Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 1, pp. 37–42, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00
Pergamon Press Ltd

Spectrophotometric determination of promethazine by flow injection analysis and oxidation by Ce^{IV}

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Abstract: A flow injection analysis (FIA) procedure is proposed for the determination of promethazine. The sample solution is directly injected into the carrier–reagent stream which comprises a solution of ceric ions in a sulphuric acid medium. The absorbance at 514 nm from the red colour developed by the oxidation of promethazine is measured. Effects of foreign substances have been investigated and the procedure has been applied to the determination of promethazine in a pharmaceutical formulation (tablets).

Keywords: *FIA; cerium(IV); promethazine hydrochloride; spectrophotometry.*

Introduction

Several *N*-substituted phenothiazines are commonly employed in medicine and therefore their determination in pharmaceutical preparations is of considerable importance. Promethazine hydrochloride, 10-(2-(dimethylamino)propyl) phenothiazine monohydrochloride, is one of those substances. This drug possesses the properties and uses of the antihistamines but it also has some anticholinergic, antiserotoninergic and marked local anaesthetic properties. It has been formulated in preparations such as elixirs, injections, syrups and tablets.

For allergic conditions promethazine is usually administered by mouth. In severe allergies it may also be given by deep intramuscular injection or, in an extreme emergency, by slow intravenous injection. The drug is given by intramuscular injection for anaesthetic premedication. It is used in cough capsules and linctuses and locally as a cream for the treatment of allergic skin conditions [1].

Previously proposed methods for the determination of promethazine include spectrophotometry. Pellerin *et al.* [2] describe a method which involves the formation of ion-pairs of promethazine with lauryl sulphate or dioctyl sulphosuccinate; these ion-pairs are extracted from aqueous solution by an immiscible organic solvent and the concentration of promethazine is determined by UV spectrophotometry. Promethazine hydrochloride can be determined photometrically after extraction as an ion-pair with methyl orange [3]. Methods involving separation before spectrophotometric determination by partition column chromatography [4], ion-exchange chromatography [5] and reverse partition column chromatography [6] have also been reported. Gas chromatography has been used to analyse promethazine and to separate it from other phenotiazines [7]. Titrimetric procedures are widely used; these are mainly based upon titration with perchloric acid in non-aqueous solution and such methods are recommended by some pharmacopoeias. The determination of some phenothiazines by amperometric titration has been proposed [8, 9]. Patriarche [10] investigated the feasibility of determining various *N*-phenothiazines by coulometric titration with electrogenerated cerium (IV), manganese (III) and bromine.

The present work is concerned with the determination of promethazine with the aid of a flow injection assembly. The method is based upon the oxidation of promethazine by means of ceric ions in a sulphuric acid medium; oxidation yields a red colour which is measured

Scheme

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at 514 nm. The procedure is simple and rapid and is proposed for the control analysis of the drug in a pharmaceutical preparation.

Experimental

Reagents and apparatus

Promethazine hydrochloride (Laboratorios B.O.I. S.A.), Ce^{III} chloride AR (Merck, Darmstadt, FRG); ammonium Ce^{IV} nitrate, AR (Merck), sulphuric acid AR (Panreac), citric acid (Probus), sodium sulphite (Probus), sucrose (Panreac), sorbitol (Scharlau), mannitol (Probus), caffeine (Eastman, Kodak, Rochester, NY) and potassium chloride AR (Panreac).

Carrier–reagent stream. 3.03×10^{-4} M Ce^{IV} in 0.2 M sulphuric acid was chosen as a suitable solution for the spectrophotometric determination of promethazine. The promethazine solutions to be injected were dissolved in 0.2 M sulphuric acid.

FIA assembly. The sample solution was directly injected into the carrier–reagent stream and the absorption was measured at 514.0 nm with the aid of a CE 2021 spectrophotometer (Cecil Instruments) fitted with a 18-μl flow-cell (Hellma) and a Rheodyne 5041 injection-valve. Teflon coils were 268 cm × 0.5 mm i.d. The flow-rate was 4.0 ml min^{-1}.

In preliminary experiments, absorption spectra under static conditions were obtained with a Hewlett–Packard 8452A diode-array spectrophotometer.

Procedures

Calibration graph for promethazine. A 226.1 μl volume of promethazine solution in 0.2 M sulphuric acid was injected into the carrier–reagent stream. Absorbance values were measured at 514.0 nm and the peak-height was recorded.

