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STABILITY-INDICATING CAPILLARY GAS–LIQUID CHROMATOGRAPHIC ASSAY OF DICYCLOMINE HYDROCHLORIDE IN SOME PHARMACEUTICAL FORMULATIONS

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SUMMARY

A stability-indicating assay method for some dicyclomine hydrochloride formulations was developed utilizing capillary gas–liquid chromatography. A methanolic extract of the sample, containing phenacetin as internal standard, was chromatographed by temperature programming on a 15 m × 0.524 mm I.D., DB-17 column with nitrogen carrier gas and flame ionization detection ($1 \cdot 10^{-10}$ A). The dicyclomine hydrochloride was well resolved from phenacetin with retention times of *ca.* 9 and 7 min, respectively. Dicyclomine hydrochloride–internal standard peak area ratio was linear over 0.1–1.0 μg of dicyclomine hydrochloride injected (r = 0.999). Under the experimental conditions the limit of detection was 0.025 μg of dicyclomine hydrochloride injected. Validation studies with synthetic capsules, tablets and injectables covering a range of 5–25 mg of dicyclomine hydrochloride per unit gave an overall percent recovery (± relative standard deviation, n = 9) of 99.9 ± 1.7%. The method was successfully applied to the assay of commercial formulations. Stability tests indicated that degradation products of dicyclomine, formed upon acid treatment, did not interfere with the dicyclomine and internal standard peaks. They further showed that dicyclomine is fairly stable in base.

INTRODUCTION

Dicyclomine hydrochloride [(bicyclohexyl)-1-carboxylic acid 2-(diethylamino)-ethyl ester hydrochloride] is a widely used anticholinergic agent. Based on its antispasmodic effect it is applied in the treatment of functional disturbances of gastrointestinal motility without affecting gastric secretion. For many years the official assay method for its pharmaceutical formulations was based on titrimetry using a chloroform–water system with sodium lauryl sulfate as titrant and methyl yellow as indicator[1]. Because the titration end point was difficult to observe and the titrant need-

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ed to be restandardized prior to use, other assay procedures have been proposed, many from drug regulatory laboratories. This has lead to the replacement of the official method by a high-performance liquid chromatography (HPLC) procedure which became official in Supplement 6 of USP XXI[2]. This new compendial method is identical to the procedure published by Jee[3,4] who utilized UV detection at 215 nm without an internal standard and did not address any stability-indicating aspects of the method. Dicyclomine does not have a distinct absorption spectrum and measurement at 215 nm is done in a region where many compounds, *e.g.* dosage form ingredients, show absorptions.

A nuclear magnetic resonance spectroscopic method[5] was published for the assay of dicyclomine hydrochloride. Because this method required an analytical sample containing 200 mg of the compound, it cannot be applied to single dose analysis for content uniformity test purposes. Dicyclomine has also been assayed by gas–liquid chromatography (GLC). The published methods, however, required the preparation of several reagents and were lengthy[6–8]. This paper reports a simple, stability-indicating assay method for dicyclomine hydrochloride in capsules, tablets and injectables based on capillary GLC.

EXPERIMENTAL

Apparatus

The following apparatus were used: Varian Model 3300 microprocessor-controlled gas chromatograph with a flame ionization detector, a Model 1040 megabore injector and Model 4270 electronic integrator (Varian Instruments, Walnut Creek, CA, U.S.A.). Injections were made with 5-μl Hamilton Series 900 syringes and gas flow-rates were measured with an Optiflow 520 digital flow meter (Fairfield, CA, U.S.A.). A Model 145 Isotemp dry bath (Fisher Scientific, Pittsburgh, PA, U.S.A.) was used for the stability tests.

Reagents and materials

The following reagents were used: dicyclomine hydrochloride (courtesy Merrell-Dow Pharmaceuticals, Cincinnati, OH, U.S.A.); phenacetin (Eastman-Kodak, Rochester, NY, U.S.A.), methanol (Optima or HPLC grade, Fisher Scientific, Fairlawn, NJ, U.S.A.), and Extrelut QE solid phase extraction cartridges (E.M. Science, Cherry Hill, NJ, U.S.A.). Ultra-high purity 99.999% nitrogen and hydrogen, and zero-grade air (total hydrocarbons < 0.1 ppm) were obtained from AGA Gas (Waumee, OH, U.S.A.). All other chemicals were analytical grade.

