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Determination of zuclopenthixol and its main N-dealkylated metabolite in biological fluids using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection

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Abstract

A highly sensitive high-performance liquid chromatographic (HPLC) method for the assay of *cis*-(Z)-clopenthixol (zuclopenthixol) in urine and plasma has been developed. Following solid-phase extraction, the samples are chromatographed using reversed-phase ion-pairing HPLC. After separation, the solutes, having a thioxanthene structure, are transformed on-line into thioxanthones in a photochemical reactor. The thioxanthones are highly fluorescent compounds, and therefore, low detection limits are obtained when using fluorescence detection. Detection limits for zuclopenthixol and its N-dealkylated metabolite, in plasma as well as in urine, using fluorescence detection with excitation at 260 nm and emission at 435 nm, were found to be 0.05 ng/ml and 0.2 ng/ml, respectively. The chromatographic system separates the *cis*-(Z)- and *trans*-(E)-isomers of clopenthixol from its main dealkylated metabolite. Furthermore, the chromatographic system is very suitable for study of the photochemical reaction, since the chloro-thioxanthone and thioxanthone are well separated from the isomers of clopenthixol.

1. Introduction

Zuclopenthixol {(Z)-4-[3-[2-chloro-9H-thioxanthen-9-yliden]-propyl]-1-piperazinethanol, (CPT)} (Fig. 1) is an antipsychotic (neuroleptic) drug [1] given in doses that result in plasma concentrations in the low ng (pico-molar) range. The metabolism of the drug has been studied in animals and humans [2–4], where the N-dealkyl-CPT (Fig. 1) was found to be the main metabolite, while only small amounts of CPT-sulfoxide and CPT-N-oxide were found. Furthermore, N-dealkyl-CPT-sulfoxide and CPT-glucuronide were found in rats and humans [2]. The determination of the drug in plasma has been performed by normal-phase HPLC with UV-detection after liquid–liquid extraction [5], with a detection limit of ca. 1 ng/ml. Using HPLC combined with mass spectrometry, a detection limit of 0.1 ng/ml has been obtained [6].

In order to obtain analytical data for the reliable pharmacokinetic evaluation of drugs

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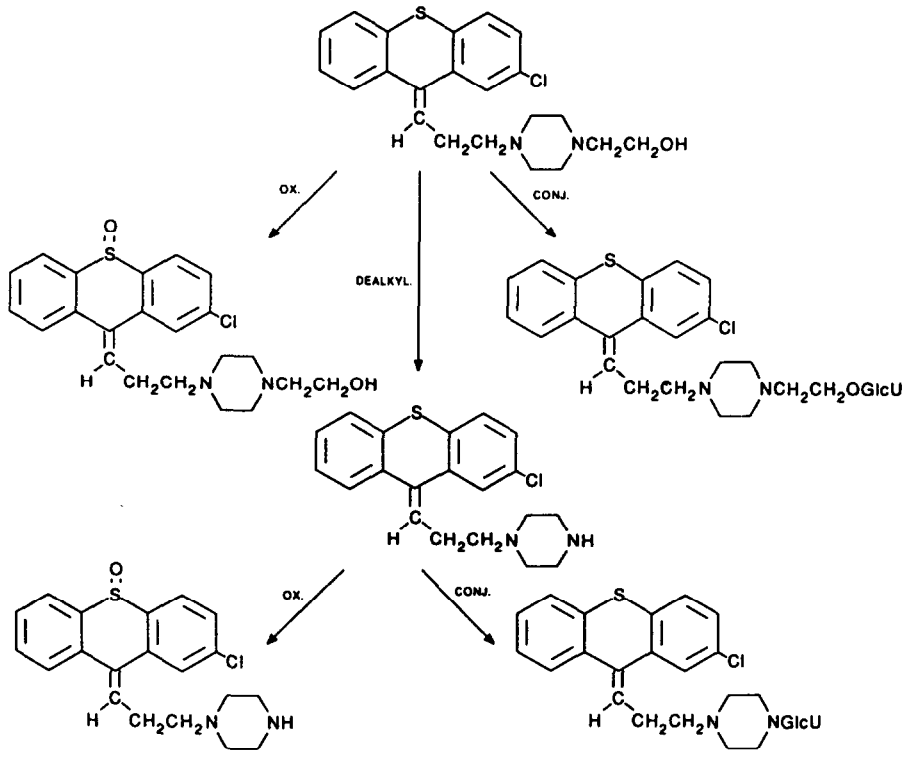

Fig. 1. Main metabolic pathway of zuclopenthixol.

containing CPT, an improved method for routine analysis had to be developed.

