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Note

Determination of reserpine in pharmaceutical formulations by gas–liquid chromatography

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The alkaloid reserpine, a constituent of *Rauwolfia serpentina* L. roots, is the active component of in a commonly used hypotensive medication.

There are many methods for the qualitative and quantitative determination of reserpine: spectrophotometric, UV and visible[1,2], colorimetric[3,4], fluorimetric[5], chromatographic[6], potentiometric[7] and hydrolysis followed by spectrophotometric determination of the resulting trimethoxybenzoic acid[8,9]. Settimj *et al.*[10] also mentioned a gas–liquid chromatographic (GLC) method based on alkaline hydrolysis followed by esterification with diazomethane of the resulting trimethoxybenzoic acid.

This paper describes an easy GLC method for the estimation of reserpine in pharmaceutical formulations.

EXPERIMENTAL

Materials and apparatus

All solvents were of analytical grade (Prolabo; Rhone-Poulenc, France). Reserpine reference standard was obtained from USP Laboratories for Standards (Rockville, MD, U.S.A.).

The GLC was performed with a Model 5720 A chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) fitted with a flame ionization detector. The column was a glass tube (182 cm × 0.4 mm I.D.) packed with 3% OV-101 on Gas-Chrom Q (100–120 mesh). Flow-rates: carrier gas (nitrogen), 20 ml/min: flame gases, hydrogen and dry air, 30 and 300 ml/min respectively. Temperatures: column, 180°C; injector and detector, 230°C. Sample volume: 2 μl injected with a Pierce liquid syringe. Recorder chart speed: 10 mm/min.

Pharmaceutical preparations

Simple preparations comprised 1-mg and 2.5-mg ampoules of serpasil (Ciba), 0.1-mg and 0.25-mg tablets of serpasil (Swiss-Pharma) and 0.25-mg tablets of ravoline (Memphis) (all containing reserpine besides excipients).

Compound preparations were as follows: Brinerdin tablets (Swiss-Pharma), containing reserpine (0.1 mg), clopamide (5 mg) and dihydroergocristine (0.5 mg); Adelphan tablets (Ciba), containing reserpine (0.1 mg) and dihydralazine (10 mg);

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Adelphan esidrex tablets (Ciba), containing reserpine (0.1 mg), hydralazine sulphate (10 mg) and hydrochlorothiazide (10 mg); Bendigon capsules (Bayer), containing reserpine (0.15 mg), baycaron (mefrusid) (15 mg) and *meso*-inostol hexanicotinate (150 mg).

Methelute

This methylating reagent comprised 0.2 *M* trimethylanilinium hydroxide in methanol (Pierce, Rockford, IL, U.S.A.).

GLC

Reserpine. About 1.2 g of reserpine were accurately weighed and then dissolved in enough chloroform–methanol (2:18) to give an exact concentration of 0.12 mg/ml. This stock solution was then diluted in the solvent to give 10, 20, up to 200 μg/2 μl in separate amber vials. 0.5 ml of Methelute were added to each vial, the contents shaken vigorously for 1 min and set aside for 10 min to attain complete *trans*-methylation. A 0.5-ml volume of methyl stearate (0.1% in dichloromethane) solution as a standard was added. Volumes corresponding to amounts of reserpine between 10 and 250 μg were injected. The magnitude of the response (peak height) at each concentration of the injected sample was studied to assess linearity (Fig. 1).

Accompanying compounds. Ten milligrams each of clopamide, baycaron (mefrusid), *meso*-inostolhexanicotinate, dihydroergocristine, hydralazine and hydrochlorothiazide (accompanying reserpine in formulations) were dissolved in 10 ml methanol in an amber vial, 2.5 ml of Methelute were added and set aside for 10 min.

A 1-ml volume of methylated reserpine (containing 0.10 mg reserpine) was mixed with 1 ml of the above methylated mixture and 0.5 ml of methyl stearate (0.1% in dichloromethane) solution as standard were added. The resulting solution was mixed and a 2-μl aliquot was chromatographed.

