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DETERMINATION OF LOW LEVELS OF PHENOTHIAZINE SULPHOXIDES IN PHENOTHIAZINE DRUG SUBSTANCES AND FORMULATIONS BY THIN-LAYER CHROMATOGRAPHY–SECOND-DERIVATIVE SPECTROFLUORIMETRY

E. O. FADIRAN*,* and A. G. DAVIDSON
Department of Pharmacy, School of Pharmacy and Pharmacology, University of Strathclyde, Glasgow G1 1XW (U.K.)
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SUMMARY

A thin-layer chromatographic–second derivative spectrofluorimetric procedure has been developed for low levels of sulphoxide (down to 0.01%, w/w) in phenothiazine drug substances and formulations. The method has been applied to prochlorperazine maleate, chlorpromazine hydrochloride and promethazine hydrochloride. It has also been applied to pharmaceutical formulations of promethazine hydrochloride and comparison of the results with those obtained by a published difference spectrophotometric procedure for promethazine sulphoxide showed that there was good agreement. The method is simple, rapid, accurate and precise and seems to have general application to the determination of low levels of sulphoxide in phenothiazine drug substances or formulations.

INTRODUCTION

Phenothiazines are among the most widely used drugs in medical practice; they are used primarily in the treatment of psychiatric disorders[1]. Other members of the phenothiazine group of drugs have other clinically useful properties, including antiemetic, antinausea and antipruritic activity and the ability to potentiate analgesics. Phenothiazines are photochemically unstable compounds and on exposure to humidity and sunlight they undergo photooxidation to form their sulphoxide derivatives, and under extreme conditions sulphone derivatives, both in the solid dosage form and, in particular, in aqueous formulations[2]. The British Pharmacopoeia (BP) monographs[3] specify limits for certain impurities and degradation products in various phenothiazine drug substances and some of their formulations. Several methods have been proposed in various compendia and by various workers for the determination of phenothiazines in pharmaceutical formulations and biological fluids[4–6]. However, a common feature of most of these methods [with the exception of indi-

* Present address: Department of Pharmaceutical Chemistry, School of Pharmacy, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria.

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vidual gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods] is their lack of specificity for parent phenothiazines in the presence of the photodegradation products. In addition, most of the published methods for the determination of the sulphoxide degradation products are not sensitive enough to determine accurately low levels of the sulphoxide in phenothiazine drug substances or in fresh aqueous phenothiazine formulations or tablet formulations.

Apart from the thin-layer chromatographic (TLC) systems prescribed in the BP[3], a number of investigators have described other TLC systems that may be useful in the identification and detection of phenothiazines and their sulphoxide metabolites[7]. A quantitative TLC–UV spectrophotometric procedure has been reported for butaperazine in pharmaceutical formulations[8,9] and trifluoperazine sulphoxide and cyanopromazine sulphoxide have been determined by means of a differential-pulse polarographic method after TLC separation from their parent compounds and pharmaceutical excipients, which interfere with the direct assay procedure[10].

This paper reports a quantitative TLC–second derivative spectrofluorimetric procedure for the determination of low levels of sulphoxides in phenothiazine drug substances and formulations.

EXPERIMENTAL

Materials

Chlorpromazine hydrochloride (CP), chlorpromazine sulphoxide (CPO), promethazine hydrochloride (PM), promethazine sulphoxide (PMO), prochlorperazine maleate (PC) and prochlorperazine sulphoxide (PCO) were obtained as gifts from May and Baker (Dagenham, U.K.). Promethazine syrup, tablet and injection formulations were manufactured by May and Baker.

All reagents and solvents were of analytical-reagent grade.

The materials and glassware used for TLC included TLC plastic sheets, silica gel 60 F_{254} (0.20 mm, E. Merck, Darmstadt, F.R.G.), a Panglas–Shandon Chromatotank lined with solvent-saturated filter-paper, a Mineralight TLC viewing box, a Rotamixer Deluxe (Hook and Tucker Instruments) and an MSE Minor S centrifuge.