Modified simplex method of optimization. The program of the modified simplex method was operated with six vertices and was written on the basis of published work [11–13]. The initial simplex was chosen according to the method of Yarbro and Deming [11] with a side length of 1 and the vertex at the origin of the coordinates. The region of the variables was normalized by the modified method proposed by Morgan and Deming [12].

Results and Discussion

Study of the reaction

A common property of all *N*-substituted phenothiazines is that they are easily oxidized, either chemically or electrolytically. Preliminary experimental results showed that oxidation occurred immediately to produce a red colour that faded continuously. The red colour with maximum absorbance at 514 nm was developed from the colourless solution of promethazine and was strongly dependent on experimental factors such as: the nature of the mineral acid; the concentration of the acid, ceric ions or promethazine; temperature; and time.

The oxidation of promethazine with Ce^{IV} was studied in different acidic media (nitric, sulphuric and perchloric acid) yielding immediately a red colour that faded slowly. The results are depicted in Fig. 1 in which absorbances at 514 nm were recorded at 15 s intervals starting from 3 min after mixing the aqueous drug solution with Ce^{IV} in the acidic medium. The intensity of the red colour was higher in the presence of sulphuric acid but the colour faded more rapidly.

The development of a red colour that fades continuously is in accordance with the work of Duchinski [14] who established that oxidation proceeds in two 1-electron stages. These two oxidation stages, typical of *N*-substituted phenothiazines, were confirmed and illustrated by chronopotentiograms [15]. The first stage results simply from the loss of an electron from the parent compound and produces the corre-

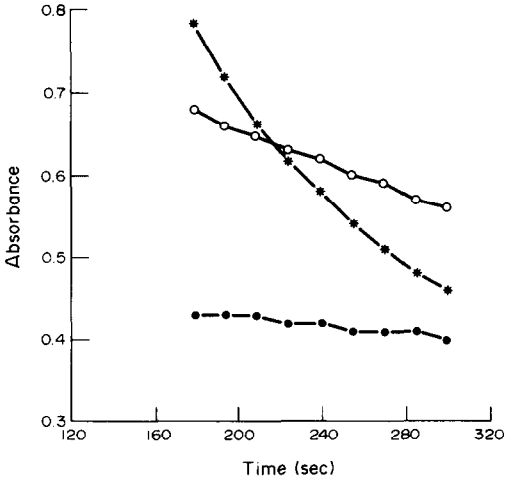

Figure 1
Influence of the acid medium on the oxidation of promethazine with Ce^{IV}. *, H_2SO_4; ○, HNO_3; ●, $HClO_4$.

sponding free radical cation which is intensely red. The second stage is a further 1-electron oxidation of the free radical to a colourless sulphoxide. The chronopotentiograms of all these compounds also showed a definite but rather attenuated third wave which evidently corresponds to further oxidation; this third reaction probably involves oxidation of side-chains rather than oxidation of the sulphoxide to the sulphone. Oxidation by ammonium persulphate also yielded a red colour which has been used for the determination of promethazine by heating the solution at 45°C in a water-bath for 15 min [16].

A study of the effects of temperature was carried out by preparing a series of solutions containing 69.7 ppm of promethazine and 2.72×10^{-4} M Ce^{IV} in 0.30 M sulphuric acid; the stock solutions were placed in a water-bath and, when thermal equilibrium was attained, the corresponding mixture was prepared and the absorption spectra were recorded at 1.5 min. The range of temperatures studied was 21.0–81.0°C. The reaction rate was greatly influenced by temperature; the red colour faded faster as the temperature was increased. Room temperature was selected for further work.

The influence of the concentration of sulphuric acid on the reaction was carried out by preparing a series of experiments in which the concentration of oxidant was kept constant at 1.66×10^{-4} M and the concentration of acid was varied over the range 0.03–5.8 M. The results were compared with those obtained from a similar series of experiments but without Ce^{IV}.

The red colour was also observed in sulphuric acid media without Ce^{IV}; a slightly red solution was immediately developed in high concentrations of the acid. However, the spectra of the solution containing 20.98 ppm of the drug in diluted sulphuric acid (0.2 and 0.4 M) were recorded over 8 days; the spectra remained constant and were very similar to those obtained from promethazine in distilled water.

The influence of sulphuric acid concentration on the oxidation of promethazine by ceric ions was examined. An increased absorbance was observed when the sulphuric acid concentration was increased from 0.03–0.1 M; then the absorbances increased slightly for concentrations up to about 1 M sulphuric acid, where a plateau was reached.

