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QUANTITATIVE GAS CHROMATOGRAPHIC DETERMINATION OF AZAPETINE AND PHENOXYBENZAMINE IN PHARMACEUTICAL PREPARATIONS*

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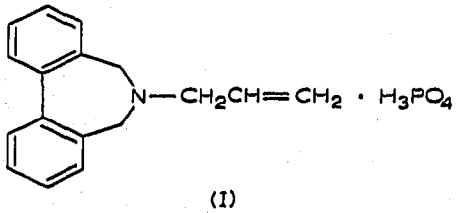
SUMMARY

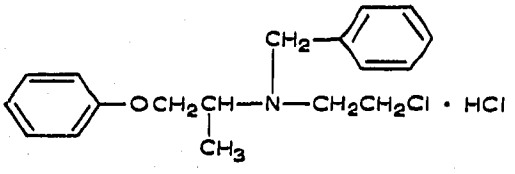
A method is described for the gas chromatographic separation and quantitative determination of azapetine phosphate and phenoxybenzamine hydrochloride in pharmaceutical products in which they may occur separately, together, or in combination with other substances. The stationary phase used was Craig polyester succinate, the internal standard was methyl stearate.

INTRODUCTION

Azapetine (6-allyl-6,7-dihydro-5H-dibenz[*c*,*e*]azepine) and phenoxybenzamine (N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)-benzylamine) are used as vasodilators of the peripheral arteries, the first generally in the form of the phosphate (I) and the second in the form of the hydrochloride (II). Pharmaceutical preparations may contain them singly, together, or in combination with other drugs.



(I)



(II)

A recent publication[1] dealt with the determination of azapetine by spectrophotometry, titration in a non-aqueous medium, and thin-layer chromatography on silica gel. However, the literature contains no specific information on the determination of phenoxybenzamine. It has now been found that gas chromatography permits the rapid determination of these two compounds singly or in mixtures.

Unlike azapetine, it is rather difficult to find the best conditions for the determination of phenoxybenzamine. Notably, the hydrochloride in methanol gives a

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series of peaks due to degradation products, while the amine itself is not sufficiently volatile and gives irreproducible chromatograms. This problem has been overcome by treating the hydrochloride with hot methanolic KOH, whereupon the following reaction takes place:

$$R_1\text{–}\underset{\displaystyle R_2}{\overset{\displaystyle |}{N}}\text{–}CH_2CH_2Cl + CH_3O^- \longrightarrow R_1\text{–}\underset{\displaystyle R_2}{\overset{\displaystyle |}{N}}\text{–}CH_2CH_2\text{–}O\text{–}CH_3 + Cl^- \qquad (1)$$

which has been confirmed by the I.R. spectrum of the methyl ether formed. The latter is sufficiently volatile and stable, and gives rise to a symmetrical and reproducible GLC peak.

EXPERIMENTAL

Apparatus

The Perkin-Elmer 881 chromatograph used was equipped with a double column and a flame ionization detector. The stainless steel columns (length, 46 cm; internal diameter, 0.3 mm) were packed with 80–100 mesh Chromosorb W impregnated with 20 % of Craig polyester succinate (Wilkens) as the stationary phase.

A Leeds-Northrup Speedomax W recorder was used at a full-scale deflection of 2.5 mV, the chart speed being about 5 cm/min. The samples (0.1–0.5 μl) were injected with a 1 μl Hamilton microsyringe.

The column, closed at one end with a suitable metal disk, was attached to a vibrator made in our laboratory. The packing was then introduced with constant shaking before the other end was closed with a similar disk. This part of the column was to be placed in the outlet of the chromatograph. This was repeated with a second column. Both were connected to the glass injection part of the chromatograph, but not yet to the detector, The columns were conditioned by heating at 220° for 12 h in a current of nitrogen (40–50 ml/min) and then for 3–4 h at the same temperature but without the nitrogen, and finally at 185° in a current of nitrogen. The detector was then connected. The internal standard, methyl stearate in petroleum ether, was found to traverse the apparatus in 2–3 min under the conditions used, namely column temperature 185°, temperature of vaporizer 230°, detector temperature 200° and flow rate of carrier nitrogen 30 ml/min.

