Journal of Chromatography, 285 (1984) 235–241
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,367

Note

High-performance thin-layer chromatographic determination of *cis*- and *trans*-chlorprothixene and two oxidation products

SEIJA TAMMILEHTO*, MATTI SYSMÄLÄINEN and PIRJO MÄKINEN
Division of Pharmaceutical Chemistry, School of Pharmacy, University of Helsinki, Fabianinkatu 35, SF-00170 Helsinki 17 (Finland)
(Received October 12th, 1983)

Thioxanthene derivatives are important neuroleptic drugs. The *cis* isomer is physiologically more active than the *trans* isomer[1–3]. Under the influence of light there is a rapid isomerization of the pure isomers, while more prolonged exposure causes the side-chain of the parent compound to split off, with formation of the corresponding thioxanthone[4]. The thioxanthone is also produced when aqueous solutions of the thioxanthenes are heated[5] or oxidized with cerium sulphate[6] or potassium permanganate[7]. Sodium metaperiodate and hydrogen peroxide primarily oxidize the sulphur atom in the ring, and sulphoxides and sulphones are formed. The principal metabolic reactions are likewise oxidative[8].

Various analytical techniques are available for the determination of the thioxanthenes. Fluorescence spectrometry provides a sensitive tool for the quantitation of the drugs after oxidation[9,10], but the method lacks selectivity. A widely used method is gas–liquid chromatography (GLC) with flame-ionization[11], electron-capture[12], nitrogen-selective[13] or mass spectrometric[14] detection. The simultaneous determination of the geometric isomers can be achieved by high-performance liquid chromatography (HPLC)[15,16].

High-performance thin-layer chromatography (HPTLC) permits separation of the *cis* and *trans* isomers of the thioxanthenes under suitable conditions[17]. The aim of the present study was to develop a specific method based on HPTLC for the quantitative analysis of *cis*- and *trans*-chlorprothixene, chlorprothixene sulphoxide and 2-chlorothioxanthone and to use the procedure to follow the photodecomposition of *cis*-chlorprothixene in aqueous solutions.

MATERIALS AND METHODS

Materials

cis-Chlorprothixene hydrochloride (*cis*-CP) was kindly supplied by Orion Oy (Espoo, Finland) and the *trans* isomer by H. Lundbeck & Co. (Copenhagen, Denmark). The compounds were used as received. All reagents and solvents were of analytical grade.

Chlorprothixene sulphoxide oxalate (CP-SO) and chlorprothixene sulphone oxalate (CP-SO_2) were synthesized by oxidizing *cis*-CP with sodium metaperiodate[18],

0021-9673/84/$03.00

and 2-chlorothioxanthone (CT) by oxidizing *cis*-CP with potassium permanganate[7]. The identity and purity of the oxidation products were verified by elemental analysis, TLC, melting point (Gallenkamp MF 370) and UV (Unicam SP 500 spectrometer), IR (Unicam SP 1000 infrared spectrometer) and ^{1}H NMR (Jeol JNM-PS-100 spectrometer)spectroscopy.

HPTLC conditions

HPTLC separations were performed on 10 × 10 cm precoated HPTLC silica gel 60 F_{254} plates (E. Merck, Darmstadt, F.R.G.). Before the analysis, the plates were developed with methanol, dried at room temperature and used immediately. Solutions (200 nl) were applied along two opposite sides of the plates, at a distance of 5 mm apart, by means of a variable-volume nano-applicator (Camag, Muttenz, Switzerland). The plates were eluted in a HPTLC linear developing chamber (Camag), the migration distance being 40 mm. Samples containing CT were first developed with the solvent system D (Table I), and thereafter with the solvent system A for quantitation of the basic compounds. The plates were dried at room temperature and stored protected from light.

