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DETERMINATION OF DIPHENHYDRAMINE IN PHOTOCHEMICALLY DECOMPOSED SOLUTIONS

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SUMMARY

For the study of the photochemical decomposition of diphenhydramine a rapid and simple method of determination is needed in which the decomposition products do not interfere.

It is shown that the method of separation by thin-layer chromatography followed by extraction of the diphenhydramine spot, reaction with methyl orange and spectrophotometric measurement of the absorbance of the complex provides reliable results. Comparison of this method with a new gas chromatographic method shows that the latter method is also satisfactory when applied to measurement of diphenhydramine in photochemically and thermochemically decomposed solutions.

Two methods, which include extractions of the photochemically decomposed solution with organic solvents, do not provide reliable results compared with the results of the gas chromatographic method. One of these two methods is a proposed modification of the U.S.P. XVIII method.

INTRODUCTION

Many methods have been reported for the determination of diphenhydramine hydrochloride. A division can be made between those methods in which diphenhydramine is directly determined and those in which an attempt is made to separate the diphenhydramine from other substances present, by means of chromatography or extractions, prior to the determination.

To study the kinetics of the decomposition of diphenhydramine it is very important to use a method of determination in which decomposition products do not interfere. For acidic hydrolysis these products are benzhydrol and dimethylaminoethanol (de Roos et al., 1963). Oxidative decomposition with hydrogen peroxide provides toluene, benzophenone, benzyl alcohol, benzoic acid and phenolic substances in addition to dimethylaminoethanol (Sasaki, 1954). The photochemically formed decomposition products are different from those thermochemically formed and for the most part unknown. However, we found experimentally that other *N*-bases besides diphenhydramine and dimethylaminoethanol are present.

The methods of determination in which diphenhydramine is directly determined are not satisfactory. When studying the kinetics of decomposition those methods which involve titration (Holcomb and Fusari, 1974) or colorimetric measurements (Bhetasiwala et al., 1975; Holcomb and Fusari, 1974; Selles, 1956) fail because several *N*-bases are present. Where direct UV-spectrophotometric methods (Holcomb and Fusari, 1974) are involved the aromatic substances also absorb at the absorption maximum of diphenhydramine. Fluorimetric measurements (Martin, 1967; Holcomb and Fusari, 1974; Wirz et al., 1974) also fail, owing to an interference of decomposition products.

The methods in which an attempt is made to separate diphenhydramine from its decomposition products by means of chromatography (Baugh and Calvert, 1976; Blaug, 1955; Holcomb and Fusari, 1974; Matsui et al., 1969) or extractions (Caddy et al., 1975; Dessouki et al., 1974; Holcomb and Fusari, 1974; Ismaiel, 1974; de Roos et al., 1963; Woo et al., 1973) are expected to be satisfactory provided that this separation is complete or that any decomposition products not separated do not affect the determination. However, for only two of these methods, which involve extractions, it was experimentally established that the decomposition products after acidic hydrolysis, benzhydrol and dimethylaminoethanol, do not interfere (de Roos et al., 1963; Woo et al., 1973). In view of its known decomposition products it can be concluded that these methods must also be suitable after oxidative decomposition.

Because the products of the photochemical decomposition are different from those thermochemically formed and for the most part are unknown, we found it necessary to examine whether these extraction methods can also be used for the determination of diphenhydramine after photochemical decomposition. In addition we examined two methods of determination which involve separation of diphenhydramine and products by means of chromatography. They are a colorimetric determination after separation by TLC and a gas chromatographic method. The colorimetric method is a revised version of the method of Matsui et al. (1969), while the gas chromatographic method is an adaption for this special type of investigation.

MATERIALS AND METHODS

Apparatus

Gas chromatography. Packard-Becker Model 420 with hydrogen flame ionization detector; column: 3% OV-225 coated in Chromosorb W.H.P. 100–120 mesh in a glass tube (1.80 m × 2 mm i.d.). Working conditions: nitrogen carrier gas, flow-rate 16 ml/min; column temperature 180°C; injection block temperature, 250°C, detector temperature, 250°C; Recorder: Kipp Dual Pen Integrating Flatbed Recorder BD 12.

