Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 5, pp. 421–426, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Short Communication

Colorimetric determination of beta-adrenergic blocking drugs with carbon disulphide and copper(I) ions

N.A. ZAKHARI,* S.M. HASSAN and Y. EL-SHABRAWY

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516 Mansoura, Egypt

Keywords: *Colorimetry; determination; beta-adrenergic blocking drugs; copper(I) dithiocarbamate complex.*

Introduction

A rapid and selective method for the trace analysis of secondary alkylamines has been sought since the early 1930s [1–2]. Primary interest was directed toward the determination of simple organic secondary amines, e.g. dimethylamine [1–6], diethylamine [5], dibutylamine [7], dibenzylamine [7] and morpholine [7] by reaction with carbon disulphide. The reaction results in the formation of a dialkyl dithiocarbamate which forms a stable complex with copper(II) ions. The copper bis(dithiocarbamate) complex can be extracted into a suitable organic solvent such as chloroform [7] or a mixture of propan-2-ol–benzene [6] and the concentration of the amine is determined directly from the concentration of the complex [3–5] or indirectly from the amount of copper remaining in the aqueous phase [7].

However, this sensitive reaction has found little application in the analysis of pharmaceutically important compounds, viz. determination of the content of ephedrine hydrochloride in compound tetracaine injections [8].

An improved version of the method was recently reported in which tetra-acetonitrilocopper(I) perchlorate was used for the spectrophotometric determination of dithiocarbamate [9]. The advantages of using this new reagent are the immediate formation of the copper(I) dithiocarbamate yellow complex which is stable for at least 24 h and the high sensitivity of the method [9].

This paper describes the analytical application of the amine–carbon disulphide reaction for the determination of some beta-adrenergic blocking drugs in pure form and in tablet formulations. The existing methods for determining this group of drugs include spectrophotometric [10–13], fluorimetric [14], colorimetric [15–17], high-performance liquid chromatographic [18–21], polarographic [22] and gas chromatographic [23] methods. Non-aqueous [13–24] and thermometric [25] titrations have also been reported.

Experimental

Apparatus

Spectra of solutions in 1-cm cells were recorded on a Perkin–Elmer 550-S double beam UV–vis spectrohotometer.

Materials

Atenolol, propranolol hydrochloride, oxyfedrine hydrochloride, pindolol, acebutolol hydrochloride and alprenolol hydrochloride as bulk substances and tablets were obtained from commercial sources and their purity was determined by UV spectrophotometry [10–13] at λ_{max} values 224, 290, 252, 264, 232 and 275 nm, respectively. Stock solutions of the reference compounds and of extracts of the

* Author to whom correspondence should be addressed.

tablets were freshly prepared as 0.4 mg ml^{-1} solutions in 0.1 N hydrochloric acid for atenolol, acebutolol hydrochloride and alprenolol hydrochloride, and as 0.4 mg ml^{-1} solutions in methanol for the other compounds.

Reagents

All the chemicals used were of analytical-reagent grade and solvents were of spectroscopic grade. Tetra-acetonitrilocopper(I) perchlorate was prepared as described by Verma *et al.* [9]. A 0.02 M solution was prepared by dissolving 0.327 g of solid tetra-acetonitrilocopper(I) perchlorate in 50 ml acetonitrile and standardized by titration with ammonium hexanitratocerate(IV) in aqueous sulphuric acid solution using ferroin as indicator. A 0.002 M solution was then prepared by quantitative dilution with acetonitrile. Aqueous solutions of 0.1 N hydrochloric acid and ammonia buffer were prepared as described by the Ph.Eur. [26]. Acetic acid 25% (v/v) solution was prepared in distilled water.

General procedure

Accurately measured volumes of stock solution, equivalent to 0.2–1.4 mg of sample followed by 5 ml ammonia buffer, 1.7 ml carbon disulphide, 5 ml of 0.002 M tetra-acetonitrilocopper(I) perchlorate solution and 10 ml of chloroform were transferred to a 125-ml separating funnel. The mixture was shaken well, allowed to separate into two layers and 4 ml of 25% (v/v) acetic acid solution were added with repeated shaking. The chloroform extract was filtered through anhydrous sodium sulphate into a 25-ml calibrated flask. The extraction was repeated twice each with 5 ml of chloroform. The combined extract was mixed well, diluted to volume with anhydrous chloroform and the absorbance was then measured at 435 nm against the corresponding reagent blank. A calibration graph was drawn or a regression equation calculated.