GLC conditions

A 15 m $\times$ 0.524 mm I.D. fused-silica, 1 μm DB-17 column (J & W Scientific, Folsom, CA, U.S.A.) was used with nitrogen carrier gas delivered at a flow-rate of 2.0 ml/min with a total make up gas of 30 ml/min. The flow-rates for hydrogen and air were about 30 and 300 ml/min, respectively. The injector port and detector were heated at 250°C. The temperature of the column oven was maintained at 160°C for 2 min, then programmed at 20°C/min for 4 min and held at the final temperature of 240°C for 5 min. A flame ionization detection (FID) current of $1 \cdot 10^{-10}$ A was used. The integrator was set at an attenuation of 16.

Internal standard solution

About 125 mg of phenacetin was transferred into a 50-ml volumetric flask, dissolved and diluted to volume with methanol.

Standard solution preparation

About 100 mg of dicyclomine hydrochloride was transferred into a 100-ml volumetric flask, dissolved and diluted to volume with methanol. Exactly 2.0 ml of the solution was transferred into a 10-ml volumetric flask and after addition of 2.0 ml of internal standard solution, the mixture was diluted to volume with methanol.

Sample solution preparation

Tablets. Twenty tablets were accurately weighed and pulverized. An aliquot of the powder, equivalent to about 20 mg of dicyclomine hydrochloride, was transferred into a 50-ml volumetric flask. After addition of 10.0 ml of internal standard solution, the mixture was diluted to volume and filtered, discarding the first 5 ml of filtrate. Exactly 5.0 ml of the filtrate was diluted to 10.0 ml in a volumetric flask.

Capsules. The contents of twenty capsules were quantitatively removed from the capsules, thoroughly mixed, and accurately weighed. An aliquot of the powder, equivalent to about 10 mg of dicyclomine hydrochloride, was transferred into a 50-ml volumetric flask and subjected to the same steps as described for tablets beginning with "addition of 10.0 ml of internal standard solution".

Injectables. A volume, equivalent to 5.0 mg of dicyclomine hydrochloride, was carefully pipetted onto a dry Extrelut cartridge. Elution of the compound was done four times with 4-ml, 2-ml, 2-ml and 2-ml portions of chloroform. The eluates were collected in a 25-ml volumetric flask. After addition of 5.0 ml of internal standard solution, the mixture was diluted to volume with methanol.

Chromatographic procedure

Using a 5-μl syringe, 2.0 μl of the sample solution or standard solution was injected into the gas chromatograph under the operating conditions described above. Quantitation was based on relating the dicyclomine hydrochloride–internal standard peak area ratio of the sample to that of the standard.

Stability tests

(a) Three samples, each containing about 15 mg of dicyclomine hydrochloride in 10 drops of water, were prepared by sonication (30 s). The solutions were heated at 80°C with 1 ml of 9 *M* sulfuric acid in separate tubes with PTFE-lined screw caps for 15, 30 and 45 min, respectively. At the end of each heating period, the mixture was poured over about 2 g of crushed ice and extracted first with ether and then with chloroform. The ether and chloroform extracts were evaporated to dryness under a gentle nitrogen stream, the residues were each reconstituted in 1.0 ml of methanol and chromatographed.

(b) Three samples were prepared and subjected to the same steps described in (a) above except that the solutions were heated with 1 ml of 1 *M* sodium hydroxide. Following the chloroform extraction, the aqueous solution was acidified with 1 *M* hydrochloric acid to pH 1 and then extracted with ether. The ether extract was evaporated to dryness under a gentle nitrogen stream, the residue was reconstituted in 1 ml of methanol, and chromatographed.