In this paper, we present an analytical method in which improvements have been made in the sample preparation, the chromatographic behavior and in the detection of CPT and its N-dealkylated metabolite.

2. Experimental

2.1. Apparatus

A Waters (Millipore, Milford, MA, USA) liquid chromatograph consisting of a 6000 A pump, a 715 WISP autosampler and a 470 fluorescence detector operated at excitation and emission wavelengths of 260 and 435 nm, respectively, was used. The collection of data was performed with Waters Maxima 820 chromatography software. A Shimadzu CTO-6A column oven was used for thermostating the column. The photochemical reactor placed between the analytical column and the fluorescence detector was constructed from teflon tubing (5 m × 0.5 mm I.D. and 1/16 O.D.) crocheted to form a cylinder in order to reduce band broadening in the reactor. The crocheted cylinder was placed around a low pressure 8-W mercury UV-light source (SterileAir UVC-9) placed in an aluminium box.

For the solid-phase extraction (SPE) a Vac Elut SPS 24 TM (Analytichem International, Cambridge, UK) was used.

2.2. Chromatographic system

A Knauer (Berlin, Germany) 120 × 4.6 mm I.D. column packed with Spherisorb S5 CN was used. The column was thermostated at 40°C. The mobile phase consisted of acetonitrile–0.2 *M* potassium phosphate pH 6.5–water (36:5:59,

v/v), containing 6 m*M* of dodecyl-N,N,N-trimethylammonium (DTMA) bromide. The flow-rate was 1.0 ml/min.

2.3. Chemicals

Zuclopenthixol, the N-dealkylated metabolite (obtained as a mixture of the *cis* and *trans* isomers) and the thioxanthones were kindly donated by H. Lundbeck A/S (Copenhagen, Denmark). All standard solutions prepared were kept refrigerated and in the dark.

Dodecyl-N,N,N-trimethylammonium bromide was obtained from Aldrich (Steinheim, Germany). Acetonitrile and methanol were from Lab-Scan (Dublin, Ireland). All other chemicals were of analytical-reagent grade from Merck (Darmstadt, Germany).

Bond Elut CN, 1 ml, SPE cartridges were obtained from Analytichem International (Cambridge, UK).

2.4. Sample preparation

All solutions prepared were protected from light. A 2.0-ml sample aliquot (plasma or urine) was loaded onto a Bond Elut CN cartridge previously activated with 2 ml of acetonitrile and washed with 2 ml of water. The cartridge was then washed with 2 ml of water and the solutes were eluted with 1.0 ml of acetonitrile containing 10% *n*-butylamine. The eluate was evaporated at 40°C to dryness under a gentle stream of nitrogen. The residue was dissolved in 200 μl of mobile phase, of which 100 μl were injected onto the analytical column.

3. Results and discussion

3.1. Chromatography

Reversed-phase chromatography of basic drugs on silica-based ODS materials is often known to cause problems due to tailing. This phenomenon has also been observed with clopenthixol [7]. To prevent tailing, a long-chain quaternary ammonium ion (DTMA) was added to the eluent. Furthermore, the less retentive cyano-propyl derivatized silica was chosen as the stationary phase. This chromatographic system gave symmetrical peaks and a good separation of all the compounds of interest.

3.2. Detection

It is known that thioxanthenes can be oxidized using potassium permanganate (MnO_4^-) or cerium ammonium sulfate (Ce^{4+}) to give the highly fluorescent thioxanthones [8]. Oxidation

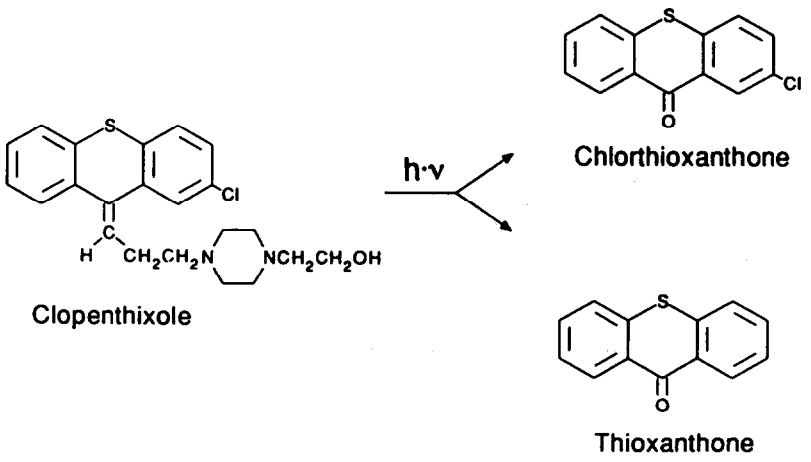

Fig. 2. Main photochemical processes of zuclopenthixol.