UV-Vis spectrophotometry. Spectrophotometer: Perkin Elmer, Model 124, double beam.

Photochemical decomposition. Rayonet Photochemical reactor RPR 208, with eight R.U.L.-2537 Å lamps.

Materials

Dipenhydramine hydrochloride complied with the standards of the Sixth Edition of the Nederlandse Farmacopee. Diphenhydramine base: an aqueous solution of diphenhydramine hydrochloride, after making alkaline with ammonia solution, was extracted with diethyl ether. The diethyl ether was then evaporated. All reagents and solvents were of Merck Analytical grade. Silica gel GF 254 for thin-layer chromatography: Merck. Buffer solution (pH 6): 10 g citric acid and 35.6 g disodium hydrogen orthophosphate in water to 1 liter.

Photochemical decomposition of diphenhydramine solutions

(I) From a solution of diphenhydramine (base) in isopropanol/ethanol 7 : 3, 1 liter was irradiated in the photochemical reactor and 15 ml samples were withdrawn at specific intervals and stored in the dark. The original strength of the solution was 3×10^{-2} M.

(II) From a solution of diphenhydramine HCl in water, 1 liter was irradiated with the photochemical reactor and 15 ml samples were withdrawn at specific intervals and stored in the dark. The original strength of the solution varied from 3×10^{-3} to 10^{-2} M.

Assay Methods

Thin-layer chromatography–colorimetry

Diphenhydramine was separated from its degradation products by thin-layer chromatography. For this purpose a 5×20 cm glass plate coated with a 0.25 mm layer of silica gel GF 254 was eluted with a mixture of chloroform, methanol and 25% ammonia solution (100 + 8 + 0.5) then dried and stored at 50°C. From a sample of irradiated diphenhydramine (base) solution in isopropanol/ethanol 7 : 3 a spot of 20 µl (2×10 µl) was directly applied to the pretreated silica gel layer (by means of a calibrated Herka micropipette). A 15 ml sample of irradiated diphenhydramine · HCl aqueous solution was made alkaline with 25% ammonia solution and then extracted with 5 ml ethyl acetate. Two separate spots of 30 (3×10) µl were applied to the pretreated silica gel layer.

After development using the same solvent mixture as above and drying the chromatogram at room temperature the diphenhydramine spots were detected under ultraviolet light of 254 nm. The area of silica gel containing diphenhydramine was scraped off the plate and transferred quantitatively to a glass column (length 15 cm, diameter 1 cm, fitted with a G4 glass filter at the base). The column was eluted with 4 ml 1% hydrochloric acid and after one night the remaining liquid in the column was forced through the filter by application of air pressure.

The silica gel was washed once with 0.5 ml of 1% hydrochloric acid (pressure was applied immediately after the acid had been added) and the eluate and washings were combined and diluted to 5.0 ml with 1% hydrochloric acid. These 5.0 ml were quantitatively transferred to a 100 ml flask with the aid of the in total 13 ml buffer solution (pH = 6). After addition of 2 ml of a 0.2% methyl orange solution the total content was shaken in a mechanical shaker for 5 min with 20 ml 1,2-dichloroethane. The organic phase was drawn off and centrifuged. The absorbance was measured at 420 nm. A blank experiment was carried out under the same conditions. Beer's law was obeyed between the maximum and minimum concentrations used (3×10^{-3} M–3×10^{-2} M). In the original publication the indicator dye bromthylmol blue was used. We found that the results after the use of this dye were not satisfactory. Therefore we used methyl orange as the indicator dye.

Gas chromatography

To a 1 ml sample of irradiated diphenhydramine (base) solution in isopropanol/ethanol 7 : 3, 1 ml of a solution of 4-*t*-butylbenzhydrol with a known concentration comparable to that of the diphenhydramine solution was added and 1 µl of this mixture was injected in the gas chromatograph.