Instrumentation

Uncorrected zero-order and second-derivative fluorescence excitation spectra were recorded simultaneously on a Lloyd Instruments CR 6255 twin-pen recorder using a Perkin-Elmer 650-40 spectrofluorimeter in series with a Hitachi analogue derivative assessory (200-0507).

Development solvent

The development system was cyclohexane–acetone–diethylamine (80:10:10). The chromatogram was developed at 20°C in the dark (to prevent photodegradation) for a distance of 15 cm (time taken, *ca.* 1 h).

Preparation of standard and sample solutions

Solutions of phenothiazine sulphoxides (100 $\mu g\ \mu l^{-1}$) in chloroform were diluted with chloroform to give final concentrations of 2, 4, 8, 12, 16 and 20 ng μl^{-1}. By

using a microsyringe (25 μl), an aliquot (20 μl) of each of the solutions was spotted on to a silica gel GF_{254} pre-coated plastic sheet such that the diameter of each of the spots did not exceed 4 mm and the spots were 2 cm apart and not closer than 2 cm to the edges of the plate.

Duplicate aliquots (20 μl) of the solution of the phenothiazine drug in chloroform (20 $\mu g\ \mu l^{-1}$) were also spotted, together with the appropriate phenothiazine sulphoxide solution (allowing at least 3 cm between the highest loading of the sulphoxide and the spots of the phenothiazine drug substance). After the development of the chromatograms, the standard sulphoxide spots and the spots corresponding to sulphoxide from the sample solution of the phenothiazine drug substance were located by examination of the plate under UV light (254 nm). A 1 × 1 cm square was marked round each spot and this was cut by using a pair of scissors and transferred to a centrifuge tube. A 5-ml volume of 0.05 *M* sulphuric acid was added to each of the centrifuge tubes, and elution of the sulphoxide from the adsorbent was effected by agitating the centrifuge tube on the rotamixer for 5 min and the extracts were centrifuged. Duplicate blank solutions were prepared by using areas of adsorbent (1 × 1 cm) between the first three sample spots at the R_F of the sulphoxide. The supernatant solution of each extract was transferred into a standard 1-cm path length silica quartz fluorescence cuvette and the second-derivative excitation fluorescence spectrum was recorded from 200 to 350 nm by using the following instrumental parameters: excitation slit, 8 nm; emission slit, 20 nm; scan speed, 400 nm min^{-1}; emission wavelength, 380 nm; and derivative mode, 5. The concentration of the sulphoxide in the sample of the phenothiazine drug substance was then determined using the regression equation obtained from standard loadings of sulphoxide and the appropriate derivative amplitude measured in the second-derivative excitation spectra.

RESULTS AND DISCUSSION

The zero-order and second-derivative excitation fluorescence spectra of CPO in 0.05 *M* sulphuric acid are shown in Figs. 1 and 2, respectively, and are typical of those of the three sulphoxides examined. The excitation spectrum was chosen in preference to the emission spectrum because of the presence in the former of four narrow excitation bands, three of which are free from Raman scatter interference while the only emission band for all the sulphoxides suffers interference from both Raman and Rayleigh scattered light when measured at the wavelength of maximum excitation, 335 nm.

The characteristics of derivative spectroscopy are that it discriminates in favour of substances with a narrow spectral bandwidth[11,12]. The derivative technique has been applied mostly to UV–visible absorption spectroscopy[13–15] but has recently been applied to fluorescence spectroscopy[13,16–18]. The use of non-optimum wavelengths of excitation or emission to reduce the distortion in the zero-order excitation or emission spectra by the scatter bands was found to result in a decrease in sensitivity of the assay. The use of second-derivative spectra for the determination of such substances as phenothiazine sulphoxides, which have narrow bandwidths, increases the sensitivity and discriminates against the broad band distortion by the reagents.

