Creatine was added to an aqueous extract solution prepared from whale meat and its aerobic and anaerobic decompositions were surveyed by using the present technique. The formation of 1-methylhydantoin from creatine was observed in an anaerobic experiment, but that of 3-methylhydantoic acid has not been ascertained yet. A detailed report on this subject will be published elsewhere.

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A rapid chromatographic procedure for the detection of some diuretics in pharmaceuticals and biological fluids

Although a few procedures for the determination of non-mercurial diuretic drugs have been developed by either paper chromatography, UV or visible spectroscopy, most methods lack rapidity, sensitivity and specificity[1–5].

PILSBURY AND JACKSON[2] described a procedure for the extraction and the identification of some thiadiazines in tablets and biological fluids by paper chromatography and UV spectroscopy. They used 1,2-naphthoquinone-4-sulphonate as detecting agent, while NEIDLEIN *et al.*[6] coloured several diuretics with sodium pentacyanoaminoferrate. By using thin-layer chromatography (TLC), DUCHÊNE AND LAPIÈRE[7] identified some diuretics by UV (254 nm).

In order to evaluate a rapid method for the screening of some of these drugs in pharmaceuticals and in urine from patients under medication, we developed a new TLC technique and a new extraction method.

Experimental

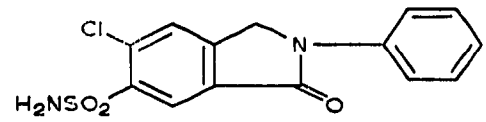
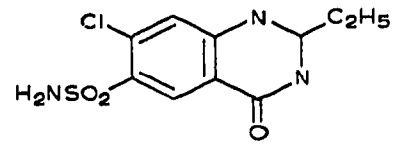
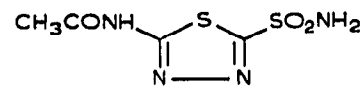
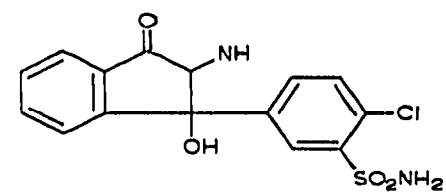
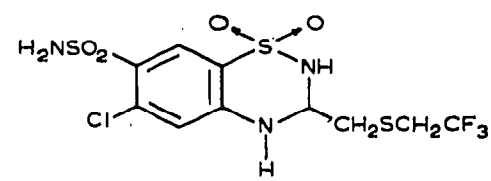
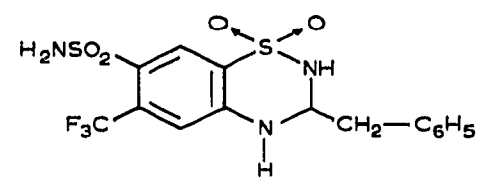
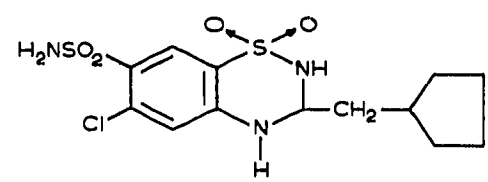
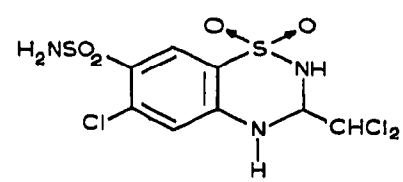
Apparatus and reagents. The following material was used.

(1) Desaga TLC set and chromatoplates.

(2) Precoated plates: Merck Alufolien Silica Gel F_{254}, 20 × 20 cm, layer thickness 0.25 mm; Macherey, Nagel & Co. MN-Polyamide TLC 6 UV_{254}, 20 × 20 cm; Macherey Nagel & Co. MN-Polyamide TLC 11 UV_{254}, 20 × 20 cm.