RESULTS AND DISCUSSION

Under the proposed experimental conditions dicyclomine hydrochloride and phenacetin eluted as fairly symmetrical sharp peaks and were well-separated from one another (Fig. 1). The approximate retention times were 7 min for phenacetin and 9 min for dicyclomine with capacity factors, k', of about 8.4 and 11.1, respectively, and height equivalent to a theoretical plate (HETP) values ($\pm$ standard deviation, S.D., $n = 10$) of 0.38 ± 0.13 mm and 0.512 ± 0.05 mm, respectively.

The relationship between dicyclomine hydrochloride–internal standard peak area ratio and amount of dicyclomine hydrochloride injected was established. Linearity was obtained between 0.1 and 1.0 μg of dicyclomine hydrochloride injected ($r = 0.999$). A typical regression equation was $A = 1.03C - 0.01$, where A = area ratio of dicyclomine hydrochloride–internal standard and C = amount of dicyclomine hydrochloride injected in micrograms.

Validation studies were performed on synthetically prepared capsules, tablets and injectables. Capsule placebos contained calcium sulfate, starch, lactose, gelatin, magnesium stearate, titanium oxide, FD&C Blue No. 1 and FD&C Red No. 3[9]. Tablet placebos contained acacia, dibasic calcium phosphate, starch, lactose, sucrose, magnesium stearate and FDC Blue No. 1[9]. The injectables contained chlorobutanol and sodium chloride. The gas chromatograms were similar to those of standard solutions. The capsule and tablet placebos did not show peaks between 1 and 10 min

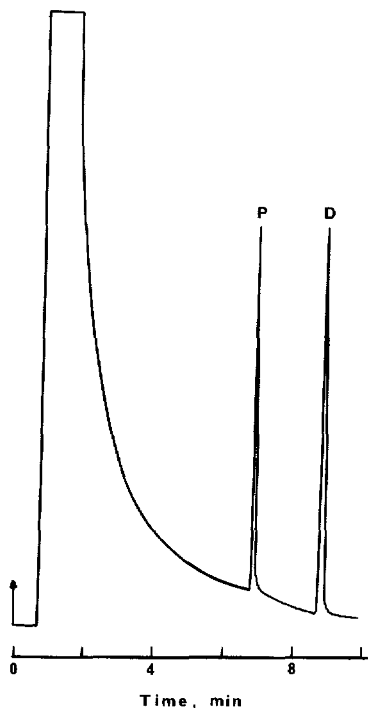

Fig. 1. Gas chromatogram of a standard solution run under conditions described in text. P = phenacetin; D = dicyclomine hydrochloride.

TABLE I

RECOVERY DATA FROM SYNTHETIC FORMULATIONS

Sample	*Dicyclomine hydrochloride*		
	Amount weighed (mg/unit[b])	*Amount found[a] (mg/unit[b])*	*Recovery (%)*
Capsule	5.3	5.4	101.9
Capsule	15.2	15.2	100.0
Capsule	25.3	25.2	99.6
Tablet	5.0	4.9	98.0
Tablet	14.5	14.2	97.9
Tablet	24.7	24.4	98.8
Injectable	4.9	5.0	102.0
Injectable	14.6	14.5	99.3
Injectable	25.1	25.6	102.0
Overall recovery (%)			99.9
R.S.D. (%)			1.67

[a] Average of duplicate assays.
[b] Unit = capsule, tablet or ml.

after injection. Table I shows the recovery data from these simulated formulations. The overall percent recovery (± relative standard deviation, R.S.D.) was (n = 9) 99.9 ± 1.7%. The validation studies were performed over a range of 5–25 mg of dicyclomine hydrochloride per capsule, tablet or ml. This corresponds to a range of 50% below and 150% above the usual label amounts in capsules and injectables of 10 mg per capsule or ml, respectively. Since tablets usually contain 20 mg per tablet, the range corresponds to 75% below and 25% above the label claim.