The importance of Ce^{IV} concentration was studied over the range 1.00×10^{-4}–2.00×10^{-3} M and for three different concentrations of sulphuric acid (0.2, 0.8 and 1.5 M). The results are depicted in Fig. 2; maximum intensity of the red colour was observed for Ce^{IV} concentrations over the range 5.00–8.00×10^{-4} M.

The development of a red colour in the reaction led to a study of a spectrophotometric method for the determination of promethazine; the transient characteristic of the colour is not a problem for a FIA procedure.

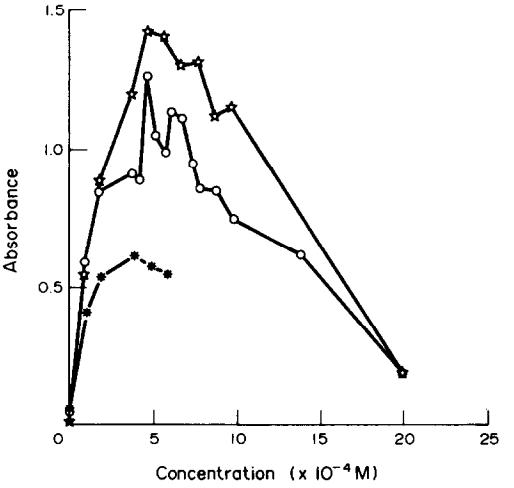

Figure 2
Influence of the concentration of Ce^{IV} at three different concentrations of sulphuric acid. *, 0.2 M; O, 0.8 M; +, 1.5 M.

Continuous-flow procedure

The chemical parameters studied for the manual method were revised for the flow injection procedure because kinetic behaviour is important in the latter. Preliminary FIA experiments were carried out by means of a single-channel assembly in which the aqueous sample solutions were directly injected into a Ce^{IV}–sulphuric acid solution as a carrier–reagent.

Positive transient signals accompanied by two negative peaks were observed; even the injection of pure distilled water gave a clear transient signal. Those results due to the optical characteristics (variation in the refractive index and optical density) of both solutions, sulphuric and pure distilled water, led to tests with a FIA procedure in which the drug was dissolved in sulphuric acid solution in a concentration similar to that of the carrier–

reagent. Experiments on optimization of the wavelength (508–520 nm) resulted in the choice of 514.0 nm as the wavelength for the procedure.

The influence of sulphuric acid on the sample solution and carrier–reagent was tested by injecting 90.0 μl of 51.48 ppm promethazine into a carrier–reagent stream of: 1.01×10^{-4} M Ce^{IV}; and sulphuric acid only. The flow-rate was 2.3 ml min^{-1} and the reaction-coil length was 150 cm plus 26 cm for the single-bed string reactor (Teflon tubing filled with glass beads). The sulphuric acid concentration was tested over the range 0.1–2.2 M; outputs from the series without Ce^{IV} were very small even for the upper concentrations of sulphuric acid. The transient signals from both series of experiments were compared; highest differences were observed for the range 0.3–1.1 M which was pre-selected for optimization of the concentration of Ce^{IV}.

Concentrations of Ce^{IV} up to 5.0×10^{-4} M were studied. Three sets of experiments with 0.3, 0.6 and 1.1 M H_2SO_4 were carried out; the FIA parameters were similar to those reported above. Figure 3 depicts the results from one series in which the influence of the concentration of Ce^{IV} in 0.3 M sulphuric acid was studied. Concentrations of sulphuric acid greater than 0.6 M resulted in no further increase in output. The working concentrations selected for further optimization were: H_2SO_4, 0.3–0.6 M; and Ce^{IV}, $1.01–2.02 \times 10^{-4}$ M.

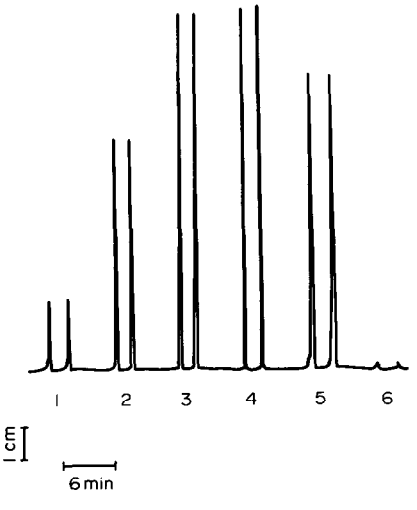

Figure 3
Influence of Ce^{IV} concentration on the transient signals. Ce^{IV} concentration $\times 10^{-4}$: 1 = 4.03, 2 = 3.02, 3 = 2.52, 4 = 2.02, 5 = 1.1, 6 = 0.0.