Analysis of tablets

Twenty tablets were weighed and powdered. An accurately weighed portion of powder equivalent to 40 mg drug was placed in a suitable vessel containing 50 ml of 0.1 N hydrochloric acid solution (methanol in the case of tablets containing propranolol hydrochloride, oxyfedrine hydrochloride and pindolol) and the active ingredients were extracted by continuous stirring for about 10 min. The extract was filtered through Whatman No. 42 paper into a 100-ml calibrated flask then washed three times each with 10 ml of solvent. The combined extract was diluted to volume with the same solvent and an accurately measured volume of this solution was assayed as described above under "General procedure".

Results and Discussion

Reaction involved

Beta-adrenergic blocking drugs possess a secondary amino group which reacts with carbon disulphide to give dithiocarbamate [1–7]. In the presence of copper(I) ions this forms a stable yellow copper(I) dithiocarbamate complex with a characteristic λ_{max} at 435 nm for all compounds studied (Fig. 1). The molar ratio of the reaction has been determined by spectrophotometric titration of 1 ml 0.002 M drug solutions with 0.002 M tetra-acetonitrilocopper(I) perchlorate solution. The results of the measurements (Fig. 2) show a 1:1 molar ratio according to the following reaction stoichiometry:

$$RR'N{-}\underset{\underset{S}{\|}}{C}{-}S^- + Cu^+ \rightarrow RR'N{-}\underset{\underset{S}{\|}}{C}{-}SCu.$$

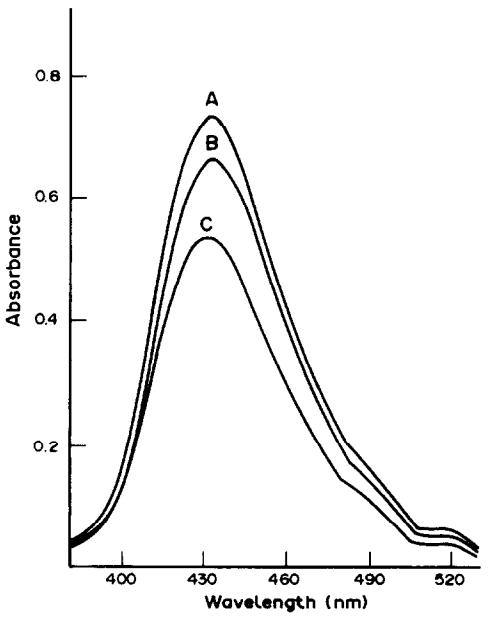

Figure 1
Absorption spectra of reaction products using 32 μg ml^{-1}. A, propranolol hydrochloride; B, oxyfedrine hydrochloride; C, atenolol.

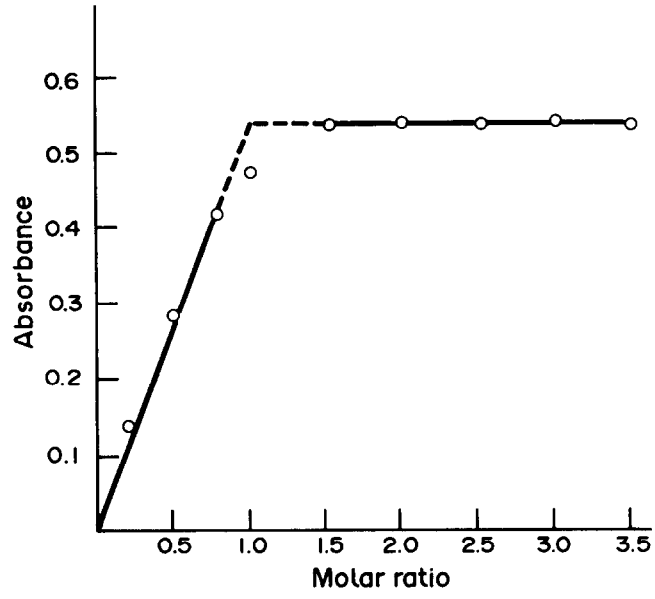

Figure 2
Spectrophotometric titration of 0.5916 mmol propranolol hydrochloride with 0.002 M tetracetonitrilo-copper(I) perchlorate solution.

