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Note

Determination of chlorthalidone and its impurities in bulk and in dosage forms by high-performance thin-layer chromatographic densitometry

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Chlorthalidone (I), 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulphonamide, is a diuretic, antihypertensive agent. Chlorthalidone has been quantitated in various media using a variety of methodologies. The major techniques for biological materials involves extractive alkylation and gas chromatography[1–3] or deamination followed by UV spectrophotometric quantitation[4,5]. For the determination of chlorthalidone in pharmaceutical dosage forms, a normal-phase chromatographic method has been reported[6], which separated the hydrolysis product (II), 4′-chloro-3′-sulphamoyl-2-benzophenonecarboxylic acid, on a polyamide column. A reversed-phase liquid chromatographic (LC) system had been used for the determination of I in tablets also containing clonidine hydrochloride[7]. The original USP procedure was a spectrophotometric determination[8], subsequently updated to an LC method[9]. In the Italian Pharmacopeia[10], chlorthalidone is determined by potentiometric titration. A study of the degradation products of chlorthalidone, II and III [2-chloro-5-(1-methoxy-3-oxo-1-isoindolinyl)benzenesulphonamide], was made using an LC system[11] to separate these products from chlorthalidone. A stability-indicating reversed-phase LC method was developed and validated for the assay of I and II in tablet formulation[12].

This paper describes a simple and rapid method for determining the chlorthalidone and its impurities II and III by scanning quenched zones on high-performance silica gel F_{254}.

EXPERIMENTAL

Reagents and materials

Chlorthalidone bulk material and related impurities were kindly supplied by pharmaceutical manufacturers. Pharmaceutical dosage forms were obtained commercially. All reagents used were of analytical-reagent grade.

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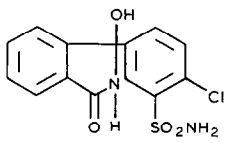
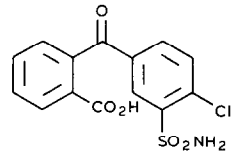


I



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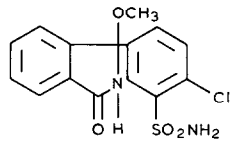

III

Standard and sample solutions were separated on pre-coated silica gel F_{254} high-performance thin-layer chromatographic (HPTLC) plates (Merck, Darmstadt, F.R.G.).

Equipment

Quantitative analysis was performed with Sigma FTR-20 TLC scanner (Biochem) in the reflectance mode using a 5-mm light beam and a shortwave (254 nm) UV source, equipped with a CR-3-A integrator printer–plotter (Shimadzu). Standards and samples were spotted on plates using a Camag Nanomat II device in order to increase the precision and reproducibility of the analysis.

Standards for calibration

The standard solutions for establish calibration graphs for compounds I, II and III were prepared in absolute methanol from concentrated solutions to give the following final concentrations: I, 5, 10, 15, 20 and 25 $\mu g/\mu l$; II, 0.0015, 0.0030, 0.0050, 0.0070 and 0.0090 $\mu g/\mu l$; and III, 0.050, 0.070, 0.090, 0.110 and 0.130 $\mu g/\mu l$.

In addition, five different methanol solutions with the same amount of chlorthalidone and different concentrations of impurities II and III were prepared as standard mixtures.

Sample preparation

Raw materials. Accurately weigh *ca.* 200 mg of chlorthalidone and transfer it into a 100-ml volumetric flask. Dissolve and dilute to volume with methanol.

Tablets. Weigh and finely powder not less than 20 tablets. Accurately weigh a portion of powder, equivalent to *ca.* 200 mg of I, transfer it into a 100-ml volumetric flask, dilute to volume with absolute methanol and mix well in an ultrasonic bath.

High-performance thin-layer chromatography

Aliquots of 1 μl of each standard and sample solution were transferred with the Camag Nanomat II onto HPTLC plates (10 × 20 cm) with alternating depositions of samples, standards and standard mixtures. The solvent used for development was dioxane–isopropyl alcohol–25% ammonia solution–toluene–xylene (30:30:20:10:10). All chromatograms were developed to about 8 cm from baseline (requiring about 10

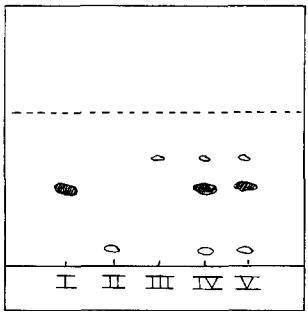

Fig. 1. Chromatogram of I (20 μg/ml), II (0.1 μg/μl), III (0.09 μg/μl), IV (standard mixture) and V (tablet extract).

min) in a chromatographic tank, previously saturated with eluent mixture, at room temperature. The layers were dried with a forced current of cool air from a hair dryer and measured at 254 nm.