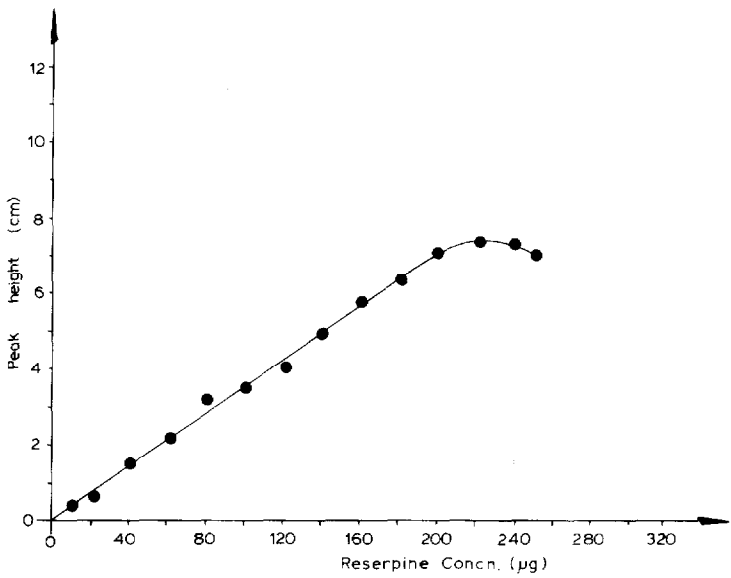


Fig. 1. Linearity of reserpine response.

TABLE I

DETERMINATION OF RESERPINE IN PHARMACEUTICAL PREPARATIONS

Sample	*Reserpine content (μg)*		*Determined amount (μg)**		
	Labelled	*Added*	*Before addition*	*After addition*	*Recovery*** *(%)*
Serpasil (0.1-mg tabl.)	100	200	98.2 ± 0.2	300 ± 0.2	100.0
Serpasil (0.25-mg tabl.)	250	200	251.6 ± 0.4	452.6 ± 0.4	100.6
Ravoline (0.2-mg tabl.)	250	200	250.4 ± 0.7	450.8 ± 0.3	100.2
Brinerdin tabl.	100	200	103.1 ± 0.5	299.1 ± 0.2	99.7
Adelphan tabl.	100	200	96.9 ± 0.3	298.7 ± 0.3	99.6
Adelphan esidrex tabl.	100	200	101.3 ± 0.4	301.6 ± 0.3	100.5
Bendigon capsule	150	200	152.1 ± 0.3	350.9 ± 0.2	100.3
Serpasil (1-mg ampl.)	1000	500	997.2 ± 0.4	1506.2 ± 0.3	100.4
Serpasil (2.5-mg ampl.)	2500	1000	2503.2 ± 0.3	3504.3 ± 0.3	100.1

* Mean of five determinations, ± S.D.
** After addition of standard.

Extraction of formulations

Ampoules. The contents of one ampoule were diluted in 10 ml of distilled water, made basic with 2 ml of a dilute solution of ammonium hydroxide (10%, v/v), extracted with chloroform (3 × 10 ml) and filtered over anhydrous sodium sulphate. The combined extracts were evaporated at 50°C under reduced pressure and the residue was dissolved in 2 ml chloroform–methanol (2:18). A 0.5-ml volume of methelute was added and on aliquot chromatographed as above.

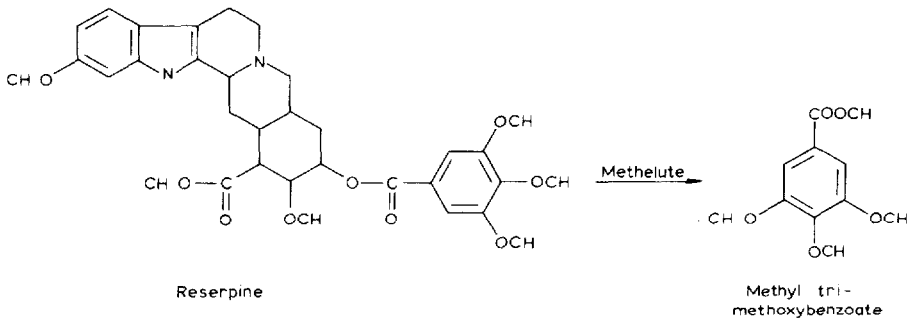
Tablets. Two tablets were finely powdered, 10 ml of distilled water were added, followed by 2 ml of 10% (v/v) ammonium hydroxide. The procedure was then as described under *Ampoules.*

Capsules. The contents of two capsules were carefully transferred to a beaker, 10 ml distilled water were added and then treated as described for *Ampoules.*

RESULTS AND DISCUSSION

Reserpine was rapidly and satisfactorily determined in various pharmaceutical preparations using the described GLC procedure. Pharmaceutical preparations were repeatedly analysed and known amounts of standard reserpine were added in order to evaluate the percentage recovery (Tables I, II). The reaction depends on the transesterification of the trimethoxybenzoic acid moiety in reserpine by Methelute.

