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GAS-CHROMATOGRAPHIC DETERMINATION OF CAMYLOFINE DIHYDROCHLORIDE IN TABLETS AND SUPPOSITORIES

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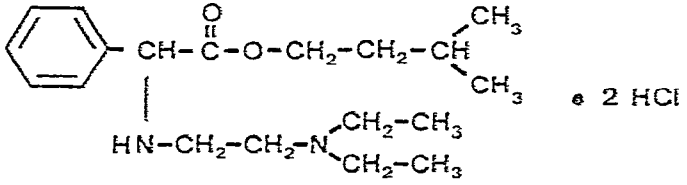
SUMMARY

A gas-chromatographic method for the quantitative determination of camylofine dihydrochloride, a spasmolytic agent, is described. The analysis is made on a porous polymer packing material, by determining the 3-methyl-1-butanol formed on alkaline hydrolysis of the drug. The method has been applied to the quantitative determination of the drug in two galenical forms, namely tablets and suppositories, in the presence of papaverine hydrochloride, codeine phosphate, novalgin and aminopyrine.

INTRODUCTION

Camylofine dihydrochloride or isopentyl N-[2-(diethylamino)ethyl]-2-phenylglycinate dihydrochloride (other names: avacan and avasina) belongs to the group of spasmolytic agents[1]. In galenical dosage forms it is often combined with other spasmolytics and analgesics like papaverine hydrochloride, codeine phosphate, novalgin and aminopyrine.



Although all of the dosage methods, described up to now, can be used for the quality control of the pure drug, they are not always suitable for its quantitative determination in a pharmaceutical preparation, due to the lack of specificity. Gravimetric determination after precipitation as silicotungstate[2] cannot be used in the presence of drugs with basic characteristics like papaverine and codeine. This method is also not suitable for small amounts because of the lack of sensitivity. Volumetric determination of the chloride content[2] or a more sensitive colorimetric chloride quantitation are also

excluded in the presence of other hydrochlorides. Moreover, this method lacks the necessary specificity for the quality control of a drug. Colorimetric determination using acid–base indicators, such as methyl orange[3,4], is based on the presence of a basic function. Although very sensitive, this method requires a preliminary separation from other interfering substances. Camylofine dihydrochloride can be separated from papaverine hydrochloride, codeine phosphate, novalgin and aminopyrine by thin-layer chromatography (TLC) on silica gel F 254. However, the results of the quantitative determination with methyl orange, after TLC separation and extraction of the drug from the plate, were not satisfactory. This may be due to poor detection of the drug on the thin-layer plate by UV light at 254 nm, as a result of the very low absorption of camylofine dihydrochloride in the UV region ($E^{1\%}_{1cm} = 6.47$ at 257 nm)[5].

The ester structure of camylofine prompted us to attempt a gas-chromatographic (GC) determination of 3-methyl-1-butanol obtained on hydrolysis of the previously isolated spasmolytic agent. *n*-Butanol appeared to be suitable as the internal standard. The alcohols 3-methyl-1-butanol and *n*-butanol were separated on Porapak Q, a porous polymer stationary phase which is very well suited to the separation of polar compounds such as alcohols[6–8]. Porapak Q has the advantage that it excludes phenomena such as "column-bleed".

EXPERIMENTAL

Apparatus and operating conditions for the chromatographic separation

A Packard series 7400 all-glass gas chromatograph with a flame-ionization detector (FID) was used. Nitrogen was used as the carrier gas (flow-rate, 30 ml/min). The hydrogen and air flow-rates were 40 and 400 ml/min, respectively. The air was dried over silica gel and filtered over molecular sieves. Other conditions were: inlet temperature, 200°; oven temperature, 180°: and detector temperature, 210°. The glass-spiral column (5 ft. × 4 mm I.D.) was filled with Porapak Q, a porous polymer packing material (Waters Ass., Milford, Mass., U.S.A.). The peak areas were integrated with a disc integrator.

The reagents used were 3-methyl-1-butanol (p.a.), *n*-butanol (p.a.), chloroform (p.a.), methanol (p.a.), light petroleum (p.a.) (b.p. 30–70°) and sodium hydroxide (p.a.).