Photometric measurements

Photometric measurements were carried out in the reflectance mode with a Zeiss PMQ II chromatogram spectrometer connected to a Servogor 310 recorder (BBC, Goerz, Austria). Spots were scanned in the direction of chromatography using a slit width of 0.8 mm and a slit length of 3.5 mm. The wavelength used for CT was 264 nm and that for other test substances was 228 nm. The scanning speed was 50 mm/min and the recorder speed 60 mm/min.

Calibration graph

To obtain the calibration graphs, a stock solution containing 1 mg/ml of *cis*-CP and CP-SO and 0.5 mg/ml of CT was prepared in ethanol–acetone (7:3). This solution was diluted to give the concentrations required for the calibration. Aliquots (200 nl) of each solution were applied in triplicate to the plate and chromatographed as described above. The calibration graphs were constructed in the ranges of 20–200 ng per spot for *cis*-CP and CP-SO and 10–100 ng per spot for CT. The calibration data were analysed using the log/log linear regression equation $\log y = A \log x + B$ where y = peak height and x = concentration.

Photodecomposition of cis-chlorprothixene

Aliquots (2 ml) of the 0.25–1.0% *cis*-CP solutions, prepared in 0.1 *M* hydrochloric acid or acetic acid (17 mg/ml) or acetate buffer at pH 4.6, were charged into clear 10-ml glass ampoules. The ampoules were exposed to ordinary daylight or to radiation from a high-pressure mercury lamp (Original Hanau TQ 150). At appropriate time intervals the contents of three parallel ampoules were diluted to exactly 10 ml in acetone. For the HPTLC analysis, aliquots (200 nl) of the sample solutions were applied to the plate alternately with a standard solution containing 0.5 mg/ml (0.05%) of *cis*- and *trans*-CP and 0.25 mg/ml (0.025%) of CT. The chromatography was performed as described above.

The amounts of individual compounds were calculated with the corresponding

regression equations. The peak heights of sample compounds were first corrected with a correction factor obtained by dividing the peak height of the 0.05% *cis*-CP solution (or 0.025% CT solution) from the calibration graph by the average peak height of all corresponding standards on the plate being analysed.

RESULTS AND DISCUSSION

Chromatography

The geometric isomers of CP as well as its oxidation products CP-SO and CT are well separated from one another with several solvent systems when conventional ascending chromatography is used (Table I). For the linear development a mixture of toluene–ethanol–water proved to be the most suitable, giving round, easily measured spots (Fig. 1). In most solvent systems CT migrated near to the solvent front, which disturbed the photometric measurement. A two-step development was therefore used when the samples contained CT. The first elution was carried out with toluene–carbon tetrachloride. After scanning of the spots of CT, a second development was performed with toluene–ethanol–water for the analysis of the basic compounds. The resolution of CP-SO and CP-SO_2 was too poor to permit their accurate simultaneous quantitation under the experimental conditions used.

TABLE I

R_F VALUES OF THE GEOMETRIC ISOMERS OF CHLORPROTHIXENE AND SOME OF ITS OXIDATION PRODUCTS

Silica gel 60 F_{254} plates (20 × 20 cm), ascending chromatography, migration distance 13 cm.

Solvent system		*R_F values*				
		cis-CP	*trans-CP*	*CP-SO*	*CP-SO_2*	*CT*
A	Toluene–ethanol–water (10:10:1)	0.51	0.44	0.25	0.33	0.91
B	Toluene–ethyl acetate–diethylamine (10:2:1)	0.73	0.67	0.56	0.48	0.91
C	Toluene–dioxane–25% ammonia (6:3.5:0.5) (upper phase)	0.54	0.44	0.33	0.29	0.92
D	Toluene–carbon tetrachloride (7:3)	0	0	0	0	0.55

Before the analysis, the plates were prewashed with pure methanol to assure a sufficiently low background for photometric measurements. The reproducibility of the photometry was estimated by scanning the same lane of spots nine times (R.S.D. = 0.25–0.4%).