For the irradiated aqueous samples of diphenhydramine · HCl the extraction procedure was the same as described earlier under the 'Thin-layer chromatographic–colorimetry' section. To 1 ml of the ethyl acetate extract, 1 ml of a solution of 4-*t*-butylbenzhydrol in ethyl acetate of comparable known strength was added and 1 µl of this mixture was injected in the gas chromatograph.

Under the used conditions diphenhydramine and 4-*t*-butylbenzhydrol have retention times of about 8 and 12 min, respectively. Quantitative results were obtained by measureing the peak area of diphenhydramine relative to the peak area of the internal standard, 4-*t*-butylbenzhydrol.

Spectrophotometry after diethyl ether extraction

This method is fully described in the literature (Caddy et al., 1975). It consists of measurement of the absorbance of the acidic solution at 258 nm after diethyl ether extraction.

Spectrophotometry after cyclohexane extraction

This method is fully described in the literature (Caddy et al., 1975). It consists of the extraction of the acidic solution with cyclohexane. After making alkaline the aqueous solution is again extracted with cyclohexane. The last step is back-extraction of diphenhydramine from the last mentioned cyclohexane layer with sulfuric acid and measurement of the absorbance at 258 nm.

RESULTS AND DISCUSSION

The reliability of the TLC–colorimetric method was tested by examination of several of its steps. No absorbance was found at 420 nm when the procedure was followed for eluates obtained from silica gel blank removed from the plate at the spot where diphenhydramine was normally located.

Further, when an irradiated solution, in which no diphenhydramine could be detected on TLC under UV light of 254 nm, was added to a flask containing a diphenhydramine solution of known strength the measured absorbance did not differ from that of the latter solution alone.

The gas chromatographic method was tested by making a calibration graph and also controlled as mentioned in the foregoing paragraph. Table 1 shows that, in view of the measured variation, the thin-layer chromatographic–colorimetric method and the gas chromatographic method provide comparable results when applied to the determination of diphenhydramine (base) in photochemically decomposed solutions in isopropanol/ethanol 7 : 3 and to the determination of diphenhydramine · HCl in photochemically decomposed aqueous solutions.

No significant difference existed between the values found at each level of decomposition. Both methods could be used for the determination of diphenhydramine in photochemically decomposed solutions in isopropanol/ethanol 7 : 3 or water. Because of its simplicity and rapidity the gas chromatographic method is preferred.

Table 2 shows that, when the spectrophotometric determination after diethyl ether extraction and that after cyclohexane extractions are used, bad results are obtained. Compared to the gas chromatographic method the spectrophotometric determination after diethyl ether extraction suggests an amount of diphenhydramine in a decomposed aqueous solution that is even higher than the amount of diphenhydramine in an undecomposed solution. The spectrophotometric determination after cyclohexane extractions also provided bad results compared with the results provided by the gas chromatographic method. It can be concluded that both methods are unreliable when applied to the determination of diphenhydramine in photochemically decomposed solutions, although the method with cyclohexane extractions is a proposed modification of the U.S.P. XVIII method. (Woo et al., 1973). With thin-layer chromatography it was found that decompo-

TABLE 1

AMOUNT OF DIPHENHYDRAMINE IN SOLUTIONS PHOTOCHEMICALLY DECOMPOSED AT 254 NM

A = gas chromatographic method. B = TLC–colorimetric method.