The FIA parameters, reaction-coil length, sample loop and flow-rate were optimized by means of the modified simplex method and on the basis of the results from the chemical parameters tested previously. The transient signal to be optimized was that giving the best compromise between peak-height, reproducibility and sample throughput.

After a stable chart-recorder base-line had been obtained, a 51.48 ppm promethazine solution was injected into the carrier–reagent of 1.64×10^{-4} M Ce^{IV} and 0.4 M sulphuric acid; the resulting absorbance at 514.0 nm was measured. This was repeated until a relative standard deviation (RSD) of <1% was obtained for the peak-height (five replicates were usually sufficient). The following variables were investigated over the ranges stated: flow-rate, 0.5–4 ml min^{-1}; sample volume, 90.0–300.0 μl; and reaction coil length, 26–400 cm.

The first vertex of the initial simplex gave a peak-height of 31.8 mm; after 33 experiments it was decided that the system did not merit further experimentation and that point 17 (123.6 mm peak-height) was the optimum that could be obtained. Zero values of peak-height were assigned to the entries which were not true (doubled peaks) or were out of the variable range. The results are shown in Table 1. The flow injection parameters corresponding to the selected point were: flow-rate, 4.0 ml min^{-1}; sample volume, 226.1 and reaction-coil length, 268 cm.

The sulphuric acid and Ce^{IV} concentrations were re-optimized on the basis of the results obtained from the modified simplex method. Former experiments resulted in the preselection of: 0.2 M H_2SO_4 and 3.03×10^{-4} M Ce^{IV}; and 0.4 M H_2SO_4 and 3.76×10^{-4} M Ce^{IV} as the optimum parameters. The final selection of concentrations was carried out by preparing calibration graphs for promethazine with six points and 10 replicates of each point. No significant difference in slope was detected but the reproducibility was most favourable for 0.2 M H_2SO_4 and Ce^{IV} 3.03×10^{-4} M as the carrier–reagent.

Analytical application

The analytical application of the continuous flow procedures was studied to establish the application range, reproducibility, detection limit and sample passage.

The calibration graph was linear over the range 10.3–51.3 ppm of promethazine and

Table 1
Modified simplex method of optimization. R = reflection, C = contraction and E = expansion. The peak height of zero is assigned to numbers outside of variable range

Cycle type	Point no.	Flow-rate (ml min^{-1})	Coil length (cm)	Samle size (μl)	Peak height (mm)	Current simplex point
	1	0.50	26.0	90	31.8	—
	2	1.32	114.2	288.0	0.0	—
	3	1.32	378.6	139.5	69.2	—
	4	3.80	114.2	139.5	118.8	1,2,3,4
R	5	2.42	231.7	42.0	0.0	—
C	6	1.60	142.9	205.5	102.7	1,6,3,4
2/R	7	3.98	398.2	233.0	119.5	—
E	8	5.72	584.8	304.4	0.0	7,6,3,4
3/R	9	4.93	57.7	245.8	0.0	—
C	10	2.23	298.4	166.1	101.0	7,6,10,4
4/R	11	4.03	137.9	219.2	122.0	—
E	12	4.93	57.7	245.8	0.0	7,6,11,4
5/R	13	6.27	290.2	189.0	0.0	—
C	14	2.77	179.7	201.4	115.6	7,14,11,4
6/R	15	5.10	253.3	193.3	0.0	—
C	16	3.35	198.1	199.3	115.6	17,18,11,19
Simplex reduct.	17	4.00	268.1	226.1	123.6	—
Simplex reduct.	18	3.40	158.8	210.3	116.0	—
Simplex reduct.	19	3.91	125.7	179.4	120.0	—
7/R	20	4.56	196.2	206.2	0.0	—
C	21	3.69	168.0	209.3	116.4	17,21,11,19
8/R	22	4.27	187.0	207.21	0.0	—
C	23	3.83	172.6	208.8	117.0	17,23,11,19
9/R	24	4.13	181.8	207.7	0.0	—
C	25	3.91	174.9	208.9	118.0	17,25,11,19
10/R	26	4.05	179.5	208.0	120.0	17,26,11,19
11/R	27	3.91	174.9	208.5	118.0	—
C	28	4.02	178.4	208.1	117.4	17,29,30,31
Simplex reduct.	29	4.02	223.8	217.1	117.1	—
Simplex reduct.	30	4.02	203.0	222.7	119.1	—
Simplex reduct.	31	3.96	196.9	202.7	120.0	—
12/R	32	3.96	221.5	217.3	112.6	—
C	33	4.01	232.2	217.1	113.0	—

could be described by the equation $h = 0.0079X + 0.0809$ (where h is the peak height in mm and X the concentration of promethazine in ppm), with a correlation coefficient of 0.995.