The calibration graphs were linear over the concentration range employed. The best fit for all calibration lines was found when the log/log linear regression technique described by Kaiser[19] was used for the analysis of the calibration data. The mean fit varied between 2.3 and 3.2% and the standard deviation between 1.8 and 2.8%. The calibration graph prepared for *cis*-CP was also used for the assay of the *trans* isomer, both compounds having similar UV absorption characteristics.

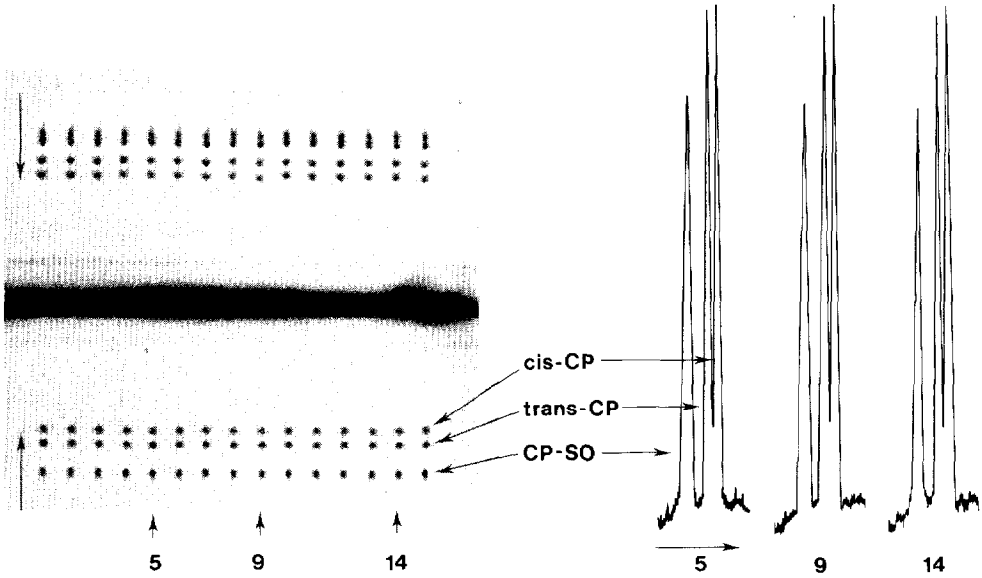

Fig. 1. Lower part, separation of *cis*- and *trans*-CP and CP-SO; upper part, separation of *cis*- and *trans*-CP, CP-SO and CP-SO_2. The densitograms on the right side were obtained by scanning the lane marked with the same number.

The accuracy and precision of the method were determined by analysing samples of known concentrations of *cis*- and *trans*-CP. The data collected in Table II show that the use of only one standard concentration on each plate gives satisfactory results in the concentration range studied.

Influence of light

Chlorprothixene is used therapeutically as the pure *cis* isomer. The influence of light on *cis*-CP was investigated in acidic milieu, since most of the commercially available parenteral solutions have a pH value between 3 and 5. Artificial radiation from a high-pressure mercury lamp caused a change of the *cis* form to a 1:1 *cis–trans* isomer mixture within a few minutes, in accordance with earlier results[4]. Further

TABLE II

ACCURACY AND PRECISION OF THE DETERMINATION OF *CIS*- AND *TRANS*-CHLORPROTHIXENE MIXTURES BY HPTLC

R.S.D. = Relative standard deviation.

Amount of cis- and trans-CP (ng per spot)	*cis-CP*		*trans-CP*	
	Found (%)	*R.S.D. (%), n = 6*	*Found (%)*	*R.S.D. (%), n = 6*
48	102.5	2.2	101.3	2.4
64	103.4	2.4	101.5	2.5
96	99.6	2.4	100.0	1.5
176	101.8	1.6	–	–

degradation of the drug was to be expected because air was not removed from the ampoules. The decrease of the total CP content followed approximately zero-order kinetics, the decomposition rate being strongly dependent on the initial drug concentration (Fig. 2). The solvent seemed to have no marked effect on the degradation rate. In hydrochloric acid as well as in acetate buffer (pH 4.6) and acetic acid the CP content of a 0.5% solution dropped to 55–60% of its initial value on exposure to radiation for 60 min. No isomerization or other decomposition was observed in *cis*-CP solutions stored in the dark for several months.