TABLE I

STRUCTURES AND CHEMICAL NAMES OF DIURETICS

No.	*Name*	*Chemical name*	*Formula*
1	Clorexolone	6-chloro-2-cyclohexyl-3-oxo-5-isoindoline-sulphonamide	
2	Quinethazone	7-chloro-2-ethyl-1,2,3,4-tetrahydro-4-oxo-6-quinazoline-sulphonamide	
3	Acetazolamide	5-acetamido-1,3,4-thiadiazole-2-sulphonamide	
4	Chlorthalidone	2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)-benzenesulphonamide	
5	Epithiazide	6-chloro-3,4-dihydro-3-{[(2,2,2-trifluoroethyl)thio]-methyl}-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide	
6	Bendroflumethiazide	3-benzyl-3,4-dihydro-6-(trifluoromethyl)-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide	
7	Cyclopenthiazide	6-chloro-3-(cyclopentylmethyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide	
8	Trichlormethiazide	6-chloro-3-(dichloromethyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide	

(3) Aluminium Oxide GF_{254} Merck for TLC.

(4) Stock solutions of 1 mg per ml in acetone were made of each studied diuretic (Table I).

(5) NQS reagent[2]: (a) 1 *N* sodium hydroxide; (b) A saturated solution of sodium 1,2-naphthoquinone-4-sulphonate in ethanol–water (1:1). By use, spray (a) followed by (b).

(6) Diazotisation and coupling reagent: After diazotisation in nitrous oxide vapours (obtained by reaction of copper curlings in nitric acid) coupling with 0.5% α-naphthylethylenediamine hydrochloride in ethanol.

(7) Fearons' reagent[6]: (A) 1% of sodium pentacyanoferrate in water; (B) 20% of sodium hydroxide in water. Before use mix 15 ml of (A), 5 ml of (B) and 1 drop of 30% hydrogen peroxide.

Thin-layer chromatography. From each compound we spotted 10 μg on a thin-layer plate. We eluted in saturated chromatotanks at room temperature to give a solvent front rise of 15 cm. As elution mixture, we tried different solvent systems of changing polarity (Table II).

Two-dimensional TLC with a mixture of *n*-hexane–acetone–diethylamine (4:4:2) as first solvent and a less polar solution of chloroform–methanol and diethylamine as second solvent (Table II, F and G of Fig. 1) was most successful. We also tried Aluminium Oxide GF_{254} and polyamide as adsorbents for the thin-layer chromatographic separation of the studied diuretics, but unsuccessfully. After the plates were eluted and dried at 100°, the drugs could be located by UV-light (254 nm) without prior treatment; a detection limit of 2–5 μg was obtained.

TABLE II

R_F VALUES (× 100) OF THE STUDIED DIURETICS ON SILICA GEL PLATES WITH DIFFERENT SOLVENTS

Solvent systems: (A) ethyl acetate–benzene (8:2) (ref. 1); (B) ethyl acetate–benzene–ammonia, 25%–methanol (80:20:1:10);(C) ethyl acetate–benzene–ammonia, 25%–methanol (60:40:2:8); (D) ethyl acetate–benzene–ammonia, 25%–methanol (50:40:2:10); (E) *n*-hexane–acetone–diethylamine (60:30:10); (F) *n*-hexane–acetone–diethylamine (40:40:20); (G) chloroform–methanol–diethylamine (80:15:5).

Diuretic No.	*Solvent systems*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
1				70	21	61	91
2				27	6.5	32	49
3				3	0	2	22
4				30	12	55	58
5	67	70	30	41	5	24	46
6	70	75	40	68	10	46	75
7	78	82	43	62	18	70	76
8	69	43	5	17	1	7	40

As spray reagents three chemicals were tested: sodium 1,2-naphthoquinone-4-sulphonate, α-naphthylethylenediamine and sodium pentacyanoaminoferrate. From our experiments we concluded sodium 1,2-naphthoquinone-4-sulphonate to be the most satisfactory. Stable weak orange spots appear within 15 min and became more

