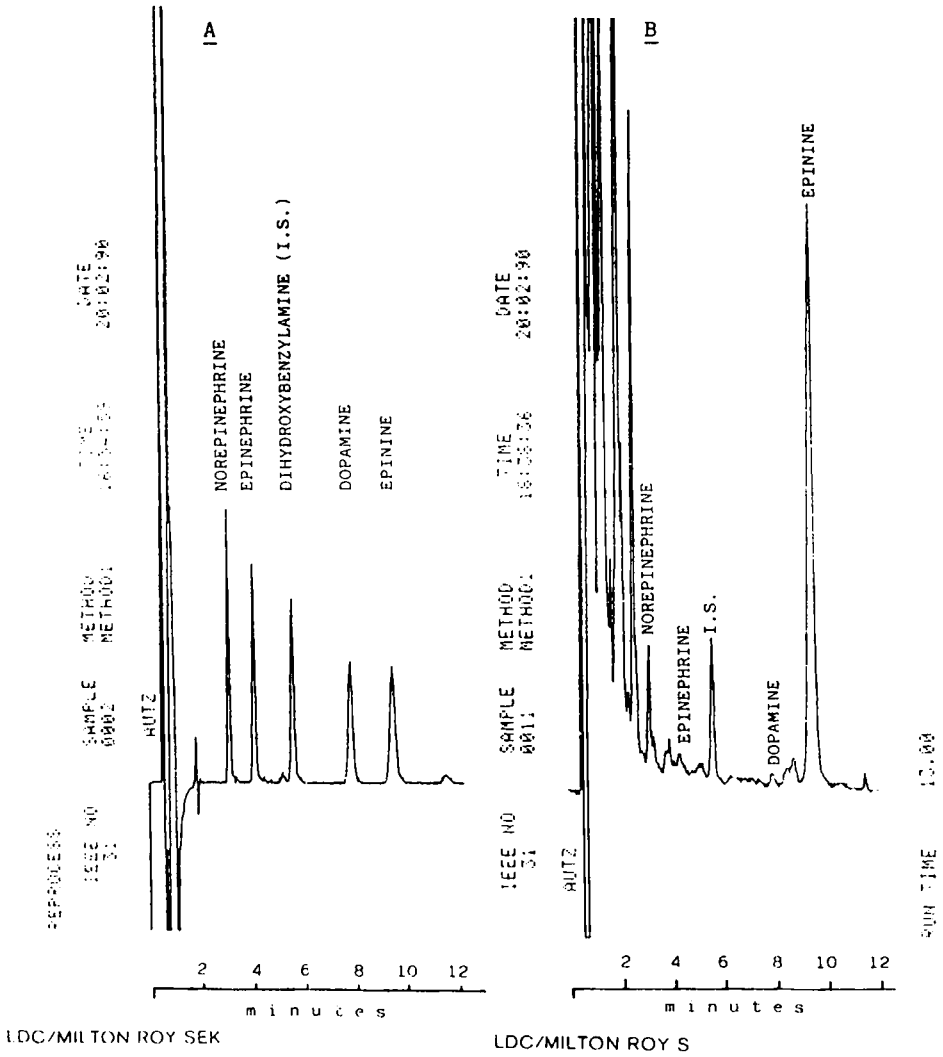

Figure 1. Chromatograms of standard (1000 pg each) after the extraction procedure (A), and plasma sample 30 minutes after the administration p.o. of ibopamine 200 mg, in a patient aged 65 with heart failure. NE 249 pg/mL, Epi 14 pg/mL, DA 58 pg/mL, Epinine 3.19 ng/mL.