The method was applied to the assay of commercial capsules, tablets, and injectables. Fig. 2 is a typical gas chromatogram for the methanolic extract from tablets. The chlorobutanol preservative in injectables did not interfere with the dicyclomine and internal standard peaks because it coeluted with the solvent. Fig. 3 shows the gas chromatogram of a solution of chlorobutanol in carbon disulfide. The recovery data (Table II) showed quantitative recovery as percent label claims of greater than 97% were obtained. The USP XXI potency limits were 93.0–107.0% of label claim for these formulations[1].

The proposed assay method was designed to allow individual dosage unit assay, particularly for the capsules and tablets, because USP XXI requires that these two formulations meet the content uniformity test. In addition, the limit of detection was determined for qualitative analysis purposes. The sensitivity, as determined by the method of Kaiser[10], was $7.2 \cdot 10^{-11}$ M or 25 ng of dicyclomine hydrochloride injected under the described experimental conditions.

Dicyclomine (I) is an ester that may undergo hydrolysis into dicyclohexylcarboxylic acid (II) and diethylaminoethanol (III) according to the reaction shown in Fig. 4. A stability test was conducted by heating the compound with 9 M sulfuric acid and with 1 M sodium hydroxide to determine if these hydrolysis products, when formed, interfere with the dicyclomine and/or internal standard peaks. The worked-

Fig. 2. Gas chromatogram of methanol extract from a commercial tablet run under conditions described in text. P = phenacetin; D = dicyclomine hydrochloride.

up ether extracts from the described 15 and 30 min 9 *M* sulfuric acid treatment gave upon gas chromatography a peak with a retention time of about 6 min. Although no identification was made, the peak was presumably that of dicyclohexylcarboxylic acid. Diethylaminoethanol and dicyclomine were not observed in the chromatogram because they were in the protonated forms and remained in the acidic aqueous phase.

TABLE II

RECOVERY DATA FROM COMMERCIAL FORMULATIONS

Sample	*Dicyclomine hydrochloride*		
	Label claim (mg/unit[b])	*Amount found[a] (mg/unit[b])*	*Label claim (%)*
Capsule A	10	9.76	97.6
Capsule B	10	9.63	96.3
Tablet A	20	19.96	99.8
Tablet B	20	19.92	99.6
Injectable A	10	10.09	100.9
Injectable B	10	10.12	101.2

[a] Average of duplicate assays.
[b] Unit = capsule, tablet or ml.

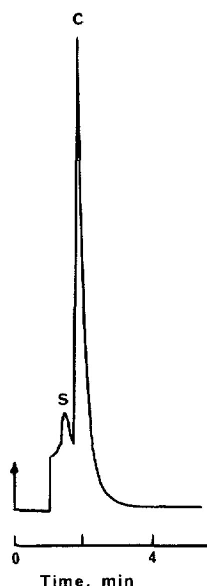

Fig. 3. Gas chromatogram of chlorobutanol (C) in carbon disulfide (S) run under conditions described in text.

Subsequent extraction with chloroform gave, however, after evaporation and chromatography of the reconstituted solution of the residue, the intact dicyclomine with a peak area somewhat smaller than that of control. Protonated dicyclomine is soluble in chloroform because of the higher polarity of chloroform compared to that of ether. The acid hydrolysis in 15 and 30 min produced only small quantities of the decomposition products. Since the FID response factor for diethylaminoethanol is low, this compound was not observed in the chromatograms. However, the worked-up ether extract from the 45-min acid treatment gave two peaks with retention times of about 5 and 6 min. The peak at 5 min was probably that of the diethylaminoethanol product which in 45 min was produced in sufficiently large quantities. Although it was protonated the large quantity was enough to distribute it into the ether phase, as dictated by the partition coefficient, in such amount that was observable in the chromatogram. The more extensive hydrolysis of dicyclomine hydrochloride in 45 min was confirmed by the appearance of a dicyclomine hydrochloride peak with a peak area much small-

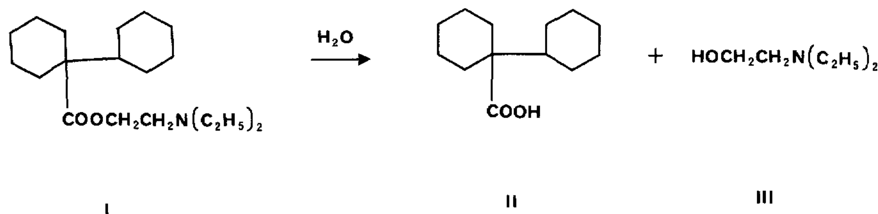

Fig. 4. Hydrolysis reaction of dicyclomine.

