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Note

Direct determination of 3-methoxy-4-sulphonyloxyphenylglycol (MHPG sulphate) in urine using gas—liquid chromatography

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3-Methoxy-4-hydroxyphenylglycol (MHPG) is a metabolite of noradrenaline. MHPG is excreted in human urine in free form and as glucuronide and sulphate conjugates. MHPG sulphate (3-methoxy-4-sulphonyloxyphenylglycol) is supposed to be primarily of cerebral origin [1, 2]. Urinary excretion of MHPG sulphate can be used as an index for central noradrenaline turnover in psychiatric and pharmacologic research.

Three methods for the determination of MHPG sulphate in urine have been described. The enzymatic method of Bond and Howlett [3] has the disadvantage of possible enzyme impurities and the absence of an internal standard. Moreover, it is not a direct method because MHPG sulphate has to be calculated after determination of free and glucuronide-conjugated MHPG. Two different non-enzymatic methods for MHPG sulphate were described by Yeh et al. [4] and Murray et al. [5]. In our hands, however, both methods resulted in low recoveries of 25% and 30%, respectively.

We think there is a need for an improved method for the isolation and determination of urinary MHPG sulphate. In this paper we describe a simple method for the separation of MHPG sulphate on Sephadex with subsequent enzymatic hydrolysis and an assay by gas—liquid chromatography (GLC). Results for 21 healthy volunteers are reported.

EXPERIMENTAL

Chemicals

All chemicals used in this procedure were pro-analysi. Ethyl acetate and acetonitrile were of Uvasol quality. Extrelut was obtained from E. Merck

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(Darmstadt, F.R.G.); heptafluorobutyric anhydride was obtained from Pierce (Rockford, IL, U.S.A.); DEAE-Sephadex A-25 was obtained from Pharmacia (Uppsala, Sweden); arylsulphatase (*Helix pomatia*) (EC 3.1.6.1) suspension 5 mg/ml was from Boehringer-Mannheim (Mannheim, F.R.G.); β-glucuronidase from *Escherichia coli* (EC 3.2.21) type VII, No. G-1758, purified soluble powder, was from Sigma (St. Louis, MO, U.S.A.). 3-Methoxy-4-sulphonyloxyphenylglycol potassium was a gift from Hoffmann-La Roche (Basle, Switzerland).

Pre-wash

Three grams of DEAE-Sephadex A-25 were saturated with distilled water for 24 h, then washed successively with 0.1 *M* hydrochloric acid, 12.5% ammonia, 100% acetic acid, 4 *M* ammonium acetate pH 7.0, with distilled water in between.

The chloride test was negative and the resin resaturated with distilled water. The anion-exchanger DEAE-Sephadex A-25 then has acetate as a counter ion.

Extraction procedure

To an aliquot (5 mg of creatinine) of a 24-h urine sample adjusted to pH 3.0 with 100% acetic acid, 20 ml of 1% acetic acid (pH 3.0) were added. This mixture was passed through a polypropylene column (4 cm × 0.7 cm I.D.) containing 200 mg of pre-washed DEAE-Sephadex A-25. Then the column was washed with 10 ml of acetic acid, twice with 10-ml portions of distilled water and the MHPG sulphate eluted with 10 ml of 1 *M* sodium acetate (pH 8.9). Finally, 0.50 ml of saturated barium chloride and 0.05 ml of arylsulphatase suspension were added to this eluate and after adjustment to pH 6.2 the volume was diluted to 20.0 ml with distilled water.

Another aliquot of urine was treated likewise after adding 40 μg of MHPG sulphate potassium as the internal standard.

The samples were incubated for 16—18 h at 37.0°C. The MHPG liberated from MHPG sulphate was then extracted with 135 ml of ethyl acetate at pH 7.5 using the Extrelut column. The eluate, dried with sodium sulphate, was evaporated to a small volume (± 1 ml), transferred quantitatively to a vial, and further evaporated to dryness in a vacuum desiccator. The obtained residue was dissolved in a mixture of 200 μl of acetonitrile, 100 μl of ethyl acetate and 100 μl of heptafluorobutyric anhydride (HFBA). The vial was tightly capped, mixed thoroughly and left standing overnight (16 h).

Next day the sample was evaporated under a stream of dry air at room temperature and the vial placed in a vacuum desiccator (1 h). Finally, the residue was dissolved in 100 μl of ethyl acetate and 2 μl were injected into the GLC column.

Gas—liquid chromatography

The analysis was carried out with a Packard Model 428 gas chromatograph equipped with a flame ionisation detector, under the following conditions: column, 2 m × 2 mm I.D.; liquid phase 5% OV-17; support, Supelcoport, 80—100 mesh (Supelco, Bellefonte, PA, U.S.A.); column temperature from 140°C to 180°C at 4°C/min; detector temperature 200°C; injection port tem-

perature 200°C; attenuation 32; supporting gas, helium, at a flow-rate of 20 ml/min.

Calculations were done using a Hewlett-Packard Model 3380A integrator.

