Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 3, pp. 287–291, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Short Communication

A colorimetric assay for dicyclomine hydrochloride using bromocresol green

M. A. RAGGI, V. CAVRINI* and A. M. DI PIETRA

Istituto di Chimica Farmaceutica e Tossicologica, via Belmeloro 6, 40126 Bologna, Italy

Keywords: *Dicyclomine hydrochloride; colorimetric assay; extraction-photometry; bromocresol green complex.*

Introduction

Dicyclomine hydrochloride,2-(diethylamino)ethyl[bicyclohexyl]-1-carboxylate hydrochloride, is a spasmolytic drug used as an adjunct in the treatment of peptic ulcer [1, 2].

Methods for the analysis of dicyclomine in pharmaceutical formulations include titrimetic [3], gas–liquid chromatographic [4–6] and colorimetric [7] procedures. The pharmacopoeial methods (USP, BP) [8, 9], according to [3], are based on a semi-micro two-phase titration using sodium lauryl sulphate as the titrant and methyl yellow as the indicator. This procedure, however, was found to be unsuitable for analysing the drug in a commercial formulation (elixir) owing to interference from the flavoured vehicle. The present work was undertaken to develop an alternative reliable and stability-indicating colorimetric method based on selective extraction of dicyclomine (free base) with cyclohexane followed by the formation of a yellow complex between the drug and bromocresol green.

Experimental

Materials

Dicyclomine hydrochloride was kindly supplied by Merrell S.p.A. (Rome, Italy) and was used as received. Diethylaminoethanol, bromocresol green (BCG), pH 3 sodium citrate–HCl buffer and all other reagents were obtained from C. Erba Strumentazione (Milan, Italy). Stock solutions were: 1 mg/ml of dicyclomine hydrochloride and 10^{-4} M bromocresol green (BCG) in water.

Equipment

Spectrophotometric analyses were performed using a digital single-beam spectrophotometer (Jasco, Model UVIDEC-4) and a double-beam spectrophotometer (Perkin–

*To whom correspondence should be addressed.

Elmer Model 402) with 1-cm cells. The pH measurements were made using a digital pH-meter (Crison Model 501).

Standard solutions

Serial volumes of 0.5–3.0 ml of dicyclomine hydrochloride stock solution were transferred into 50-ml separation funnels; to each solution, 5.0 ml of chloroform, 5.0 ml of pH 3 buffer solution and 15 ml of 10^{-4} M BCG were added. The yellow complex was extracted by vigorous shaking for 1 min. After 5 min the chloroformic layer containing the dicyclomine–dye complex was separated and the aqueous layer was extracted again with successive portions of 3.0 ml and 2.0 ml of chloroform. The extracts were combined into a 10-ml volumetric flask and diluted to 10 ml with chloroform. After 20 min the absorbance of the chloroformic solution was measured at 415 nm against a blank prepared similarly. A calibration curve of absorbance against concentration was drawn.

Sample preparation

A commercially available dosage form (elixir) containing dicyclomine hydrochloride (20 mg/ml) in a flavoured vehicle was analysed as follows: 1.0 ml of the sample was diluted to 20 ml with water and 1.0 ml of the resulting solution was transferred to a centrifuge tube; 1.0 ml of water, 2 drops of 8% (m/v) sodium hydroxide solution, 100 mg of sodium chloride and 3.0 ml of cyclohexane were added. After shaking for 1–2 min and subsequent centrifugation for 5 min, the upper cyclohexane layer was accurately transferred into a 10-ml volumetric flask. A further two extractions were performed similarly and the combined extracts were diluted to volume with cyclohexane. A 2.0-ml aliquot of the resulting solution was transferred by pipette to a 50-ml separation funnel and 3 ml of chloroform was added; subsequently the procedure described for standard solutions was followed, beginning with ". . . 5.0 ml of pH 3 buffer solution and 15 ml of 10^{-4} M BCG were added".

Results and Discussion

Dicyclomine reacts with bromocresol green (BCG) to form a yellow complex, which is extractable with chloroform and exhibits a maximum absorption at 415 nm. The factors affecting the intensity and the stability of the colour (pH, time and molar ratio of dye to drug) were studied and controlled at room temperature, so that the method could be made quantitative. The optimal range of pH for complex formation and chloroformic extraction was found to be 2.3–3.2 (Table 1). For quantitative work pH 3 was chosen and the absorbance readings (415 nm) were made after 20 min.