The analysis conditions were as follows: width, 5 mm; attenuation, 5; slit, 5.8 × 0.4 mm. The scanning was performed at 40 mm/min with a time constant of 200 ms. The areas of peaks I, II and III were measured directly by the integrator printer–plotter. The amounts of chlorthalidone and its impurities in a spotted sample were calculated using the equation

$$\frac{\text{average area of standard peaks}}{\mu\text{g of standard spotted}} = \frac{\text{average area of sample peaks}}{\mu\text{g of I (or II or III) in a 1-}\mu\text{l aliquot}}$$

RESULTS AND DISCUSSION

Fig. 1 shows a representative chromatogram for a single standard, standard mixture and tablet extract. The R_F values allow quantitative densitometric analysis (Fig. 2). Fig. 3 shows the linearity for standard solutions that was established by spotting different concentrations. The linearity correlation coefficient was between 0.97 and 0.98 for II and III but always above 0.99 for I.

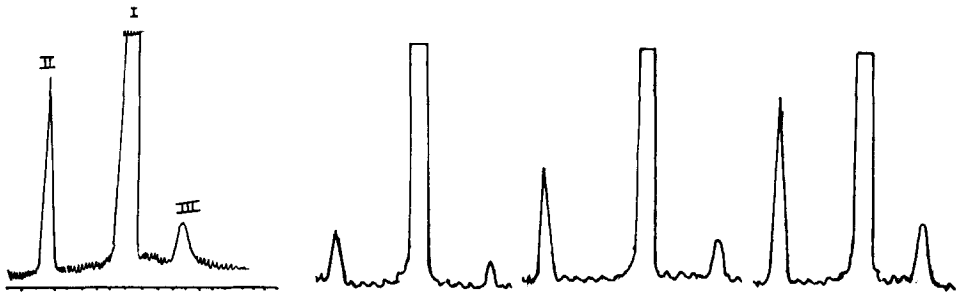

Fig. 2. Scanning of a chromatogram with I (20 μg/μl), II (0.1 μg/μl) and III (0.09 μg/μl).

Fig. 3. Linearity for standard solutions.

TABLE I

RECOVERY OF CHLORTHALIDONE AND IMPURITIES II AND III FROM STANDARD MIXTURES

a = Added; f = found; R = recovery.

I			*II*			*III*		
a (mg/ml)	*f (mg/ml)*	*R (%)*	*a (mg/ml)*	*f (mg/ml)*	*R (%)*	*a (mg/ml)*	*f (mg/ml)*	*R (%)*
5	4.97	99.4	0.0015	0.0012	–	0.050	0.051	102.0
10	9.87	98.7	0.0030	0.0028	93.3	0.070	0.069	98.6
15	15.10	100.7	0.0050	0.0047	94.0	0.090	0.090	100.0
20	19.91	99.6	0.0070	0.0071	101.4	0.110	0.106	96.4
25	25.12	100.5	0.0090	0.0089	98.9	0.130	0.129	99.2
		S.D. 0.82			S.D. 3.9			S.D. 2.04

TABLE II

DETERMINATION OF I, II AND III IN BULK MATERIAL AND TABLETS

Sample No.	*Bulk materials*			*Tablets*		
	I	*II*	*III*	*I*	*II*	*III*
1	98.7	0.030	0.11	99.1	–	0.13
2	98.9	0.027	0.09	97.9	0.040	0.10
3	98.0	0.032	0.12	98.4	0.035	0.097

The recovery of chlorthalidone and the two impurities from artificial mixtures prepared in order to validate the method are summarized in Table I. The results obtained from the analysis of samples are reported in Table II.

A study was made of the reproducibility of the HPTLC procedure by spotting eight times the same amounts and volumes of I, II and III standards on a single plate. The zones were scanned after development and the relative standard deviation of the peak areas was 1.9%.

The proposed quantitative HPTLC method proved to be accurate, reproducible and selective for the determination of chlorthalidone and its two impurities. Impurity II below 0.0015 μg is not observed, but the densitometric analysis of this concentration is possible. The ability to spot multiple samples together with a standard of each product and standard mixtures of three compounds on the same plate allows a high sample throughput to be achieved.

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