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Quantitative analysis of tropane alkaloids in pharmaceutical preparations*

The problem of tropane alkaloid determination in mixtures is familiar to the drug analyst. The present paper describes procedures for the determination of scopolamine in injection solutions containing, in addition to scopolamine hydrobromide, morphine hydrochloride and sodium bisulphite and for the determination of atropine sulphate in coated tablets containing phenobarbitone and ergotamine tartrate. To our knowledge the few analytical methods elaborated so far for these mixtures are based on the determination of tropane alkaloids only after double extraction procedures in separation funnels or by ion exchange.

For the determination of scopolamine hydrobromide in a mixture with morphine hydrochloride, SVENSEN[1] proposed UV spectrophotometric assay after double shaking-out from the solution. SJÖSTRÖM AND RANDALL[2] separated scopolamine from morphine by passing the solution through a column filled with the strongly basic Dowex resin. The phenolic morphine was quantitatively retained on the column while the non-phenolic alkaloid passed through the resin and was determined in the efluent by colorimetry.

To obtain a rapid separation we thought that TLC separation known for its simplicity, high separation efficiency and accuracy might be useful for solving this problem. It must be borne in mind that in medical preparations the tropane alkaloids are usually present in microamounts in relation to other components present, which was another reason to choose TLC for this purpose.

Experimental

Reagents. Dragendorff–Munier reagent was made by mixing 1 part of a solution containing 17 g of bismuth nitrate and 200 g of tartaric acid in 800 ml of water with 1 part of a solution containing 160 g potassium iodide in 400 ml of water. Before use 100 g tartaric acid and 500 ml of water were added to 50 ml of this mixture. Hydrochloric acid was 10%; ethanol was 10%; sodium nitrite solution was a freshly prepared 1% water solution; ammonium sulphamate solution was a 2.5% water solution; N-naphthylethylenediamine dihydrochloride solution was a 1% water solution. The standard solution consisted of 7.5 mg of scopolamine hydrobromide (B.P. 1968) dissolved in 50 ml of water; the solvent system was chloroform–25% ammonia (100:0.15).

Apparatus. The TLC apparatus used was fitted with a thickness regulation spreader (Desaga, Heidelberg, G.F.R.).

Preparation of plates. The layer thickness was 0.5 mm. Activation required 3 h at 130°. The adsorbent was Aluminium Oxide G (Merck A.G., Darmstadt, G.F.R.).

Determination of scopolamine hydrobromide in scopolamine–morphine injection solutions

By means of a micropipette twice 150 μl of the injection solution (about 45 μg

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of scopolamine·HBr) and 150 μl of the standard solution were applied separately along the starting line as 4-cm horizontal bands. The chromatogram was run by the ascending technique in a chamber previously saturated with solvent for 1 h. After 1 h, the plate was removed from the chamber and dried, and the alkaloid was located by means of the guide chromatogram sprayed with the Dragendorff–Munier reagent. The corresponding zones containing the alkaloid on the sample chromatogram and that of the standard were marked and quantitatively scraped off the plate into 50-ml flasks. The alkaloid was extracted by stirring for 30 min with 10 ml of chloroform, and the solution was centrifuged (5 min at 5000 r.p.m.). 5 ml of the clear supernatant solutions were each pipetted into 50-ml beakers and evaporated to dryness on a boiling water bath. The residues were allowed to cool and thereafter 1 ml of fuming nitric acid was added and evaporated to dryness in the same way as before. The residues were dried for 10 min at 100°. After cooling to room temperature, 2 ml of hydrochloric acid, 0.1 g of zinc powder and 10 ml of ethanol were added. After heating for 10 min at 100° and cooling on ice, each solution was filtered through a white band filter paper ($2r = 4.5$ cm) into a 25-ml volumetric flask, and each residue on the filter was thereafter washed with three 2-ml portions of water. To the filtrates 1 ml of sodium nitrite solution and 1 ml of ammonium sulphamate solution were added. The solutions were stirred for 10 min, *i.e.* until liberated oxygen was completely expelled. After addition of 1 ml of N-naphthylethylenediamine dihydrochloride solution the reacting solution was mixed well, filled up to the mark with water and after 30 min the absorbances were measured at 545 nm in a 2-cm glass cell against water as a blank. The percentage of scopolamine hydrobromide was calculated from the mean value of the absorbances of both samples relative to that of the standard.

Determination of atropine sulphate in coated tablets containing ergotamine tartrate and phenobarbitone

Reagents. Dragendorff–Munier reagent, sodium nitrite solution, ammonium sulphamate solution, N-naphthylethylenediamine solution were as prescribed for the determination of scopolamine. Tartaric acid solution was a 1% water solution. Sulphuric acid–ethanol was a mixture of 100 ml of ethanol (96%) and 1 ml of sulphuric acid (16%).

Preparation of plates. Layer thickness was 0.5 mm. Activation required 3 h at 130°. The sorbent was Kieselgel G (Merck A.G., Darmstadt, G.F.R.).

Standard solution. 6.0 mg of atropine sulphate (B.P. 1968) was dissolved in the mixture of chloroform–methanol (4:1). The solvent was used to bring the volume to 25 ml. Solvent system was benzene–chloroform–ethanol (1:4:2).