The composition of the drug–dye complex was studied by the method of continuous molar variations [10]. A series of pH 3 buffered solutions containing the drug and BCG at various molar fractions for a constant total concentration of 5×10^{-5} M was prepared and the absorbance of each solution was measured at 415 nm. As shown in Fig. 1 (a Job's plot) maximum absorbance occurs at a molar fraction of 0.5, indicating a complex of the 1:1 type. Furthermore, the molar ratio method was applied by adding different amounts of 10^{-4} M BCG to a standard dicyclomine solution (10 μg/ml) and the drug–dye complex was determined. As shown in Fig. 2, the ratio of drug to dye in the complex was 1:1, but the required ratio for complete complexation and quantitative extraction was about 1:3.

Table 1
Effect of pH and time on the formation of the dicyclomine-BCG complex*

	Absorbance (415 nm)				
pH	t = 1 min	t = 5 min	t = 15 min	t = 20 min	t = 60 min
2.3	0.612	0.590	0.582	0.581	0.580
3	0.611	0.593	0.583	0.580	0.580
3.2	0.603	0.588	0.580	0.578	0.578
3.4	0.597	0.574	0.568	0.567	0.567
3.7	0.590	0.575	0.563	0.563	0.563
4.2	0.577	0.568	0.557	0.554	0.552

* Dicyclomine hydrochloride: 10 μg/ml.

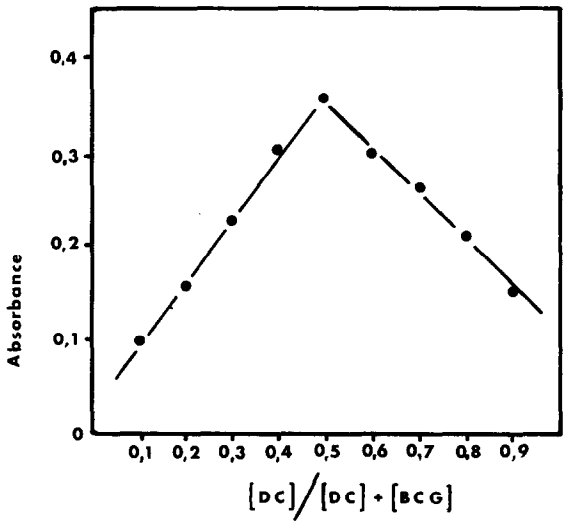

Figure 1
A Job's plot [10] for the dicyclomine (DC) and bromocresol green (BCG) system. The total concentration of drug and dye was 5×10^{-5} M.

Under the experimental conditions described the colour was stable for at least 3 h and a linear relationship between absorbance and concentration of dicyclomine was found over the concentration range of 2.6–15.6 μg/ml ($y = 0.0618x + 0.0397$; $r = 0.9994$; $n = 7$). The relative standard deviation was 0.45% ($n = 8$). The molar absorptivity of the drug–BCG complex solution in chloroform was 2.0×10^4.

For the determination of dicyclomine in the commercial formulation preliminary separation of the drug as free base from its vehicle by solvent extraction was necessary. By suitable choice of the parameters (solvent, pH) which control extraction, the required recovery and selectivity were obtained. With cyclohexane as the extracting solvent, the optimal pH range was 11–12.3; either sodium hydroxide or ammonium hydroxide solution could be used for the appropriate pH adjustment. Under these conditions, the dicyclomine extraction was quantitative, since a calibration curve obtained by the assay procedure (i.e. involving an extractive step with cyclohexane) was essentially identical