Several solvent systems were screened for their abilities to separate the sulphoxide from their undegraded parent compound and other possible degradation

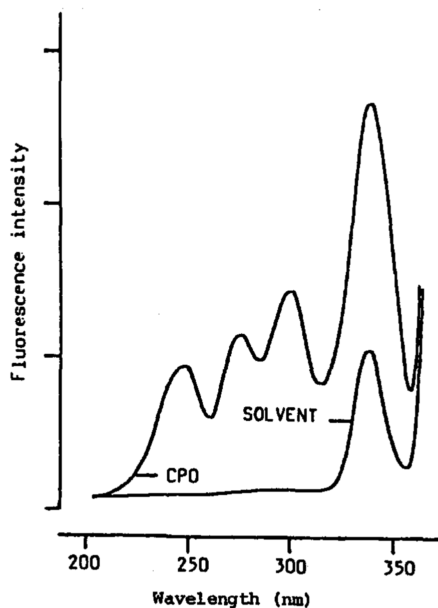

Fig. 1. Zero-order excitation spectrum of chlorpromazine sulphoxide (100 ng ml^{-1}) superimposed on that of the blank (0.05 *M* sulphuric acid). λ_{em} = 380 nm; excitation slit = 8 nm; emission slit = 20 nm.

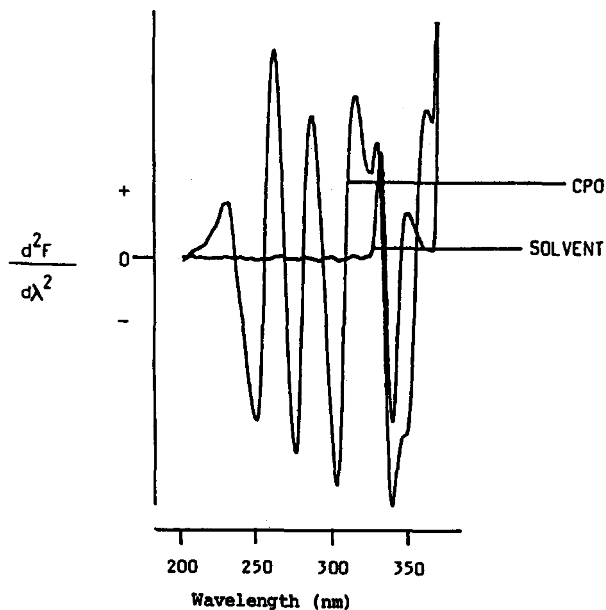

Fig. 2. Second-derivative excitation spectrum of chlorpromazine sulphoxide (100 ng ml^{-1}) superimposed on that of the blank (0.05 *M* sulphuric acid). λ_{em} = 380 nm; excitation slit = 8 nm; emission slit = 20 nm; derivative mode, 5; scan speed, 400 nm min^{-1}.

products or impurities such as phenothiazine sulphone. The best resolution was obtained by using cyclohexane–acetone–diethylamine (80:10:10), in which the R_F values of the sulphoxide derivatives of CP, PCP and PM were 0.11, 0.09 and 0.09, respectively, those of the sulphone derivatives were 0.17, 0.15 and 0.16, respectively, and those of the parent drugs were 0.51, 0.41 and 0.42, respectively. The maximum possible loading of the parent phenothiazine was found to be greater than 500 μg but a maximum load of 400 μg was used. The minimum detectable concentration of the phenothiazine sulphoxide was less than 20 ng, which represents a detection limit of 0.005% (w/w) or 50 $\mu g\ g^{-1}$ (50 ppm).

The choice of the eluting solvent was based on the recovery of the sulphoxides (100 ng) spotted on to the chromatographic plate and extracted into various solvent systems without developing the chromatogram. The precision of extraction into organic solvents such as chloroform and 1,2-dichloroethane was very poor. The recoveries in acidic solutions (hydrochloric and sulphuric acids) appear to be dependent on the strength of the acid, with 0.05 and 0.1 *M* sulphuric acid giving the highest recoveries. 0.05 *M* sulphuric acid was chosen as the eluting solvent because the fluorescence quantum yield of the sulphoxides was found to decrease with increasing acid strength.