Quantitative analysis

Principle of the method. Mixtures of known amounts of compounds I, II, and the reference methyl stearate (Q_I, Q_{II}, and Q_{Ref}, in mg) were repeatedly chromatographed, the peak areas (A_I, A_{II}, and A_{Ref}) were measured, and the unit area corresponding to 1 mg of a substance was calculated for all three compounds [A_I/Q_I ($= A_I^u$); A_{II}/Q_{II} ($= A_{II}^u$); and A_{Ref}/Q_{Ref} ($= A_{Ref}^u$)]. This was followed by the calculation of the ratios A_I^u/A_{Ref}^u ($= f_I$) and A_{II}^u/A_{Ref}^u ($= f_{II}$). The mean values of these f-values for a number of runs (*i.e.* f_I and f_{II}) were then used as calibration factors. The amounts of azapetine and phenoxybenzamine could then be determined by chromatographing them with a known amount of the internal standard, measuring the peak areas, and using the calibration factors.

Preparation of the calibrating mixtures. Each of five calibrating mixtures was

prepared by weighing out accurately about 150 mg of pure azapetine phosphate and about 100 mg of pure phenoxybenzamine hydrochloride. These were placed in a 100 ml ground glass flask, 15 ml of 99.9 % pure methanol were added, and the solution was refluxed for 5–6 min. Then 20 ml of a freshly prepared methanolic solution saturated with KOH in the cold were introduced, and the solution was refluxed for 1 h. The solution was cooled, transferred with the addition of water into a 250 ml separating funnel, and extracted three times with 80, 40 and 20 ml portions of diethyl ether. The extracts were combined, and an accurately weighed-out portion (*ca.* 50 mg) of methyl stearate was added as a reference substance. The solution was shaken with three 15 ml portions of water, and the aqueous layer was discarded on each occasion. The ethereal solution was dried over anhydrous sodium sulphate, filtered through paper into a 250 ml round-bottom flask, reduced in volume to 1–2 ml, and 0.5 ml of toluene added. The sample, which was now ready to be chromatographed, contained methyl stearate, azapetine base, and the methyl ether of phenoxybenzamine formed according to reaction 1.

The resulting chromatogram (see Fig. 1), obtained under the conditions mentioned above, exhibits perfectly symmetric peaks appearing in the following order: (a) solvent, (b) methyl stearate, (c) azapetine, and (d) phenoxybenzamine. Each of the five calibrating samples was chromatographed three times.

Calculation of the calibration factors. The peak area was calculated in mm^2 as height times width at half-height on all 15 charts. The factors f_I and f_{II} were calculated on each chart, and the mean values f_I and f_{II} were found by averaging over

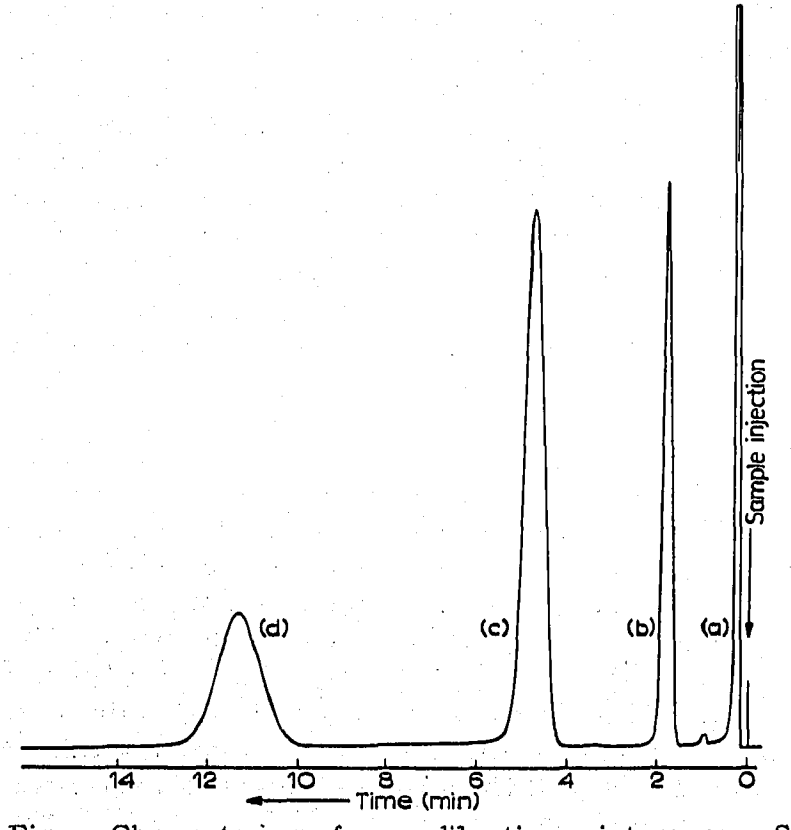

Fig. 1. Chromatogram for a calibrating mixture. a = Solvent; b = methyl stearate; c = azapetine; d = phenoxybenzamine (methyl ether).