Different parameters of the methylation process were studied, including the Methelute volume and methylation time. Volumes of Methelute ranging between 0.1 and 1 ml were added to 200 μg reserpine solution; 0.5 ml was found to be sufficient for complete methylation. When the methylation of reserpine was repeated using 0.5 ml Methelute, set aside for 10, 20 and 30 min, the same results were obtained. This indicates that complete methylation occurs upon injection. The retention times of the separated components were 0.7, 1.3, 2.6, 3.0, 3.7, 5.4, 6.7 and 7.7 min for *meso*-inostol hexanicotinate, reserpine, hydrochlorothiazide, baycaron, dihydroergocristine, hydralazine, clopamide and methyl stearate respectively; *i.e.*, these compounds



Reserpine

Methyl tri-methoxybenzoate

do not interfere with the GLC determination of reserpine (Fig. 2). The response of reserpine was found to be linear over the range 10–200 μg (Fig. 1).

The recent pharmacopoeial assay method for reserpine, depending on colorimetric determination at 390 nm, is tedious and lengthy because reserpine solution is very sensitive to light[12] and the accompanying substances in the formulation may interfere, giving false results. Our GLC method saves much time and eliminates other tedious steps. It is preferred to that of Settimj *et al.*[10] which is more lengthy and involves refluxing reserpine with sodium hydroxide in absolute ethanol for 90 min, cooling the hydrolysate to 0°C and methylating with diazomethane for another 25 min at 0°C.

The presence of any degradation products of reserpine (mainly oxidation products) or adulterants, mainly trimethoxybenzoate, can be detected and eliminated by thin-layer chromatography (TLC) using *n*-butanol–methyl ethyl ketone–water (65:25:25) as a developing system[13].

A further advantage of our method is that rescinnamine and other similar

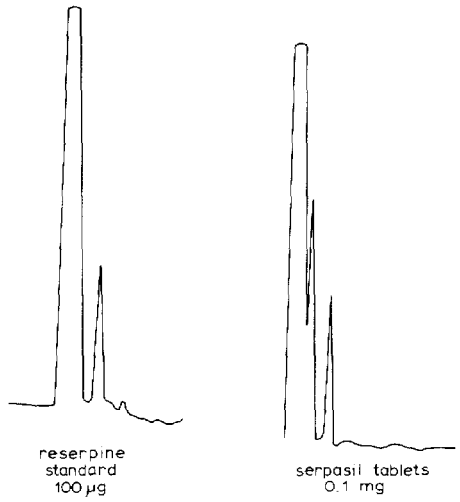


Fig. 2. Chromatograms of reserpine standard and serpasil tablets (0.1 mg).

TABLE II

DETERMINATION OF RESERPINE IN PHARMACEUTICAL PREPARATIONS BY THE PROPOSED GLC METHOD AND THE USP METHOD

Sample	*Reserpine content (mg)*		*Recovery (%)*	
	Labelled	*Added*	*GLC method*	*USP method*
Serpasil (0.1-mg tabl.)	100	200	100.0	99.5
Serpasil (0.25-mg tabl.)	250	200	100.6	100.1
Ravoline (0.2-mg tabl.)	250	200	100.2	98.9
Brinerdin tabl.	100	200	99.7	99.8
Adelphan tabl.	100	200	99.6	99.0
Adelphan esidrex tabl.	100	200	100.5	100.6
Bendigon capsule	150	200	100.3	98.5
Serpasil (1-mg ampl.)	1000	500	100.4	99.4
Serpasil (2.5-mg ampl.)	2500	1000	100.1	98.9
Mean recovery ($P = 0.05$)			100.16 ± 0.37	99.40 ± 0.63
Variance			0.137	0.397
$t_{0.975}$				3.09 (2.12)
F				2.89 (3.44)

alkaloids (sometimes present with reserpine) will not react with the Methelute reagent and thus can be separated.

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