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Note

Rapid determination of urinary oxalic acid by gas—liquid chromatography without extraction

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The existing methods for the gas—liquid chromatographic (GLC) analysis of urinary oxalic acid have until now necessitated separation of oxalic acid prior to derivatization [1]. We have previously developed a method [2] using an ethanol—diethyl ether mixture for extraction, and now describe a new procedure which avoids this time-consuming step and the potential hazard of using diethylether.

EXPERIMENTAL

Reagents

Hydrochloric acid and absolute ethanol (R.P. Normapure) were from Prolabo (Paris, France) and sulphuric acid (1.84 density, Suprapure) was from Merck (Darmstadt, G.F.R.). A stock solution of 140 mg oxalic acid $\cdot 2\ H_2O$ (analytical grade, Prolabo) in 100 ml distilled water was prepared. This corresponds to a 1000 μg/ml solution of anhydrous oxalic acid.

Apparatus

A rotating vacuum evaporator (Büchi Rotavapor), Jouan centrifuge, 3-ml graduated pyrex test-tubes (65 mm, ϕ 10 mm) and ground-glass stoppered pyrex test-tubes (90 mm, ϕ 10 mm) were used.

GLC Specifications

A Hewlett-Packard Model 7610 gas—liquid chromatograph was equipped with a flame ionization detector and a temperature programming device. A glass column (1.50 m × 1/8 in. I.D.) was packed with 15% DEGS on Chromo-

sorb W AW (80—100 mesh) as the stationary phase. Injector temperature was 230°, and the detector temperature 200°. The oven temperature programme was 10 min at 110°, increased by 10°/min to 180°, then kept constant for 10 min.

Procedure

When necessary the volume of the 24-h urine sample is made up to 2 l in order to improve the recovery of the dissolved dry residue (see below). The urine sample is then acidified with 1% HCL and a 30-ml aliquot is evaporated to dryness under reduced pressure at 65° with a rotating vacuum evaporator. The dry residue is dissolved in 1 ml ethanol and the evaporating vessel washed twice with 1 ml ethanol. The three extracts are transferred to a graduated test-tube and the volume adjusted to 3 ml with ethanol. Sulphuric acid (0.1 ml) is added to catalyse the ethylation. The test-tube is centrifuged at 300 *g* and the supernatant immediately transferred to a ground-glass stoppered test-tube and placed in an incubator at 37° overnight.

Two aliquots of 1000 μg (1 ml) and 2000 μg (2 ml) of the stock solution of oxalic acid undergo the same procedure and are used as standards. Three microlitres of each sample are injected into the GLC column for analysis.

RESULTS AND DISCUSSION

Table I compares the data obtained by GLC of urine samples with and without extraction. The results of the two methods are in excellent agreement. The Student's t-test has been performed on paired series. For normal subjects $t = 0.15$; from the Table t is 1.298 for $\alpha = 0.10$ and degree of freedom, d.f. = 66. For subjects with lithiasis $t = 0.10$; from the Table t is 1.301 for $\alpha = 0.10$ and d.f. = 52. Moreover, Fig. 1 shows identical chromatographic profiles for two samples of the same urine, one corresponding to an ethanol—diethyl ether extract, the other to the direct evaporation product.

Recovery of [^{14}C]oxalic acid is 70% with the extraction technique and 90%

TABLE I

GLC OF URINE SAMPLES WITH AND WITHOUT EXTRACTION

	No. of subjects	Oxalic acid (mg/24 h)		Range for both methods (mg/24 h)
		Extraction method	Direct method	
Normal subjects	34	27.14 ± 8.5*	27.44 ± 8.54	10—45
Subjects with lithiasis	27	45.59 ± 20.67	45.04 ± 20.61	10—105

*Mean ± 1σ.

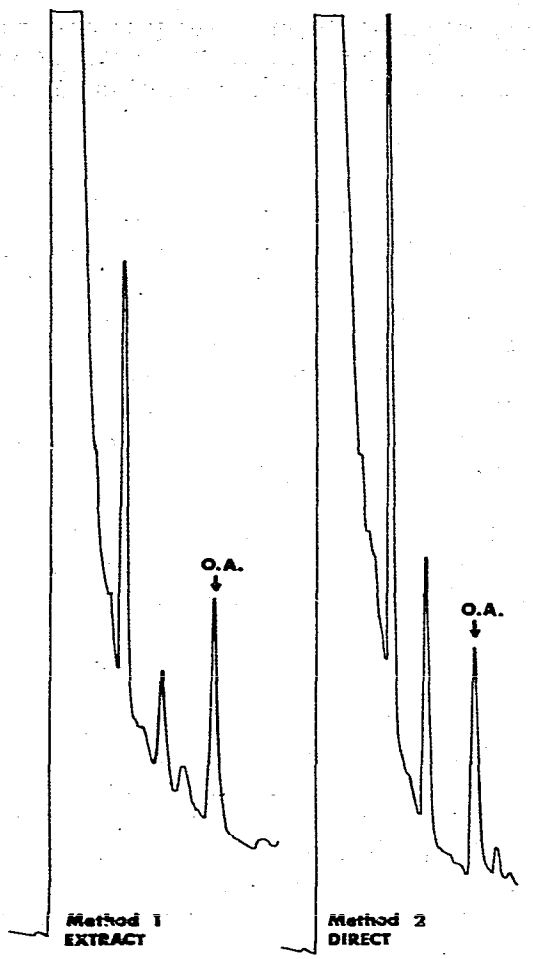

Fig. 1. Oxalic acid (O.A.) obtained from a sample analysed by each method. Injection at 110° on a 15% DEGS column. Retention time, 8 min.

using the direct method. The ethylation rate for both methods, compared with the standard ethyl oxalate, is near 99%.

In order to test the specificity of the direct method we checked the possible interference from another compound at the locus of the peak characteristic of ethyl oxalate. Thirty ml of urine were concentrated to 2 ml and oxalic acid was specifically extracted with 2 ml tributyl phosphate [3]. The remaining aqueous phase underwent total evaporation and ethylation and after injection of the product no peak corresponding to ethyl oxalate could be detected within the limits of sensitivity of the chromatographic technique.

CONCLUSION

The direct determination of oxalic acid on a urine sample by GLC without prior extraction gives results identical to those obtained with the extraction method. Moreover, it has several technical advantages. The extraction rate prob-

lem no longer occurs neither does the usual loss of oxalic acid during the distillation of the ethanol—diethyl ether mixture. The technique is time- and solvent-saving, a factor which has to be taken into account for routine clinical determinations. Safety for routine applications is increased by avoidance of the use of diethyl ether. Finally, a point which should be emphasized: repeated injections of a non-purified urine extract does not shorten the life of the chromatographic column.

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