TABLE I

EXAMPLE OF THE DETERMINATION OF THE CALIBRATION FACTORS

	Q_I (mg)	Q_{II} (mg)	Q_{Ref} (mg)	A_I (mm^2)	A_{II} (mm^2)	A_{Ref} (mm^2)	f_I	f_{II}
1	152.15	101.15	50.70	307.85	189.00	110.00	0.860	0.805
2				370.70	228.20	142.50	0.867	0.803
3				393.97	243.30	152.30	0.862	0.801
4	150.70	100.30	50.05	878.90	567.00	393.00	0.802	0.794
5				885.50	542.70	364.00	0.808	0.744
6				726.13	477.90	298.10	0.809	0.800
7	150.30	100.90	50.50	922.00	603.00	370.50	0.836	0.810
8				850.11	547.40	342.90	0.833	0.799
9				465.08	293.70	188.50	0.829	0.780
10	150.55	100.50	49.80	285.25	188.65	111.00	0.850	0.842
11				326.25	205.66	125.20	0.862	0.814
12				707.37	465.67	270.20	0.866	0.854
13	150.18	101.66	50.20	370.00	247.95	151.50	0.817	0.809
14				476.46	296.10	194.70	0.818	0.751
15				492.61	322.86	201.30	0.818	0.792
Mean values (f_I and f_{II})							0.836	0.800
Standard deviation							0.023	0.089

TABLE II

Preparation	*Amount of azapetine phosphate per tablet (mg)*		*Amount of phenoxybenzamine hydrochloride per tablet (mg)*	
	Found	*Deviation %*	*Found*	*Deviation %*
Azapetine phosphate tablets (35.4 mg per tablet = 25 mg of pure base per tablet)	34.2	−3.4	—	—
	34.1	−3.6	—	—
	35.5	+0.4	—	—
Phenoxybenzamine hydrochloride[a] capsules, each containing 10 mg	—	—	10.1	+1.0
	—	—	10.2	+2.0
	—	—	10.5	+5.0
Vadigen Gentili[b]	15.4	+2.6	10.3	+3.0
	15.7	+4.6	10.1	+1.0
	15.1	+0.7	10.4	+4.0
	15.6	+4.0	9.8	−2.0
	14.8	−1.3	10.2	+2.0
	15.2	+1.3	9.9	−1.0

[a] Phenoxybenzamine hydrochloride capsules are not produced in Italy.

[b] Each tablet of Vadigen Gentili contained 15 mg of azapetine phosphate, 10 mg of phenoxybenzamine hydrochloride, 50 mg of cyclandelate, and 1 mg of ajmalicine.

all the runs. The more numerous the latter, the better the resulting calibration factors. These must, of course, be determined experimentally whenever the conditions are changed, *e.g.* using a new column or a new batch of methyl stearate. An example of the calculation is shown in Table I.

Analysis of pharmaceuticals. A known amount (P) of a pharmaceutical containing azapetine and/or phenoxybenzamine was treated, if necessary after grinding *e.g.* a tablet of mean weight M, in exactly the same way as the calibrating mixtures, and at least three chromatograms were recorded for each sample. The amount of azapetine phosphate and phenoxybenzamine hydrochloride (mg) present in one tablet was then calculated with the aid of the formulae:

$$\frac{A_{\mathrm{I}} \cdot M \cdot Q_{\mathrm{Ref}}}{f_{\mathrm{I}} \cdot A_{\mathrm{Ref}} \cdot P} \text{ and } \frac{A_{\mathrm{II}} \cdot M \cdot Q_{\mathrm{Ref}}}{f_{\mathrm{II}} \cdot A_{\mathrm{Ref}} \cdot P}$$

where A_{I} is the area of the azapetine peak on a given trace, A_{II} is the area of the phenoxybenzamine peak, A_{Ref} is the area of methyl stearate peak and Q_{Ref} is the amount of methyl stearate introduced (in mg). The resulting values were then averaged over equivalent chromatograms, and some of the data are listed in Table II.

DISCUSSION

The results indicate that the method is suitable for the quantitative determination of azapetine and phenoxybenzamine in drugs, whether they are present in these singly or in mixtures. As in all such work, care is needed in connection with the preparation of the columns, avoiding saturation of these with the compounds to be analysed during the runs, and with the necessity of frequently checking the efficiency of the columns. When care is taken, the results are reproducible with an error of not more than $\pm$ 5 %.

REFERENCES

1 S. SILVESTRI, *Farmaco (Pavia), Ed. Prat.*, 23 (1968) 84.