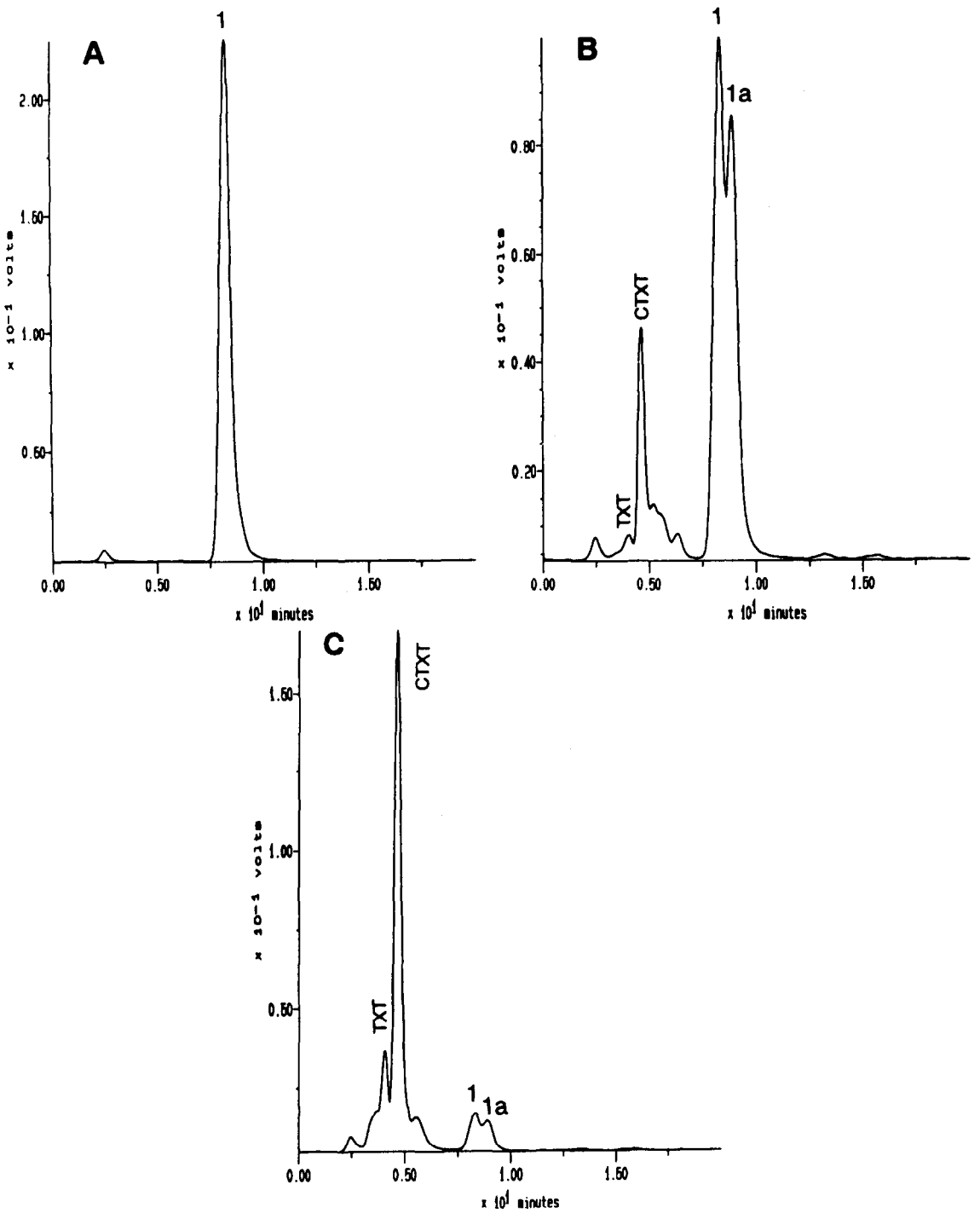

Fig. 3. Chromatograms of photochemical reaction mixtures of zuclopenthixol (10 μg/ml in mobile phase) (A) prior to, (B) after 1 min, and (C) after 10 min of photochemical reaction. Peaks: TXT = thioxanthone; CTXT = chlorothioxanthone; 1 = *cis*-(Z)-clopenthixol, and 1a = *trans*-(E)-clopenthixol.

may also be performed by photochemistry (UV-light), and this technique is used to perform on-line derivatization of the thioxanthenes into thioxanthones.

The chromatographic system developed may be used for study of the photochemical process since all the compounds of interest are separated simultaneously.