Diphenhydramine base (%) in isopropranol/ ethanol 7 : 3 [a]		Diphenhydramine · HCl (%) in water [b]	
A	B	A	B
100 [d]	100 [d]	100 [d]	100 [d]
79.5 ± 0.5 [c]	78.8 ± 1.2 [c]	89.2 ± 2.2 [c]	89.0 ± 0.1 [c]
70.3 ± 2.1	69.5 ± 1.5	81.3 ± 1.2	82.4 ± 0.3
65.4 ± 1.0	66.5 ± 0.5	76.4 ± 0.5	76.0 ± 0.0
49.9 ± 0.1	49.5 ± 2.2	71.4 ± 1.3	69.1 ± 3.2
40.1 ± 1.0	39.2 ± 1.3	59.5 ± 0.8	60.4 ± 2.0
36.3 ± 0.5	35.6 ± 1.0	56.3 ± 1.1	57.4 ± 0.0
31.7 ± 1.3	31.1 ± 1.0	50.4 ± 2.0	51.3 ± 3.3
25.9 ± 0.2	26.1 ± 1.3	40.9 ± 0.7	40.1 ± 2.0
23.1 ± 0.9	21.5 ± 1.0	31.2 ± 0.5	31.9 ± 0.3

[a] Original strength 3×10^{-2} M.

[b] Original strength 3×10^{-3} M.

[c] Measured variation (for A: n = 2; for B: n = 3).

[d] Figures refer to various time intervals of irradiation.

TABLE 2

AMOUNT OF DIPHENYDRAMINE · HCl IN SOLUTIONS PHOTOCHEMICALLY DECOMPOSED AT 254 NM

I = Comparison of the spectrophotometric determination after diethyl ether extraction (A) with the gas chromatographic method (B). Original strength of the solution: 3 × 10^{-3} M.
II = Comparison of the spectrophotometric determination after cyclohexane extractions (C) with the gas chromatographic method (D). Original strength of the solution: 10^{-2} M.

Diphenhydramine content (%)			
I		II	
A	B	C	D
100 [c]	100 [c]	100 [c]	100 [c]
154.9 ± 1.4 [a]	82.8 ± 0.5 [a]	101.4 ± 1.2 [a]	90.6 ± 0.1 [a]
182.2 ± 4.1	73.8 ± 0.9	96.0 ± 0.5	83.8 ± 0.5
b	15.5 ± 0.0	67.9 ± 1.1	40.1 ± 0.1
b	2.6 ± 0.0	27.8 ± 0.6	19.0 ± 0.2

[a] Measured variation (for A, B, C and D: n = 2).
[b] Not measurable; absorbance too high.
[c] Figures refer to various intervals of irradiation.

sition products were still present after extractions with diethyl ether or cyclohexane as applied in the two last described methods. Because these decomposition products have an absorption at 258 nm they interfere in the determination.

Table 3 shows the results obtained from this-layer chromatography for the method with diethyl ether extraction. In addition to the spot of diphenhydramine other spots

TABLE 3

R_f VALUES AND DETECTION DATA OBTAINED FROM TLC OF A SAMPLE BEFORE AND AFTER DIETHYL ETHER EXTRACTION

A: before diethyl ether extraction. B: after diethyl ether extraction. Solvent: chloroform-methanol-ammonia (100 + 8 + 0.5). Dragendorff's spray for detection of alkaloids and other *N*-compounds complied with the 7th edition of the Nederlandsche Faramcopee.

A			B		
R_f value	Visible under 254 nm	Detection with Dragendorff's spray	R_f value	Visible under 254 nm	Detection with Dragendorff's spray
0.8–1.0	+	–	0.8–1.0	+	–
0.73	+	–	0.73	–	–
0.47 [a]	+	+	0.47 [a]	+	+
0–0.2	+	+	0–0.2	+	+

[a] Diphenhydramine spot.

could also be detected with Dragendorff's spray. This indicates that other *N*-compounds besides diphenhydramine are present in the photochemically decomposed solution.

Conclusions

The results of our research justify the conclusion that the gas chromatographic method and the thin-layer chromatographic–colorimetric method can be used for the determination of diphenhydramine in photochemically decomposed solutions. Of these two methods the gas chromatographic method is preferred on account of its simplicity and rapidity. Because the two methods which include extractions give incorrect values with photochemically decomposed diphenhydramine they cannot be used for the quality control as it is not always possible to be sure that no photochemical decomposition has occurred.

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