Forty different samples containing 35.0 ppm of promethazine were injected into the carrier–reagent stream in order to determine the RSD and sample passage; the results obtained were: RSD = 1.2%; and 122 samples per h.

The tolerance of the method to foreign compounds which can be found in typical pharmaceutical preparations containing promethazine was investigated by using solutions similar to those used for the reproducibility studies and adding various concentrations of the interfering compounds. The results (concentration and relative error) obtained for various interfering compounds were: citric acid, 101 ppm, 3.3%; sodium sulphite, 5 ppm, 4.3%; sucrose, 302 ppm, 0.0%; sorbitol, 106 ppm, 3.3%; mannitol, 26 ppm, 2.1%; caffeine, 300 ppm, 3.1%; and ascorbic acid, 3 ppm, 2.5%. Ascorbic acid and sodium sulphite gave high errors; however, as aqueous solutions of promethazine can be gently heated without risk, both sources of interference can be previously eliminated.

In order to minimize the volume of the carrier–reagent solution required per sample unit (high price of Ce^{IV} salts and environmental caution about sulphuric acid waste) recycling of the carrier stream was attempted by preparing an aliquot of the carrier–reagent solution, which was continuously re-used, and injecting 35.0 ppm of promethazine in sample solutions. The spectrophotometric measurements resulted in a RSD of 2.5% for 132 replicates and 500 ml of carrier–reagent.

The promethazine content of Fenergan tablets (Rhone-Poulenc Farma S.A.E.) was determined. The tablets were powdered by grinding in an agate mortar and pestle. Aliquots of the powdered tablets were transferred to a beaker and shaken with distilled water. The resulting mixture was filtered and diluted

to an adequate volume. Up to 15 replicate injections were performed with the soluble extract from each aliquot. At least five different preparations were analysed and the results were compared with those provided by the manufacturer. Declared content 25 mg per tablet. Found: 25.3 mg per tablet (RSD = 1.0%).

References

[1] Martindale, *The Extra Pharmacopoeia*, 27th Edn, pp. 1289–1291. The Pharmaceutical Press, London (1978).
[2] F. Pellerin, D. Mancheron and D. Demay, *Ann. Pharm. Franc.* **30**, 429–432 (1972).
[3] L. Nyberg, *J. Pharm. Pharmacol.* **22**, 500 (1970).
[4] A.R. Sperling, *J. Pharm. Sci.* **56**, 98–101 (1967).
[5] D.J. Smith, *J. Ass. Off. Analyt. Chem.* **55**, 596–598 (1972).
[6] K. Wahlund and K. Gronengsson, *Acta Pharm. Suecica.* **7**, 615–618 (1970).
[7] A. Deleenheer, *J. Chromat.* **74**, 35–39 (1972).
[8] H. Beral, L. Murea, M. Madgearu and E. Cuciureanu, *Acta Pharm. Yugoslav.* **15**, 77–82 (1965).
[9] G.J. Patriarche, *J. Pharm. Belg.* **24**, 533–534 (1969).
[10] G.J. Patriarche, *Mikrochim. Acta* **65**, 950–954 (1970).
[11] L.A. Yarbro and S.N. Deming, *Analyt. Chim. Acta* **73**, 391–396 (1973).
[12] S.L. Morgan and S.N. Deming, *Analyt. Chem.* **46**, 1170–1181 (1974).
[13] J.A. Nelder and R. Mead, *Computer J.* **7**, 1308–1310 (1965).
[14] G. Duchinski, *Die Pharmazie* **13**, 478–480 (1958).
[15] G.J. Patriarche and J.J. Lingane, *Analyt. Chim. Acta* **49**, 25–33 (1970).
[16] C. Fossoul, *J. Pharm. Belg.* **7**, 308–331 (1952).

[*Received for review 17 October 1990*]