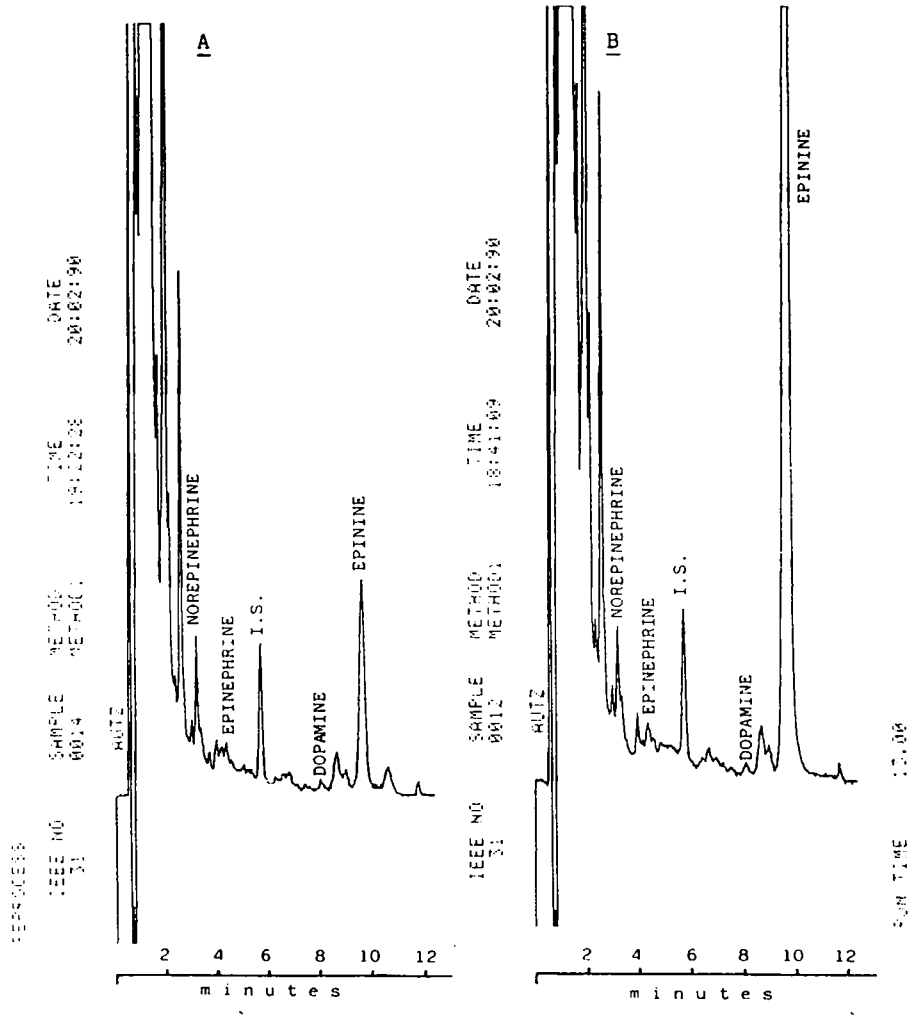

Figure 2. Chromatograms of plasma extracts. A) 120 minutes and B) 60 minutes after the oral administration of ibopamine 200 mg. A) NE 267 pg/mL, Epi 28 pg/mL, DA 42 pg/mL, Epinine 1.24 ng/mL. B) NE 213 pg/mL, Epi 18 pg/mL, DA 68 pg/mL, Epinine 7.23 ng/mL.

dards and Figure 1B and 2 plasma samples after ibopamine administration p.o. (200 mg).

Fifty plasma samples can be assayed in a working day.

DISCUSSION

Some analytical procedures involving HPLC with electrochemical detection (ECD) have been developed for the assay of epinine and other catecholic compounds (6). However, most of them were addressed to the determination of epinine and its metabolites and few Authors attempted a simultaneous measurement of epinine and CA (3). These methods are limited to NE and Epi, lacking data about plasma DA. Recently the renal epinine synthesis in the rat was studied by radioenzymatic assay (2). The advantages of HPLC ECD methods for catechols over the radioenzymatic techniques are their speed, the easy preparation of samples, the low cost per analysis, and lack of use of radiolabelled compounds. However, usual HPLC ECD assay cannot determine the low levels of circulating DA; moreover elution times of the epinine peak is often

delayed to 20 minutes or more (6), and the peak broadening and tailing can affect the quantitation of this molecule. The method presented here shows adequate separations of CA and epinine from low physiological levels to the pharmacological ones. The reliability (intra- and inter-assay CVs < 9 %) and the validity (peak height with pg correlations), together with the high analytical recovery, the low cost per sample analyzed (less than 7 US$), and the number of runs per day make this method useful both in research and in routine analysis.

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