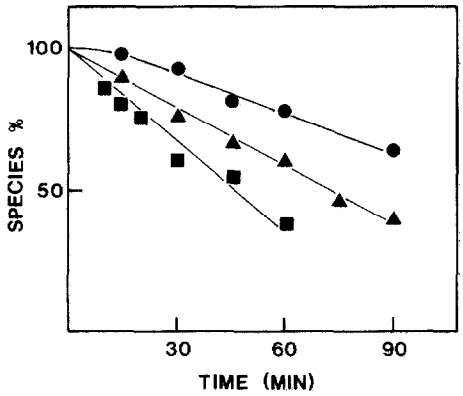

Fig. 2. Effect of drug concentration on the degradation of CP at pH 4.6: ●, 1%; ▲, 0.5%; ■, 0.25% CP solution. Radiation source: mercury lamp.

Qualitative TLC experiments on irradiated solutions with different solvent systems revealed several degradation products, most of them present only in trace quantities. CT was formed in small amount, but CP-SO and CP-SO_2 were not detected. The discolouring of the solutions and a light brown precipitate suggested polymer formation.

Similar decomposition behaviour of *cis*-CP was observed in solutions exposed

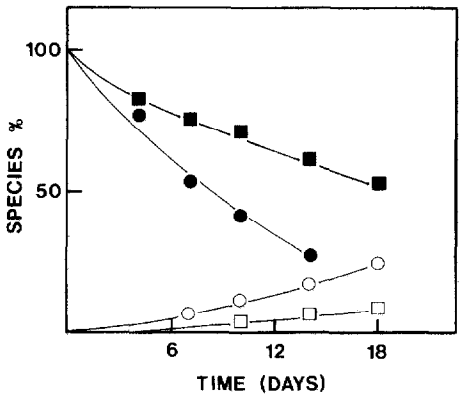

Fig. 3. Time course for the degradation of CP in 0.1 *M* hydrochloric acid (■) and at pH 4.6 (●), and for the formation of CT in 0.1 *M* HCl (□) and at pH 4.6 (○). Radiation source: daylight.

to ordinary daylight. The equilibrium ratio between the *cis* and *trans* isomers was achieved within a few days in all solutions studied. The overall degradation of CP and the formation of CT were more pronounced in acetate buffer than in hydrochloric acid (Figs. 3 and 4).

