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## Note

# Determination of a wide range of urinary amine metabolites using a simple high-performance liquid chromatographic technique

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The determination of catecholamine and indoleamine metabolites in body fluids plays an important role in both psychopharmacological research and clinical diagnosis, particularly in diseases such as phaeochromocytoma or carcinoid syndrome [1]. Qualitative screening can be performed by thin-layer chromatography; alternatively (or in addition) the quantitative determination of single compounds or small groups of compounds can be performed by gas chromatography [2], fluorimetry [3] or high-performance liquid chromatography (HPLC) [4, 5]. We report here a procedure for urinary metabolites which, although a simple screening method, has the adequate sensitivity and accuracy otherwise exhibited by the more complex HPLC separation and detection systems. It involves a simple solvent extraction, HPLC separation and UV spectroscopic detection of *p*-hydroxyphenylpyruvic acid (*p*-HPPA), 3-methoxy-4-hydroxyphenyl glycol (MHPG), 2-hydroxyphenylacetic acid (2-HPAC), 3,4-dihydroxyphenylacetic acid (DOPAC), hippuric acid (HA), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), homovanillic acid (HVA), vanillic acid (VA) and indoleacetic acid (IAA).

## EXPERIMENTAL

### *Urine extraction*

An aliquot from a 24-h urine sample collected in 25 ml of concentrated hydrochloric acid (pH 2—3) containing 15 mg of creatinine was diluted to 30 ml with water and extracted with two 30-ml volumes of diethyl ether. The pooled organic phase was dried over anhydrous sodium sulphate for 30 min and then evaporated to dryness under vacuum at 30°C. The dry deposit was dis-

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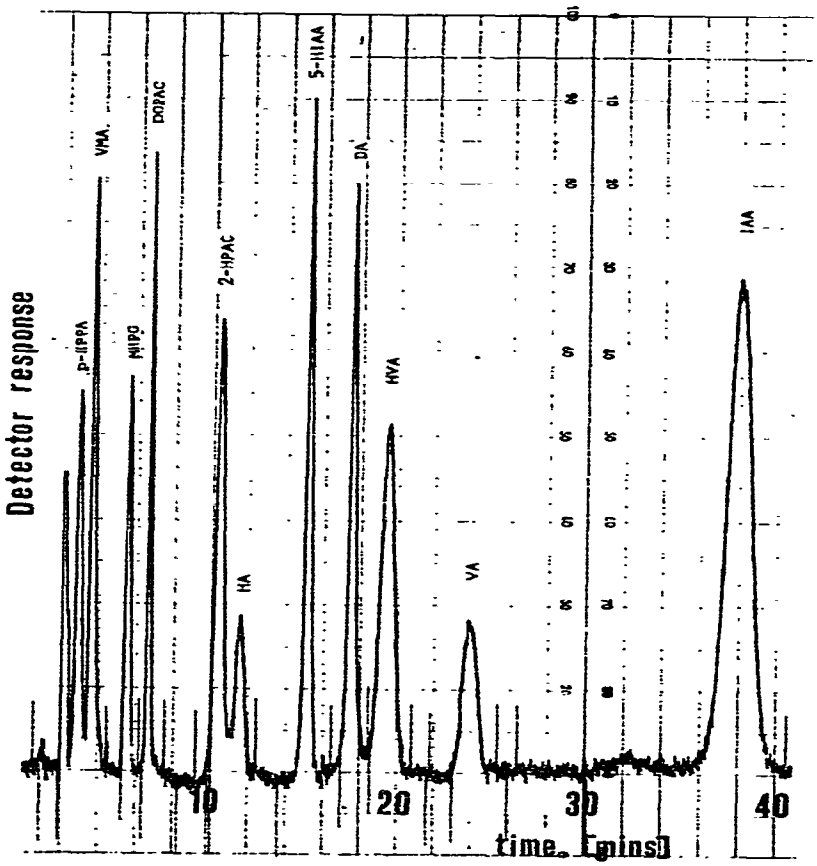


Fig. 1. Qualitative chromatographic separation of a mixture of standard compounds.