Effect of concentration of copper

Figure 3 shows that 5 ml of 0.002 M tetra-acetonitrilocopper(I) perchlorate solution provided the optimum concentration of copper. Other copper(I) and copper(II) salts produced lower colour intensity.

Effect of concentration of carbon disulphide

The optimum volume of carbon disulphide for colour development was between 1.4 and 1.7 ml in a total volume of 27–30 ml (Fig. 4).

Effect of pH

Several experiments were carried out to study the effect of pH on the formation and stability of the complex. The complex was formed only in alkaline solution and its extracts from aqueous solution with pH greater than 7.5 showed decreasing absorbance with increasing pH. A similar behaviour has been observed with copper(II) bis(dialkyldithiocarbamate) [27]. Extracts from aqueous solution with pH less than 7.5 gave an almost constant absorbance over a period of at least

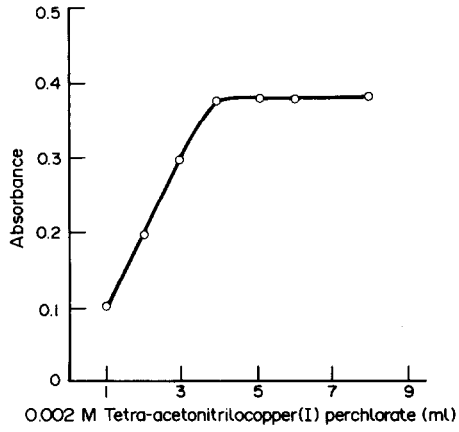

Figure 3
Effect of copper(I) ions on the reaction product of 24 μg ml^{-1} atenolol.

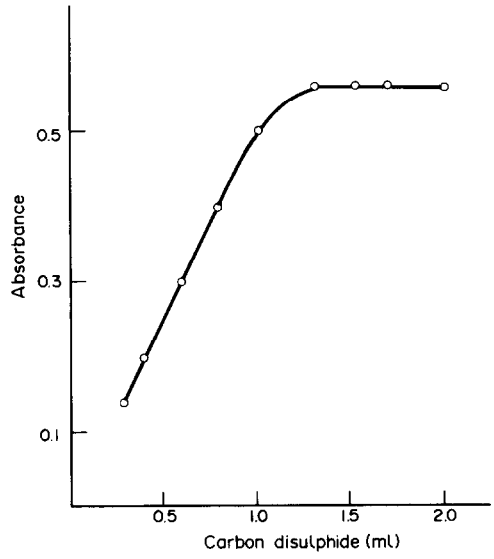

Figure 4
Effect of carbon disulphide on the reaction product of 32 μg ml^{-1} atenolol.

24 h. Figure 5 shows the effect of acetic acid on the stability of the copper(I) dithiocarbamate complex. The optimum volume of 25% (v/v) acetic acid necessary to maintain the pH in the range 7–7.5 was found to be 3–5 ml.

Performance characteristic

Figure 6 shows that there is a linear correlation between absorbance and final concentration over the range 8–56 μg ml^{-1} with apparent molar absorptivities 4.44 × 10^3, 6.60 × 10^3, 6.32 × 10^3, 6.65 × 10^3, 6.13 × 10^3 and 5.81 × 10^3 for atenolol, propranolol hydrochloride, oxyfedrine hydrochloride, pindolol, acebutolol hydrochloride and alprenolol hydrochloride, respectively. The correlation coefficients were between 0.99999 and 0.99985.

The proposed method has been applied to the determination of six beta-adrenergic blocking drugs in bulk drug form and in commercial tablets. The results are both accurate and precise (Table 1). To investigate the possibility

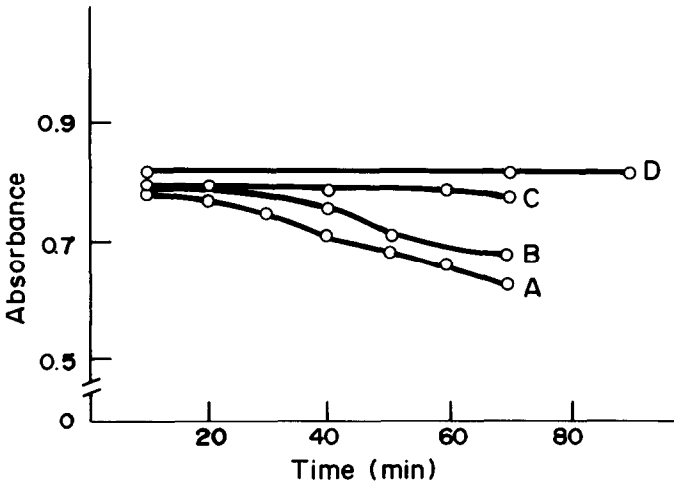

Figure 5
Effect of pH on the reaction product of 48 μg ml^{-1} atenolol. A, without acetic acid; B, using 1 ml 25% acetic acid; C, using 2 ml 25% acetic acid; D, using 3–5 ml 25% acetic acid.