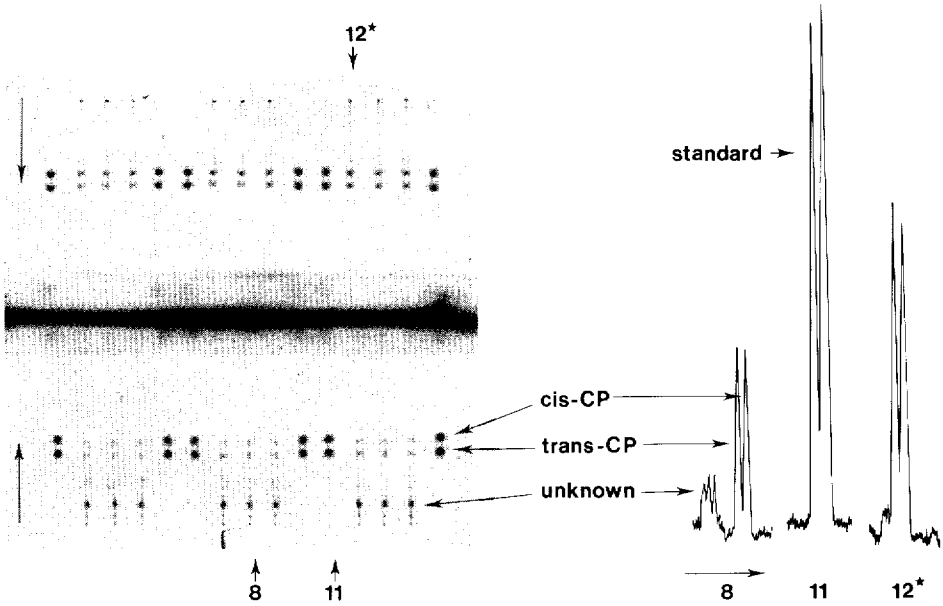

Fig. 4. HPTLC of *cis*-CP solutions exposed to daylight for 14 days. Upper part, 0.1 *M* hydrochloric acid; lower part, acetate buffer pH 4.6.

In conclusion, the present results show that HPTLC can provide a valuable alternative technique for the determination of *cis*- and *trans*-CP and some of the oxidation products of the drug. The simple sample preparation and the simultaneous separation of several samples make the method particularly attractive for the analysis of complicated systems. The accuracy and precision of the assay procedure compare well with those of other chromatographic methods.

REFERENCES

1 J. D. Dunitz, H. Eser and P. Strickler, *Helv. Chim. Acta*, 47 (1964) 1897.
2 M. L. Post, O. Kennard and A. S. Horn, *Acta Crystallogr.*, B30 (1974) 1644.
3 M. L. Post, O. Kennard, G. M. Sheldrick and A. S. Horn, *Acta Crystallogr.*, B31 (1975) 2366.
4 A. Li Wan Po and W. J. Irwin, *J. Pharm. Pharmacol.*, 32 (1980) 25.
5 R. P. Enever, A. Li Wan Po and E. Shotton, *J. Pharm. Sci.*, 68 (1979) 169.
6 S. P. Agarwal and M. I. Blake, *J. Pharm. Pharmacol.*, 21 (1969) 556.
7 S. A. Tammilehto, *J. Pharm. Pharmacol.*, 32 (1980) 524.
8 J. Raaflaub, *Arzneim.-Forsch.*, 17 (1967) 1393.
9 H. D. Dell, J. Fiedler and R. Kamp, *Z. Anal. Chem.*, 253 (1971) 357.
10 T. Mjörndal and L. Oreland, *Acta Pharmacol. Toxicol.*, 29 (1971) 295.
11 B. Kämpe, *Arch. Pharm. Chem., Sci. Ed.*, 2 (1974) 145.
12 P. Hartvig, N. O. Ahnfelt and K. E. Karlsson, *Acta Pharm. Suecica*, 13 (1976) 181.
13 A. Cailleux, A. Turcant, A. Premel-Cabic and P. Allain, *J. Chromatogr. Sci.*, 19 (1981) 163.
14 D. C. Hobbs, W. M. Welch, M. J. Short, W. A. Moody and C. D. van der Velde, *Clin. Pharmacol. Ther.*, 16 (1974) 473.

15 T. Aaes-Jørgensen, *J. Chromatogr.*, 183 (1980) 239.
16 A. Li Wan Po and W. J. Irwin, *J. Pharm. Pharmacol.*, 31 (1979) 512.
17 A. Li Wan Po and W. J. Irwin, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 623.
18 S. A. Tammilehto, R. Pere and M.-L. Hämäläinen, in preparation.
19 R. E. Kaiser, in W. Bertsch, S. Hara, R. E. Kaiser and A. Zlatkis (Editors), *Instrumental HPTLC, Proceedings of the First International Symposium on Instrumentalized High Performance Thin-Layer Chromatography (HPTLC), Bad Dürkheim*, Dr. Alfred Hüthig Verlag, Heidelberg, Basle, New York, 1980, p. 165.