TABLE I

HPLC OF URINARY METABOLITES

| Compound | Retention time (min) | Sensitivity of the method (μg per 30 ml) |
|---|---|---|
| *p*-HPPA | 4.04 | 15 |
| VMA | 4.73 | 1.0 |
| MHPG | 6.53 | 3.5 |
| DOPAC | 8.12 | 0.2 |
| 2-HPAC | 12.45 | 0.1 |
| HA | 13.08 | 2.0 |
| 5-HIAA | 17.15 | 0.1 |
| DA | 19.3 | 0.2 |
| HVA | 22.4 | 0.1 |
| VA | 25.4 | 0.1 |
| IAA | 41.38 | 0.1 |

solved in 2 ml of 0.2 *M* acetic acid and 20 $\mu$l of the solution were injected into the HPLC system.

*Chromatography and detection*

HPLC was performed using a reversed-phase column (25 × 0.4 cm, 5-$\mu$m Spherisorb ODS; LDC, Riviera Beach, FL, U.S.A.) with 0.2 *M* acetic acid as eluent delivered by a reciprocating double-piston pump at 1 ml/min. Samples were introduced by a 20-$\mu$l loop injector. Detection was effected using a UV spectrometer (Spectromonitor II; LDC) set at 280 nm. An electronic integrator (minigrator; Spectra Physics, Santa Clara, CA, U.S.A.) was used to simplify peak identification and quantitation. The compounds present were determined by an external standard method; together with each batch of 5—10 samples a standard mixture was extracted and the peak areas were compared. The validity of this method was checked from time to time by addition of standards to a duplicate urine sample.

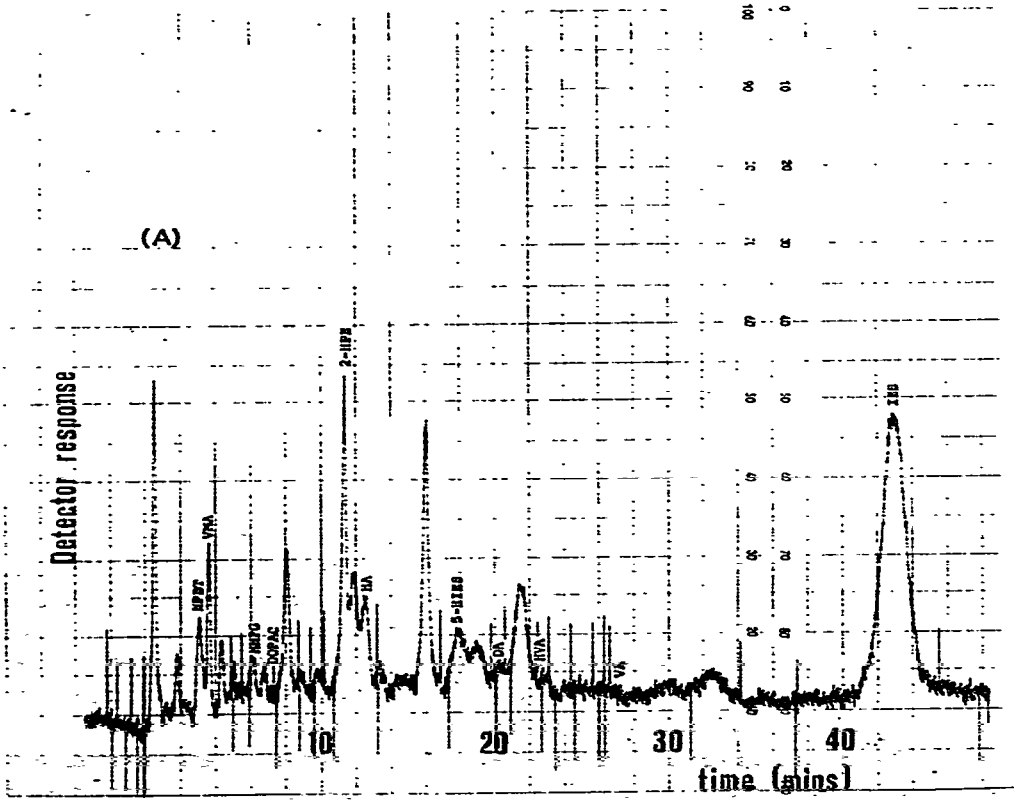


Fig. 2A.