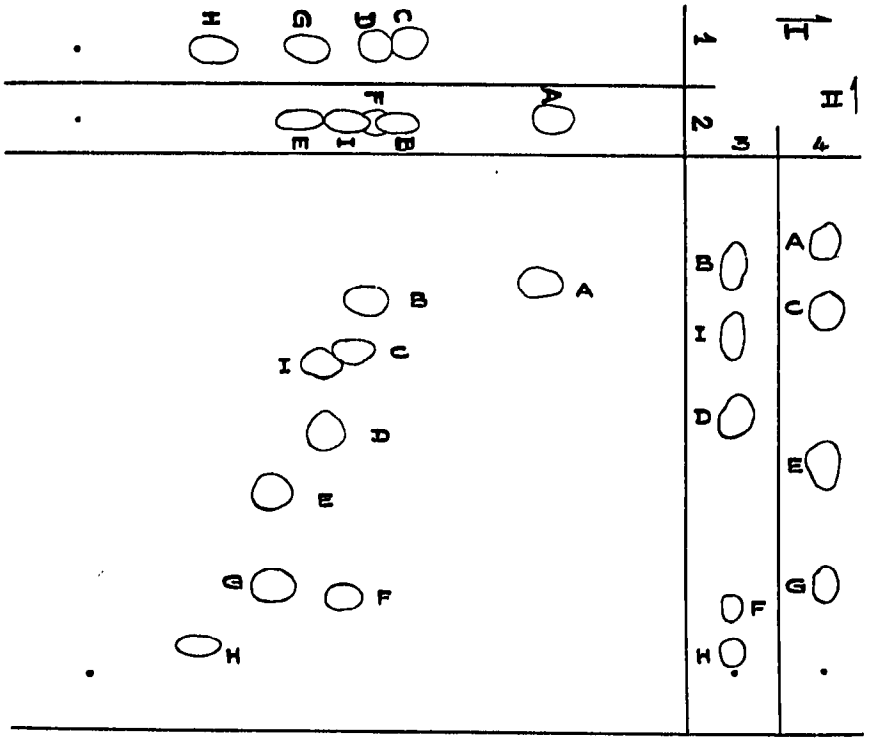


Fig. 1. Two-dimensional chromatography of the studied diuretics. Solvent systems: (I) chloroform–methanol–diethylamine (80:15:5); (II) *n*-hexane–acetone–Diethylamine (40:40:20). A = Clorexolone, B = Cyclopenthiazide, C = Bendroflumethiazide, D = Quinethazone, E = Epithiazide, F = Impurity spot from quinethazone, G = Trichlormethiazide, H = Acetazolamide, I = Chlorthalidone.

pronounced after storing in the dark for one or two days. The sensitivity limit was 5 μg for each drug.

Extraction of the diuretics from tablets or from urine. 30 ml of urine were deproteinised with 5 ml of 10% of lead acetate and acidified with diluted hydrochloric acid (pH 2–3) after which the mixture was filtered through glass-wool.

The tablet was ground in a mortar with 20 ml of distilled water, acidified with diluted hydrochloric acid (pH 2–3) and the mixture was filtered through glass-wool.

The filtrate was extracted with 60 ml of ethyl acetate by shaking in a separatory funnel for 5 min. The aqueous layer was discarded while the ethyl acetate extract was washed twice with 25 ml of a freshly prepared saturated sodium bicarbonate solution. After separation, the ethyl acetate phase was washed twice with 10 ml of sodium hydroxide 0.25 *N*. Separately, the bicarbonate as well as the sodium hydroxide washings were reacidified with concentrated hydrochloric acid and both were extracted again with 30 ml of ethyl acetate. The separated ethyl acetate extracts were dried over anhydrous sodium sulphate and evaporated in a rotavapor at 20°.

The two residues were dissolved in 0.2 ml of acetone and separately spotted on a thin-layer chromatographic Silica Gel GF_{254} plate for identification (Table III).

Conclusions

From the results of the Tables II and III, the TLC determination of the studied diuretics is best performed on Silica Gel GF_{254} plates by two-dimensional chromato-

TABLE III

RESULTS OF THE URINE EXTRACTS AFTER INGESTION AND PASSAGE THROUGH THE BODY OF SOME THIADIAZINES

Diuretic No.	*Oral dose (mg)*	*Time (h) after intake*		
		1	*2*	*3*
5	4	+	+	++
6	3	+	+	++
7	0.5	−	−	+
8	4	+	++	+++

graphy by combination of systems F and G. For general use, the NQS spray reagent is preferred.

Because the extraction procedure of PILSBURY AND JACKSON[2] is rather time consuming, we tried to develop a shorter method. This method provided a good qualitative and semi-quantitative analysis as well as a rapid and sufficient clean-up of urine samples.

This screening may be important to the clinical and toxicological chemist, since some diuretics are frequently used in the treatment of barbiturate poisoning in order to increase their excretion, so they may interfere in the determination of barbiturates and salicylates.

Two different ethyl acetate extracts are observed due to the different acidity of the studied compounds. Therefore, the thiazides were preferentially extracted at higher pH than the sulphonamide derivatives.

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