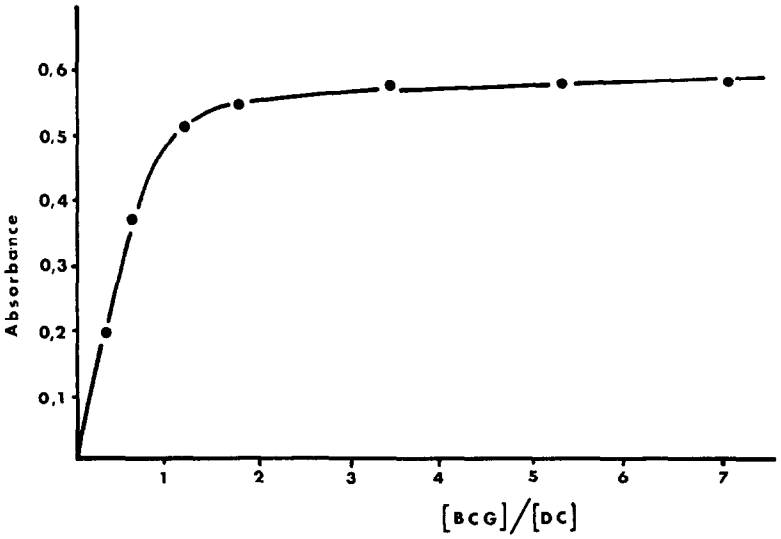

Figure 2
Plot of absorbance *against* the molar ratio of bromocresol green (BCG) to dicyclomine (DC). The dicyclomine concentration was 10 μg/ml.

with that obtained as described for standard solutions. Moreover, cyclohexane was found to be a solvent capable of separating the intact dicyclomine from 2-diethylaminoethanol, a potential interfering degradation product; thus the assay was shown to be a stability-indicating procedure. As shown in Table 2, the addition of different amounts of diethylaminoethanol to a known concentration of dicyclomine did not interfere with the colorimetric assay. With chloroform, however, diethylaminoethanol was coextracted and the procedure was not specific for dicyclomine.

The proposed method was applied to the determination of dicyclomine hydrochloride in a commercial dosage form containing the drug (20 mg/ml) dissolved in a flavoured syrup (invert sugar, saccharin sodium, anise oil and cherry flavour). The results were in good agreement with the label claim (recovery: 100.67%; RSD = 0.56 from five determinations). The excipients did not interfere with the analysis. Interference was observed when dicyclomine was associated with an additional basic drug (e.g. pyridoxine hydrochloride). Recovery studies were performed by adding known amounts of dicyclomine hydrochloride; quantitative recoveries (100.40%, RSD = 1.14 from five

Table 2
Recovery of dicyclomine hydrochloride in the presence of 2-diethylaminoethanol

Dicyclomine HCl content (mg)	2-ethylaminoethanol added (mg)	Dicyclomine HCl found (mg)	Dicyclomine HCl recovery (%)
10.16	4.03	10.30	101.37
10.09	2.15	10.19	100.99
10.05	1.03	10.14	100.89
10.00	1.05	10.03	100.30
Mean			100.89
SD			0.44

determinations) from the commercial formulation were obtained. Drug extraction from the alkaline medium was easily performed on a microscale in centrifuge tubes, but attempts to use larger samples in separation funnels, in view of the possible application of a titrimetric procedure, were unsuccessful owing to emulsion formation.

In summary, the described colorimetric method meets the requirements of a feasible and sensitive procedure for the determination of dicyclomine in a single-component pharmaceutical formulation; it may have advantages over a previously proposed method [7] because of the simplicity of the procedure. The whole analytical procedure (extraction with cyclohexane and drug–dye complex formation) is precise, accurate and suitable for the stability-indicating assay of dicyclomine.

Acknowledgement: The authors thank Mr F. Collina for his valuable technical assistance.

References

[1] A. J. Lewis (Ed.), *Modern Drug Encyclopedia*, 15th Edn. Yorke Medical Books, New York (1979).
[2] R. R.Miller and A. J. Greenslatt (Eds), *Handbook of Drug Therapy*, Elsevier, New York (1979).
[3] F. Pellerin, J.A. Gautier and D. Demay, *Ann. Pharm, Fr.* **22**, 559–565 (1964).
[4] F. L. Fricke, *J. Ass. Off. Anal. Chem.* **55**, 1162–1167 (1972).
[5] E. R. Kaplan and A. A. Spark, *S. Afr. J. Sci.* **71**, 241–243 (1975).
[6] C. R. Brownell and L. L. Alber, *J. Ass. Off. Anal. Chem.* **62**, 1116–1123 (1979).
[7] M. A. Abdel Kader and A. M. Taha, *J. Pharm. Pharmacol.* **30**, 68 p. (1978).
[8] *United States Pharmacopeia XX and the National Formulary XV*, United States Pharmacopeial Convention, Rockville, MD (1980).
[9] *British Pharmacopoeia* 1980, Vols I and II.
[10] P. Job, *Ann. Chim.* **9**, 113–203 (1928).

[*Received for review 2 February 1984; revised manuscript received 27 March 1984*]