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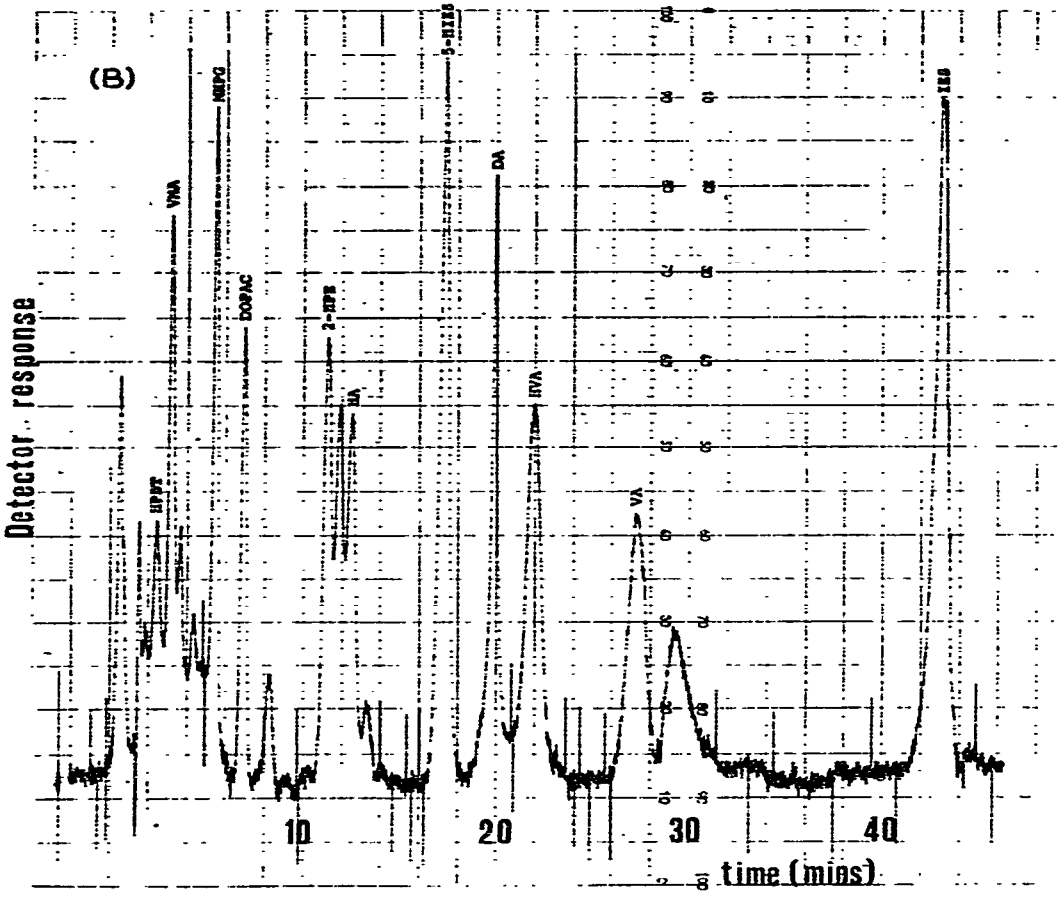


Fig. 2. Chromatograms of a normal diluted urine sample extracted as described in the text, (A) without or (B) with added standards.

## RESULTS AND DISCUSSION

The sensitivity of the method with respect to each urinary metabolite is shown in Table I. The high variation between different samples reflects the variation in both the percentage extracted and the molar absorptivity at 280 nm. The sensitivity is, however, adequate for all amine neurotransmitter metabolites measured here (Fig. 1). The reproducibility of the method was established by multiple extraction from a single urine sample; this provided a mean coefficient of variation of 6% for the range of compounds studied, which compares favourably with other techniques [2—5].

It takes approximately 1 h to run a chromatogram; this time could perhaps be reduced when not all of the metabolites listed here need to be determined.