Procedure

Extraction of camylofine dihydrochloride from tablets. A quantity of ground and homogenized tablet powder, corresponding to 50 mg of camylofine dihydrochloride, was extracted with *ca.* 50 ml of chloroform. The resulting suspension was stirred for 15 min and filtered on a sintered glass filter (G 3). The residue on the filter was washed several times with small amounts of chloroform. The collected chloroform fractions were evaporated *in vacuo*. A few millilitres of methanol were then added to the residue and the solution was evaporated to dryness *in vacuo*. The extraction may also be made with smaller quantities of tablet powder, corresponding to 10 mg of camylofine dihydrochloride. In this case, the extract was dissolved in 1 ml of the hydrolysis mixture and hydrolyzed on a microscale. A quantitation for unit dose is possible.

Extraction of camylofine dihydrochloride from suppositories (lipophilic base). A quantity of homogenized suppository mass, corresponding to 50 mg of camylofine dihydrochloride, was treated with light petroleum and stirred until the lipophilic mass dissolved. The drug is practically insoluble in light petroleum. The suspension obtained was filtered on a sintered glass filter (G 3) and washed several times with light petroleum until the lipophilic mass was completely removed. The powder retained on the filter was moistened twice with 25 ml of chloroform and stirred (manually) in order to dissolve the drug. After filtering, the residue was washed several times with small amounts of chloroform. The chloroform solution was evaporated *in vacuo* and treated as described above for tablets.

Hydrolysis. 2.5 ml of 1 *N* sodium hydroxide solution and 2.50 ml of methanol, containing a known quantity of *n*-butanol (300 mg of *n*-butanol in 100.0 ml of methanol), were added to the extract obtained as described above. The mixture was heated to boiling on a hot plate, under reflux, for 1 h. After cooling, the hydrolyzate was centrifuged at 1300 *g* for 30 min in order to obtain a clear solution, ready to be used in the chromatographic analysis.

Chromatography. 3 μl of the clear solution, obtained on hydrolysis, were used for the chromatographic analysis. The composition of the solutions used for the calibration graph is given in Table I. The solvent mixture consisted of equal volumes of water and methanol.

TABLE I

COMPOSITION OF SOLUTIONS USED FOR CONSTRUCTION OF THE CALIBRATION GRAPH

Amount of 3-methyl-1-butanol (mg)	*Amount of n-butanol (mg)*	*Volume of solvent (ml)* *
120	125	100
170	125	100
220	125	100
270	125	100

* Water–methanol (1:1).

RESULTS AND DISCUSSION

The efficiency of the hydrolysis and the chromatographic procedure was tested, not only on a known quantity of pure camylofine dihydrochloride, but also on a powder mixture of the same composition as the tablets. The results given in Table II confirm that the hydrolysis is complete and the extraction from the powder is also quantitative. The analysis was also carried out on two galenical dosage forms, having the following composition: novalgin (100 mg), papaverine hydrochloride (50 mg), codeine phosphate (10 mg), aminopyrine (300 mg) and camylofine dihydrochloride (10 mg) (average tablet weight, 625 mg); novalgin (300 mg), papaverine hydrochloride (80 mg), codeine phosphate (40 mg), aminopyrine (500 mg) and camylofine dihydrochloride (40 mg) (average suppository weight, 3.75 g). The results are given in Table III.

TABLE II

GC ESTIMATION OF 3-METHYL-1-BUTANOL FORMED AFTER EXTRACTION AND HYDROLYSIS OF CAMYLOFINE DIHYDROCHLORIDE

Amount of drug (mg)	*Amount of 3-methyl-1-butanol (mg)*		*Recovery (%)*
	Theoretical	*Experimental*	
106.7*	23.87	24.65	103.3
92.8*	20.76	21.06	101.4
101.9*	22.80	23.41	102.7
48.9**	10.93	10.63	97.3
50.3**	11.24	11.17	99.4
51.7**	11.57	11.70	101.1

* Pure camylofine dihydrochloride.

** A powder mixture of the following composition: camylofine dihydrochloride (402.4 mg); codeine phosphate (406.5 mg); papaverine hydrochloride (804.8 mg); novalgin (2.992 g) and aminopyrine (5.007 g) (total, 9.613 g).