RESULTS AND DISCUSSION

The use of DEAE-Sephadex A-25 for isolation of MHPG sulphate was first introduced by Yeh et al. [4], who used 0.15 *M* hydrochloric acid for elution of the sulphate. Replicating their procedure we had a yield of MHPG sulphate of only 25%. This can be explained by the fact that MHPG sulphate was unstable under this acid condition [5].

The method described by Murray et al. [5] includes the use of Amberlite XAD-2 for extraction of all three MHPG variants. They mention a yield of only 30% in this procedure, which we confirm. Sephadex LH-20 was subsequently used for the isolation of MHPG sulphate. This method, however, is very laborious and time-consuming.

The reproducibility of our analytical procedure was tested by determination of MHPG sulphate in ten aliquots of one 24-h urine sample. Determinations were implemented on different occasions and carried out by different laboratory assistants. In the test samples we consistently found a recovery of more than 80%. The mean value obtained was 0.82 mg per g of creatinine with a standard deviation of 0.16. Recovery appears to be "urine-dependent" to a certain extent (age, diet, drugs) and varies between 60 and 95% according to the internal standard.

After adding free MHPG to the sample no free MHPG could be detected in the eluate. The eluate of urine samples was tested for the presence of MHPG glucuronide by incubating with purified β-glucuronidase. No MHPG could be

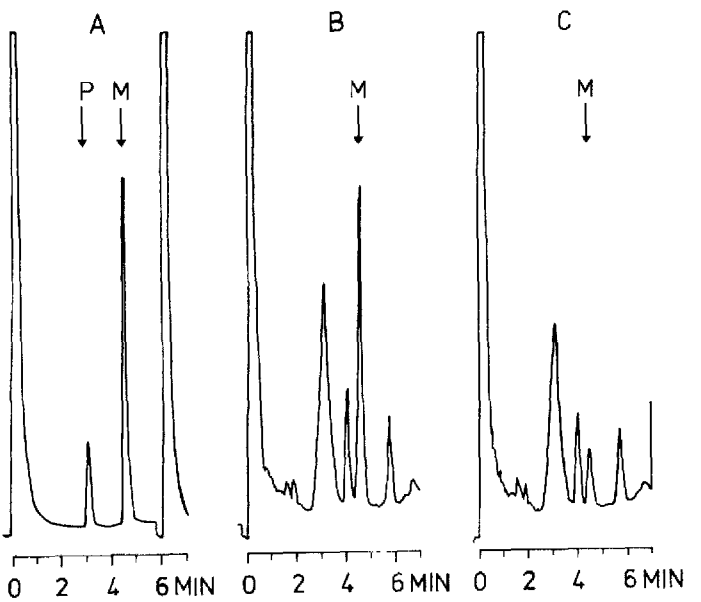

Fig. 1. Chromatograms of (A) reference standard (495 ng), (B) blank urine plus MHPG sulphate standard, serving as a recovery standard (452 ng), and (C) blank urine (77.5 ng) (recovery value 76.4%). P = piperazine (unfluoridated) and M = MHPG (fluoridated).

TABLE I

EXCRETION OF MHPG SULPHATE IN HEALTHY VOLUNTEERS COMPARED WITH VALUES GIVEN BY BOND AND HOWLETT AND MURRAY ET AL.

Values are expressed as mean ± S.D., *n* in parentheses.

	mg MHPG sulphate per g creatinine*	
	Males	Females
Present paper	0.60 ± 0.16 (9)	0.69 ± 0.17 (12)
Bond and Howlett [3]	0.85 ± 0 34 (7)	0.98 ± 0.37 (6)
Murray et al. [5]	0.67 ± 0.15 (7)	0.65 ± 0.21 (3)

*Calculated as free MHPG.

detected in the eluate afterwards. This demonstrates a complete purification of MHPG sulphate by DEAE-Sephadex A-25 in acetate at pH 3.

In the final stage of their analytical procedure Murray et al. [5] claimed "quantitative conversion in a single step" of MHPG sulphate by trifluoroacetic anhydride and ethyl acetate at room temperature to MHPG tris-trifluoroacetic acid. In our replication we found only 25% conversion after 2 h of incubation and 42% after 16 h. When we used HFBA in the same procedure there was almost 60% conversion of MHPG sulphate while fluoridation was complete. Enzymatic hydrolisis of MHPG sulphate appears to be necessary for a high yield. We used an arylsulphatase preparation from Boehringer which did not contain MHPG itself. MHPG sulphate potassium was only poorly soluble in ethylacetate, and we therefore added acetonitrile to increase its solubility and to serve as a catalyst in the fluoridation reaction.

Fig. 1 shows a typical chromatogram. It concerns a depressed male patient without medication. The excretion was 0.61 mg per g of creatinine, and the recovery 76.4%.

In a group of 21 healthy university students using a catecholamine-restricted diet, MHPG sulphate excretion was estimated in 24-h urine samples. In Table I these values are compared to those given by Bond and Howlett [3] and Murray et al. [5] for a normal population. Neither of these latter studies mention dietary conditions. We think ours is an improved method for the estimation of MHPG sulphate in urine.

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