Two methods of eluting the sulphoxides from the chromatographic plate were investigated. The first involved scraping an area of the silica gel (1 × 1 cm) containing the sulphoxide (100 ng) spot into a centrifuge tube and then extracting with 5 ml of 0.05 *M* sulphuric acid and the second involved cutting an area of the plastic-backed plate (1 × 1 cm) containing the sulphoxide spot (100 ng), transferring it to a centrifuge tube and extracting it with 5 ml of 0.05 *M* sulphuric acid. After centrifugation the supernatant liquids were assayed for sulphoxide and the percentage recoveries were determined. The mean and the relative standard deviation (R.S.D.) of six replicate recoveries of CPO from the chromatographic plate by using the first procedure were 77.82% and 5.26%, respectively, and those obtained by using the second method were 79.86% and 4.98%, respectively. Although the two procedures gave similar CPO recoveries and precision, the second method was adopted because it is more convenient and simpler. There was no improvement in the recovery of CPO when the extraction period was increased to 15 min. A 5-min extraction period was therefore regarded as optimum and was used.

Calibration graph

Derivative amplitudes were measured from the minimum in the derivative spectrum at the specified wavelength to the shorter (D_S) or longer (D_L) wavelength satellite. Fig. 3 shows the calibration graphs obtained when the amplitude 272_S in the second-derivative excitation spectrum of CPO and amplitudes 272_L and 297_S in the second-derivative excitation spectrum of PCO were plotted against the loadings of CPO and PCO, respectively. Similar graphs were obtained for amplitudes 272_L and 297_S in the second-derivative excitation spectrum of CPO, for amplitudes 272_S and 297_S in the second-derivative excitation spectrum of PCO and for amplitudes 268_S, 268_L and 290_S in the second-derivative excitation spectrum of PMO. The results obtained indicate that there is a linear relationship between the measured derivative amplitudes and the loading of the sulphoxide. Because of the significant intercepts obtained, a six-point calibration graph was used rather than a single-point standard-

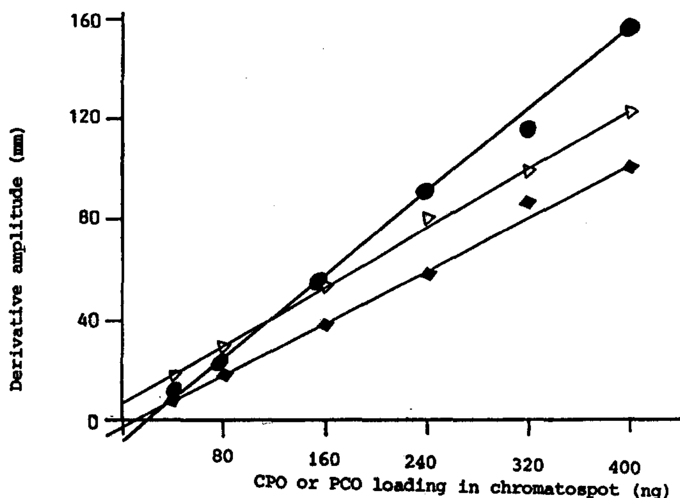

Fig. 3. Calibration graphs of derivative amplitude against the loading of chlorpromazine sulphoxide or prochlorperazine sulphoxide in the chromato spots. (▽) 297_S (PCO), $y = 0.29x + 7.68$, $n = 6$, $R = 0.9989$; (●) 272_S (CPO), $y = 0.40x - 6.68$, $n = 6$, $R = 0.9960$; (◆) 272_L (PCO), $y = 0.27x - 4.13$, $n = 6$, $R = 0.9977$.

ization. The concentration of the sulphoxide in the sample was then determined by using the appropriate regression equation for the measured derivative amplitude. In each instance the amplitude that gave the smallest intercept was considered to be the amplitude of choice. The regression coefficients for each amplitude were found to be reproducible. It was generally observed that the method could be readily used to quantify 40 ng of the sulphoxide, which represents 0.010% (w/w) of a 400-μg load of the parent phenothiazine drug substance.