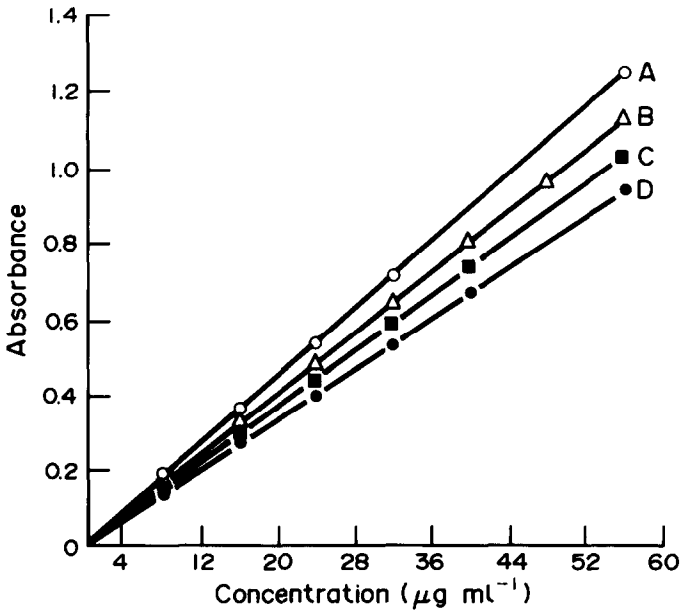

Figure 6
Calibration graphs of the reaction products. A, propranolol hydrochloride and pindolol; B, oxyfedrine hydrochloride and alprenolol hydrochloride; C, acebutolol hydrochloride; D, atenolol.

Table 1
Assay results of beta-adrenergic blocking drugs by the carbon disulphide method and UV-spectrophotometric method in pure and tablet forms

Sample*	Found† (% ± SD)	
	Carbon disulphide method	UV-spectrophotometric method
Atenolol		
Drug substance	100.20 ± 0.77	99.94 ± 0.44
Tablet (i)	101.11 ± 0.59	100.63 ± 0.44
	t = 0.647 (2.306) and F = 3.038 (6.39)‡	
Propranolol HCl		
Drug substance	100.69 ± 0.68	100.26 ± 0.50
Tablet (ii)	99.16 ± 0.30	98.73 ± 0.49
	t = 1.235 (2.306) and F = 1.821 (6.39)	
Oxyfedrine HCl		
Drug substance	99.97 ± 0.34	100.03 ± 0.48
Tablet (iii)	98.35 ± 0.34	98.51 ± 0.42
	t = 0.227 (2.306) and F = 1.991 (6.39)	
Pindolol		
Drug substance	100.04 ± 0.39	99.81 ± 0.75
Tablet (iv)	100.33 ± 0.50	101.14 ± 0.75
Tablet (v)	99.15 ± 0.56	101.54 ± 0.20
	t = 1.588 (2.306) and F = 3.653 (6.39)	
Acebutolol HCl		
Drug substance	99.99 ± 0.88	99.96 ± 0.64
Tablet (vi)	98.20 ± 0.51	98.42 ± 0.36
	t = 0.061 (2.306) and F = 1.875 (6.39)	
Alprenolol HCl		
Drug substance	100.04 ± 0.39	100.03 ± 0.57
	t = 0.032 (2.306) and F = 2.130 (6.39)	

*The samples were (i) Tenormin tablets (Kahira Co., Egypt) labelled to contain 100 mg atenolol; (ii) Inderal tablets, (Kahira Co., Egypt) labelled to contain 10 mg propranolol hydrochloride; (iii) Ildamine fort tablets (Kahira Co., Egypt) labelled to contain 24 mg oxyfedrine hydrochloride; (iv) Visken tablets (Sandoz) labelled to contain 5 mg pindolol; (v) Viskaldix tablets (Swiss Pharma) labelled to contain 10 mg pindolol and 5 mg clopamide; (vi) Sectral tablets (Alexandria Co., Egypt) labelled to contain 200 mg acebutolol hydrochloride.
†Average of five experiments, recovery from the nominal or added drug content.
‡Values in parentheses are the theoretical values at P = 0.05.