TABLE III

GC ESTIMATION OF 3-METHYL-1-BUTANOL FORMED ON EXTRACTION AND HYDROLYSIS OF CAMYLOFINE DIHYDROCHLORIDE IN TABLETS AND SUPPOSITORIES

Galenical form	*Amount (g)*	*Amount of drug (mg)*	*Amount of 3-methyl-1-butanol (mg)*		*Recovery (%)*
			Theoretical	*Experimental*	
Tablets	0.621	9.94	2.22	2.1	95.4
Suppositories	4.392	46.72	10.45	9.64	92.2
	4.374	46.53	10.41	9.62	92.4
	4.541	48.31	10.81	10.03	92.8

The separation of *n*-butanol (internal standard) and 3-methyl-1-butanol was very good under the given chromatographic conditions (Fig. 1). The retention times were 675 and 1290 sec, respectively, and the resolution was 1.95. Because of this very good resolution, it is possible to reduce the analysis time by using a shorter column. Increasing the temperature in order to decrease the analysis time is not recommended because of the increasing instability of the baseline when approaching the maximum temperature of Porapak Q (230–240°). The other compounds present in the galenical forms are sufficiently removed and no interfering peaks were observed in the chromatograms. It is necessary to remove the chloroform completely before hydrolysis, because this solvent is eluted very closely before *n*-butanol. When it is present in a large amount, it gives a tailing peak so that the separation from *n*-butanol is not complete (Fig. 2).

The determination of camylofine dihydrochloride, by determining the hydrolysis product, requires a blank, namely a chromatographic analysis on Porapak Q of the extract before hydrolysis, in order to ensure that there is no free 3-methyl-1-butanol present in the sample. The amount of camylofine dihydrochloride was calculated from the measured area ratio of the drug and the internal standard, and the corresponding concentration ratio was derived from the calibration graph.

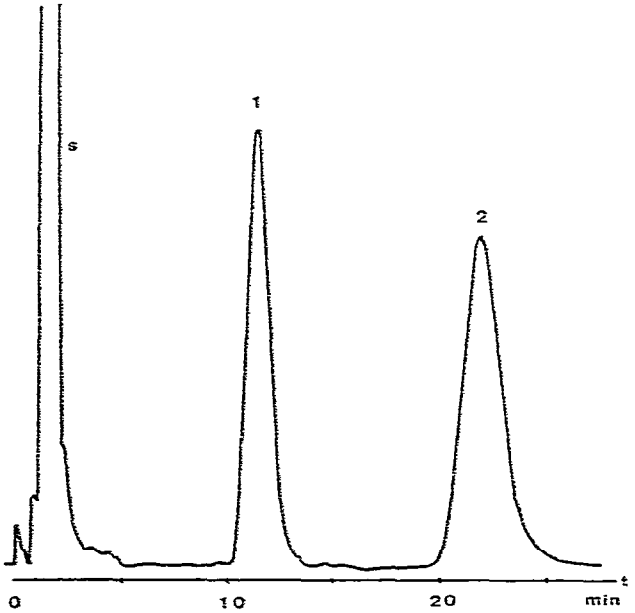


Fig. 1. Separation of *n*-butanol and 3-methyl-1-butanol on Porapak Q. s = Solvent (methanol); 1 = *n*-butanol and 2 = 3-methyl-1-butanol. Conditions, as given under Experimental.

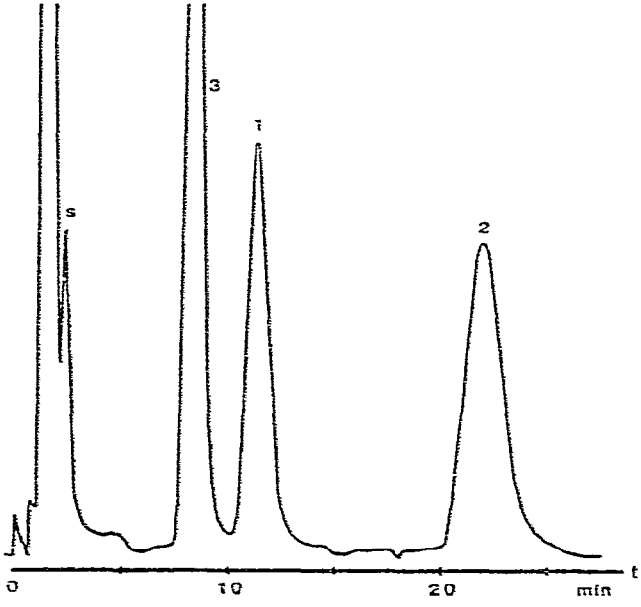


Fig. 2. Chromatogram of a suppository extract after hydrolysis, with incomplete removal of chloroform. s = Solvent; 1 = *n*-butanol; 2 = 3-methyl-1-butanol and 3 = chloroform. Conditions, as given under Experimental.

CONCLUSION

The proposed GC procedure allows a quantitative determination, even for unit dose, of camylofine dihydrochloride present in small amounts in pharmaceutical preparations and in the presence of other compounds.

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