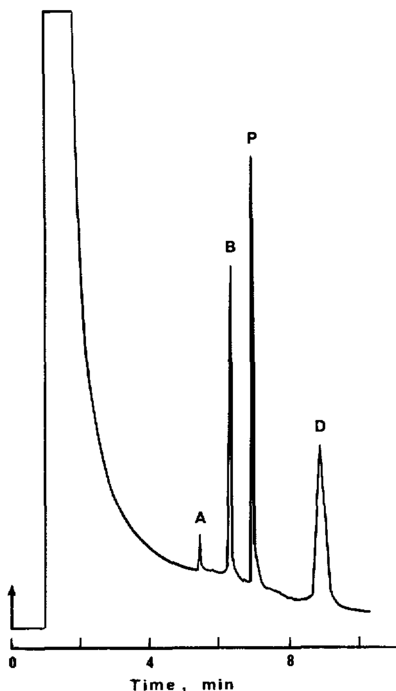

Fig. 5. Gas chromatogram of a mixture of acid decomposition products (A and B) with dicyclomine (D) and phenacetin (P).

er than that of control in the chromatogram of the subsequent chloroform extract. Fig. 5 is the chromatogram of the reconstituted solution in methanol from the 45-min ether extract which was spiked with a relatively large amount of dicyclomine hydrochloride and phenacetin to determine the locations of the hydrolysis products, the dicyclomine hydrochloride and phenacetin peaks. This chromatogram clearly shows that the hydrolysis products do not interfere with the peaks of interest.

Surprisingly, dicyclomine hydrochloride remained fairly stable upon heating with 1 *M* sodium hydroxide. Because dicyclomine is an ester, the compound is expected to hydrolyze also upon heating with aqueous base. The chromatograms of the worked-up ether extracts after 45 min heating showed only the peak of intact dicyclomine with an area of similar size as that of control. The subsequent chloroform extract showed only trace quantities of dicyclomine because this compound was already removed in the ether extraction. When the remaining basic solution was acidified with hydrochloric acid to pH 1 and then extracted with ether, the worked-up ether extract showed only a trace of the purported dicyclohexylcarboxylic acid.

The above results indicate that the assay method is stability-indicating for dicyclomine as far as interference by hydrolysis decomposition products are concerned. The method is simple, no reagents are necessary, the assay is quantitative, and it is applicable for single unit dosage assay.

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REFERENCES

1 *The United States Pharmacopeia,* Mack Publishing Co., Easton, PA, 21st rev., 1985, p. 316 ff.
2 *The United States Pharmacopeia,* The United States Pharmacopeial Convention, Rockville, MD, 21st rev., Suppl. 6, p. 2558 ff.
3 J. M. Lee, *FDA Lab. Info. Bull.,* 1 (1985) 2943.
4 J. M. Lee, *FDA Lab. Info. Bull.,* 2 (1986) 3083.
5 G. M. Hanna, *J. Assoc. Off. Anal. Chem.,* 67 (1984) 222.
6 F. L. Fricke, *J. Assoc. Anal. Chem.,* 55 (1972) 1162.
7 C. R. Brownell and L. L. Alber, *J. Assoc. Off. Anal. Chem.,* 62 (1979) 1116.
8 T. Daldrup, F. Susanto and P. Michalke, *Fresenius' Z. Anal. Chem.,* 308 (1981) 413.
9 *Physicians' Desk Reference,* Medical Economics Co., Oradell, NJ, 42nd ed., 1988, p. 1119.
10 H. Kaiser, *Anal. Chem.,* 42 (1970) 26A.