of other constituents in commercial tablets interfering, the proposed method was compared with conventional UV-spectrophotometric methods [10–13]. When evaluated by the t and F-tests (Table 1) similar results were obtained. The proposed method can therefore be used for the quality control and routine analysis of those drugs investigated and possibly can be extended to the determination of other beta-adrenergic blocking drugs.

References

[1] E. Katcher and M. Voroshilova, *Anilinokrascohnaya Prom.* **4**, 39–41 (1934).
[2] H.C. Dowden, *Biochem. J.* **32**, 455–459 (1938).
[3] L. Nebbia and F. Guerrieri, *Chim. Ind. (Milan)* **35**, 896 (1953); *Chem. Abstr.* **48**, 3869C (1954).
[4] E.L. Stanley, H. Baum and J.L. Gove, *Anal. Chem.* **23**, 1779–1782 (1951).
[5] F.E. Critchfield and J.B. Johnson, *Anal. Chem.* **28**, 430–436 (1956).
[6] G.R. Ubreit, *Anal. Chem.* **33**, 1572–1573 (1961).
[7] D.H. Karweik and C.H. Meyers, *Anal. Chem.* **51**, 319–320 (1979).
[8] M. Qiu, *Yaoxue Tongbao* **21**, 144 (1986); *Anal. Abstr.* **49**, 1E37 (1987).
[9] B.C. Verma, S. Chauhan, A. Sood, D.K. Sharma and H.S. Sidhu, *Talanta* **32**, 139–143 (1985).
[10] C. Vetuschi, G. Rango, P. Mazzeo and A. Mazzeo-Farina, *El Farmaco Ed. Prat.* **40**, 215–224 (1985).
[11] M.E. Mohamed, M.S. Tawakkol and H.Y. Aboul-Enien, *Spectrosc. Lett.* **15**, 609–621 (1982).
[12] N.M. Sanghavi and N.G. Jivani, *Talanta* **27**, 591–592 (1980).
[13] *British Pharmacopoeia 1980*, pp. 374 and 815. HMSO, London (1980).
[14] B. Flouvat, M. Bazin, M. Lucsko, A. Roux and J. Guedon, *Ann. Biol. Chim.* **36**, 339–346 (1978).
[15] M. Jovanovic, D. Radulovic and L. Zivanovic, *Acta Pol. Pharm.* **44**, 322–326 (1987).
[16] M.A. Korany, M.H. Abdel-Hay, S.M. Galal and M.A. El-Sayed, *J. Pharm. Belg.* **40**, 178–184 (1984).
[17] D. Radulovic, M. Jovanovic and R. Milosevic, *Acta Pharm. Jugosl.* **34**, 169–175 (1984).

[18] S. Decourt and B. Flouvat, *J. Chromatogr.* **174**, 258–263 (1979).
[19] J. Poey and P. Puig, *Analusis* **14**, 421–426 (1986).
[20] V. DasGupta, *Drug Dev. Ind. Pharm.* **11**, 1931–1937 (1985).
[21] M.A. Lefebvre, J. Girualt and J.B. Fourtillan, *J. Liq. Chromatogr.* **4**, 483–500 (1981).
[22] H. Salomies and J. Halmekoski, *Acta Pharm. Fenn.* **91**, 113–118 (1982).
[23] H. Ehrsson, *J. Pharm. Pharmac.* **28**, 662–663 (1976).
[24] H. Auterhoff and R. Stank, *Dt. Apoth. Ztg.* **116**, 1596–1597 (1976).
[25] U.M. Abbasi, F. Chand, M.I. Bhanger and S.A. Memon, *Talanta* **33**, 173–175 (1986).
[26] *European Pharmacopoeia 1969*, Vol. I, pp. 220 and 212. Maisonneuve S.A. 57-Sainte Ruffine, France (1969).
[27] K.S. Tung and D.H. Karweik, *Anal. Chem.* **52**, 1387–1389 (1980).

[*Received for review 12 October 1989*]