After removing the coating from 20 tablets, the dried tablets were finely powdered. The quantity of the powder equivalent to about 1 mg of atropine sulphate, 3 mg of ergotamine tartrate and 200 mg phenobarbitone were mixed thoroughly for 2 min with 5 ml of 0.1 N H_2SO_4 and then with 7 ml of 0.1 N NaOH. The mixture was quantitatively transferred into a 100-ml separatory funnel with 3 times 3-ml portions of water. Extraction was carried out with six 10-ml portions of chloroform and the extract was filtered through a white band filter paper over anhydrous Na_2SO_4. After washing the filter with 5 ml of chloroform, the chloroform extracts were evaporated *in vacuo* at 40° to dryness. The residues were dissolved in 5 ml of a mixture of 40 ml

of chloroform and 10 ml of methanol (sample solution). By means of a micropipette, 200 μl of about 40 μg of atropine sulphate of the sample solution and 200 μl of the standard solution were applied twice as about 4-cm horizontal bands along the starting line. The chromatogram was run by the ascending technique until the solvent moved 10 cm from the start. The plate was removed from the chamber, air dried and the alkaloid detected on the guide chromatogram by spraying with the Dragendorff–Munier reagent. The corresponding zones with atropine on the samples and standard chromatogram were marked and quantitatively scraped off the plate into 50-ml flasks. To each flask 10 ml of sulphuric acid–ethanol were added, and the contents of the flask were mixed for 20 min and thereafter centrifuged (5 min at 5000 r.p.m.). 5 ml of the clear supernatant solution were pipetted each into 50-ml beakers and processed as directed for the determination of scopolamine. The percentage of atropine sulphate in the sample was calculated relative to the concentration of the alkaloid in the standard solution.

Results and discussion

For the separation of scopolamine hydrobromide from morphine hydrochloride, various solvent systems were checked. Owing to the phenolic character of morphine, various alkaline solvent systems were primarily used such as chloroform–diethylamine (90:10), chloroform–ammonia (100:0.15), cyclohexane–chloroform–diethylamine (50:40:10), using Aluminium Oxide G coated plates. Best separation was obtained with the solvent system chloroform–ammonia (100:0.15).

In choosing an appropriate solvent system for the separation of atropine sulphate from ergotamine tartrate and phenobarbitone, special care had to be taken to use a solvent system which would quantitatively separate atropine from ergotamine and phenobarbitone but cause no isomerisation of ergotamine which was then determined separately off the same plate. The solvent mixture chloroform–benzene–ethanol (40:10:20) met these requirements. Plates were coated with Silica Gel G and visualisation of the separated alkaloids was carried out using the Dragendorff–Munier reagent.

TABLE I

ANALYSIS OF A STANDARD SOLUTION OF SCOPOLAMINE–MORPHINE

Analysis No.	*Scopolamine hydrobromide found (mg) (0.3 mg present)*
1	0.281
2	0.276
3	0.300
4	0.279
5	0.310
6	0.292
7	0.301
Mean	0.291
Standard deviation ($P = 0.05$)	0.014
Limits of error	± 4.8%

TABLE II

ANALYSIS OF ATROPINE–ERGOTAMINE TARTRATE–PHENOBARBITONE TABLET

Analysis No.	*Atropine sulphate found (mg) (0.1 mg present)*
1	0.095
2	0.098
3	0.104
4	0.106
5	0.093
6	0.093
7	0.104
Mean	0.099
Standard deviation ($P = 0.05$)	0.052
Limits of error	± 5.2%

In spite of various methods published so far, the quantification of minute quantities of tropane alkaloids seems still to be a problem. Methods described in the literature for this purpose make use mainly of UV spectrophotometric[1], colorimetric[3–6] or IR[7] procedures of determination. The most frequently used method is that making use of the Vitali–Morin colorimetric test[3]. For the purpose of attaining stability of the violet colour formed, this method has been submitted for years to various investigations and modifications. However in spite of all precautions proposed and technical improvements introduced, such as the strict control of the water content of the solvent or introducing solvents other than acetone as for instance pyridine[8], isopropylamine[9], dimethylformamide[10], ethyl methyl ketone[11], the quick fading of the colour caused poor reproducibility of the results.

TABLE III

ANALYSIS OF COMMERCIAL TROPANE ALKALOID SAMPLES

Atropine sulphate (0.1 mg quantity claimed)		*Scopolamine hydrobromide (0.3 mg quantity claimed)*	
Found (mg)	*Difference (%)*	*Found (mg)*	*Difference (%)*
Sample A			
0.105	+5	0.100	+3.3
0.103	+3	0.295	−1.6
Sample B			
0.095	−5	0.300	—
0.100	—	0.310	+3.3
Sample C			
0.111	+11	—	—
0.112	+12		
0.113	+13		

For all these reasons the modified Bratton–Marshall colorimetric method[3,12] seemed to be the method of choice. This method includes nitration of the alkaloid, subsequent reduction of the obtained nitroproduct to the corresponding amino compound that is coupled after diazotisation with N-naphthylethylenediamine dihydrochloride to produce a red-violet colour having a maximum at 550 nm. Although this reaction includes four operations, the reaction product is stable and results are reproducible.

To confirm the accuracy and reproducibility of the procedures, determination of scopolamine hydrobromide and atropine sulphate, respectively, were carried out with standard mixtures containing all components in the same quantities as those of preparations commonly used in therapy. Results were statistically treated and as can be seen in Tables I and II satisfactory limits of error were obtained. Results of determinations with commercial samples of both mixtures are given in Table III.

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