The method was applied to the determination of low levels of sulphoxide in phenothiazine drug substances. Table I summarizes the results obtained for samples of CP, PM and PC. The precision of the method (RSD ≈5%) was considered to be satisfactory for such low levels of sulphoxide impurities. To assess the accuracy of the procedure, aliquots of CPO (0.08, 0.20, 0.40 μg) were spotted on top of a CP (400 μg) spot and the chromatograms were developed in the dark as described above. Table II gives the recoveries of the added CPO in CP after appropriate correction had been made for the sulphoxide content of the latter (0.014%, Table I). The results

TABLE I

CONCENTRATION OF SULPHOXIDE IN PHENOTHIAZINE DRUG SUBSTANCES

Parameter	*Chlorpromazine*	*Prochlorperazine*	*Promethazine*
R_F of sulphoxide	0.11	0.09	0.09
R_F of parent drug	0.51	0.41	0.42
Concentration of sulphoxide (%, w/w)	0.014	0.020	0.019
RSD (n = 6)	5.33	4.97	4.85

TABLE II

RECOVERY OF ADDED PROCHLORPERAZINE SULPHOXIDE (PCO) IN PROCHLORPERAZINE MALEATE (PC) SPOT

Composition of spot		$\frac{PCO}{PC} \times 100$ (%)	*Recovery of PCO in PC spot (%)*
PCO (μg)	*PC (μg)*		
0.08	400	0.02	97.00
0.20	400	0.05	95.68
0.40	400	0.10	96.28

obtained showed a satisfactory recovery of the added sulphoxide in CP and confirmed the accuracy of the method.

The method was also applied to the determination of sulphoxide in degraded PM formulations for which a direct second-derivative spectrofluorimetric assay could not be applied as a result of the instability of the parent promethazine on exposure to the high intensity UV radiation at the wavelength of determination[19,20]. An amount of the tablet or syrup formulation was weighed, diluted with water, basified with 5 *M* sodium hydroxide solution and extracted with chloroform. The chloroform extracts were then subjected to the chromatographic procedure described above. In addition to the PMO (R_F = 0.09) and the PM (R_F = 0.42), the chloroform extracts of the formulations gave two additional spots (R_F = 0.26 and 0.34, respectively). The compound with R_F = 0.34 has a different spectrum to that of PMO. The compound with R_F = 0.26 was found to have an identical second-derivative excitation spectrum with that of PMO; it is not the sulphone derivative of PM, which has an R_F value of 0.16 in this solvent system and which also has an identical spectrum with PMO. It was therefore decided that this compound (R_F = 0.26) should be quantified relative to PMO, as its real identity was not known. Hence a "total sulphoxide" content was determined for each of the formulations using the TLC–second derivative spectrofluorimetric procedure. The results obtained are shown in Table III. The

TABLE III

CONCENTRATIONS OF PROMETHAZINE SULPHOXIDE (PMO) IN DEGRADED PROMETHAZINE (PM) FORMULATIONS

Formulation	*Declared amount per unit dose*	*Storage conditions*	*PMO (as PM; % of label claim)**			
			D^2			ΔA
			a	*b*	*a + b*	
Syrup	5 mg/ml	Full bottle; fresh	0.2	0.1	0.3	N.D.
Syrup	5 mg/ml	2/3 full bottle; 5 years	13.1	N.D.	13.1	12.8
Injection	25 mg/ml	1-ml ampoule; 10 years	2.7	0.6	3.3	2.9
Tablet	25 mg	13 years	0.3	0.1	0.4	N.D.

* *a* = Spot with R_F = 0.09; *b* = spot with R_F = 0.26. D^2 = TLC–second derivative spectrofluorimetric procedure. ΔA = Difference spectrophotometric procedure for PMO. N.D. = None detected.

sulphoxide contents of the degraded PM formulations were also determined using a published difference spectrophotometric technique[21] and the results obtained are also given in Table III. The results obtained by using the difference spectrophotometric technique (limit of detection is 0.5% sulphoxide in the presence of the undegraded parent phenothiazine) showed reasonable agreement with those obtained for the "total sulphoxide" using the TLC–second derivative spectrophotometric procedure and confirm that the sulphoxide is the main degradation product of PM.

CONCLUSION

The proposed TLC–second derivative spectrofluorimetric procedure for the determination of low levels of sulphoxide in phenothiazine drug substances has been shown to be simple, rapid, accurate and precise. The method has obvious advantages over the BP[3] limit test, which involves the visual comparison of the intensity of colours of chromatographic spots of a known concentration of the parent phenothiazine with those of the sulphoxide impurities. The proposed method, which has also been applied to promethazine preparations, should be applicable to other degraded phenothiazines.

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