We have applied the method to the routine analysis of urine samples (Fig. 2) in the diagnosis of carcinoid syndrome, phaeochromocytoma and similar

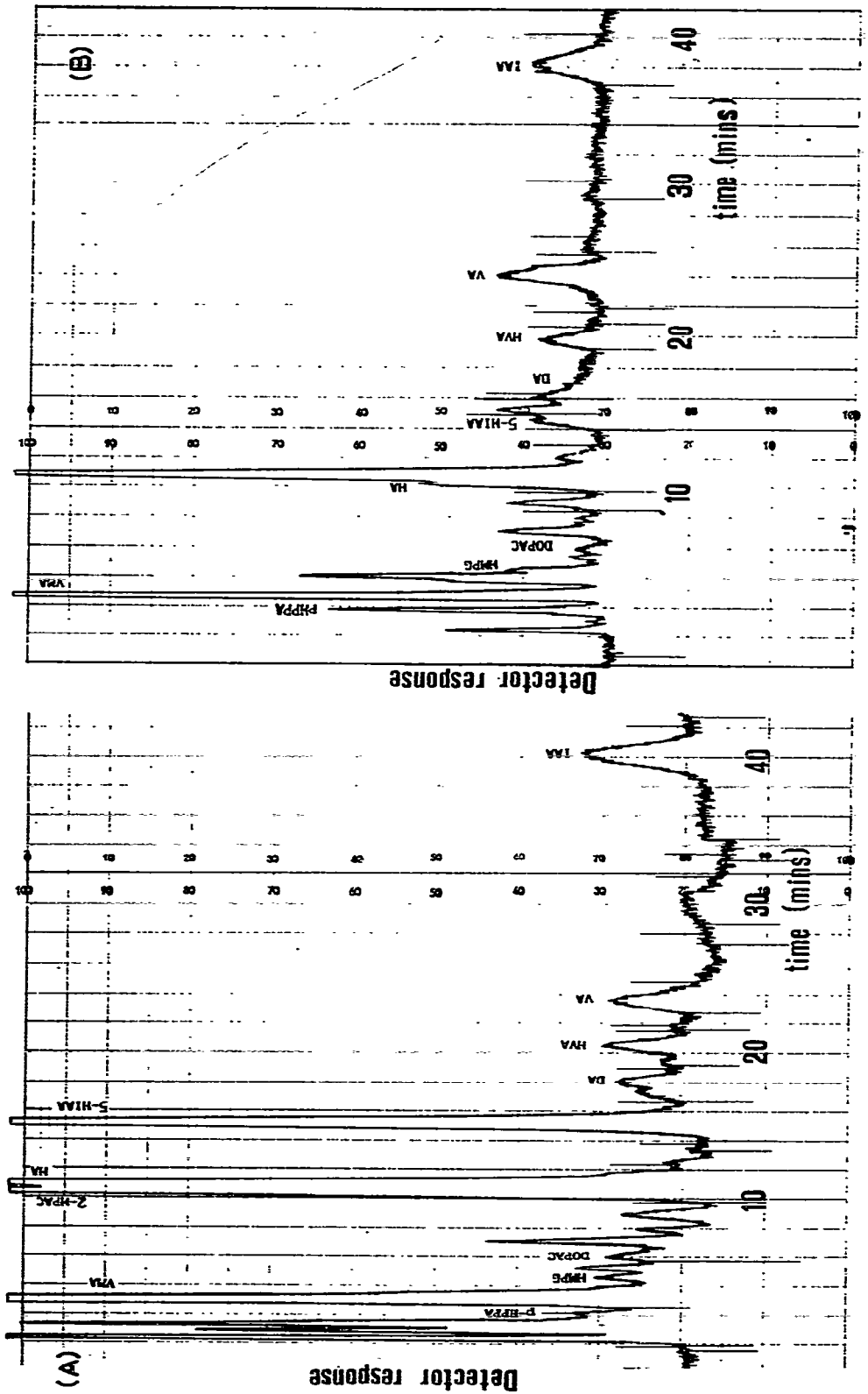


Fig. 3. Typical chromatogram of (A) a carcinoid urine sample and (B) another showing the urinary pattern of amine metabolites from a patient with anorexia nervosa.

diseases [1]. In addition, an investigation into amine metabolism in anorexia nervosa has been undertaken using this system [6]. Examples of pathological analyses are shown in Fig. 3.

In conclusion we have found this method to be a simple and accurate procedure for the determination of a wide range of urinary metabolites.

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