Halogen atoms bound to the chromophore are often known to quench the fluorescence. On the other hand, halogen atoms are often lost by photolysis in photochemical processes. Thus, it was interesting to investigate whether CPT was transformed into chlorothioxanthone (CTXT) or into thioxanthone (TXT) (Fig. 2). An experiment involving UV-irradiation of CPT dissolved in the mobile phase and placed in a quartz cuvette in the photochemical reactor, followed by HPLC analysis, showed that CPT was derivatized into a mixture of the two thioxanthones (Fig. 3). Furthermore, it was found that CPT isomerises to a mixture of *cis*-(Z)- and *trans*-(E)-CPT in less than one minute due to the UV-irradiation. After 10 min only small amounts of CPT remain. However, as the two thioxanthones were found to exhibit exactly the same molar fluorescence intensity, a possible change in the relative amounts of the two thioxanthones formed will not influence the response in the on-line analytical system.

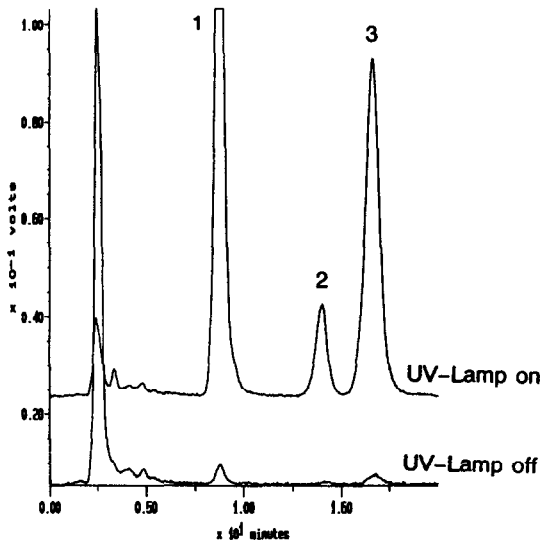

Fig. 4. Chromatogram of a standard mixture of zuclopenthixol and its N-dealkylated metabolite with the power of the UV-lamp in the photochemical reactor switched on or switched off. Peaks: 1 = *cis*-(Z)-clopenthixol; 2 = *cis*-(Z)-N-dealkyl-clopenthixol; and 3 = *trans*-(E)-N-dealkyl-clopenthixol.

The photochemical reaction was optimized using reactors with various lengths (0.75, 2.0, 5.0 and 15.0 m) and using flow-rates of 0.5 and 1.0 ml/min, thus providing different reaction times. It was found that the fluorescence response reached a constant level, expressed as the peak height after 1 min of reaction time in the on-line system with the chosen eluent. A reactor with dimensions 5 m × 0.5 mm I.D. used at a flow-rate of 1 ml/min corresponded to a 1 min reaction time.

The fluorescence of the solutes was ca. 60 times more intense when the UV-lamp of the photochemical reactor was switched on compared to when it is switched off (Fig. 4).

3.3. Sample preparation

In order to avoid a laborious liquid–liquid extraction procedure, a SPE procedure was developed. It was found that the solutes were adsorbed to the cartridge packing material by hydrophobic as well as cation-exchange mechanisms. To elute the solutes from the cartridges, it was necessary to use either a concentrated ammonia (> 5%) or an aliphatic amine. Thus, the ion-exchange interactions are the main reason for not obtaining 100% recovery using SPE.

In the final sample preparation procedure, Bond Elut CN cartridges were chosen for the SPE, and the solutes were eluted with acetonitrile containing 10% of *n*-butylamine.

3.4. Internal standardisation

The drug chlorprothixen was evaluated for use as internal standard. The compound chromatographed well in the HPLC system, and eluted with a retention time between those of CPT and the dealkylated metabolites. However, addition of the internal standard did not improve the precision of the analysis, probably due to differ-

ent extraction characteristics. Also the unknown characteristics of the kinetics of the photochemical process of chlorprothixen may cause increased imprecision.

3.5. Validation

The developed method was validated using spiked human plasma and urine. The validation of recovery (accuracy) and precision was performed at three concentration levels in plasma and two concentration levels in urine using peak-height measurements. The concentration levels, the recoveries and the coefficient of variation (C.V.) of the intra-day determinations are given in Table 1. Some sample chromatograms obtained are shown in Fig. 5.

The fluorescence response of the solutes was investigated in the range from 1 to 500 ng/ml (0.1–50 ng injected onto the column) in the final sample solutions with 7 data points in duplicates, and were found to be linear, with a correlation coefficient higher than 0.999. The slope of the standard curve determined on different days varied within ± 6%, but was stable throughout a given analysis series. The minimum detectable

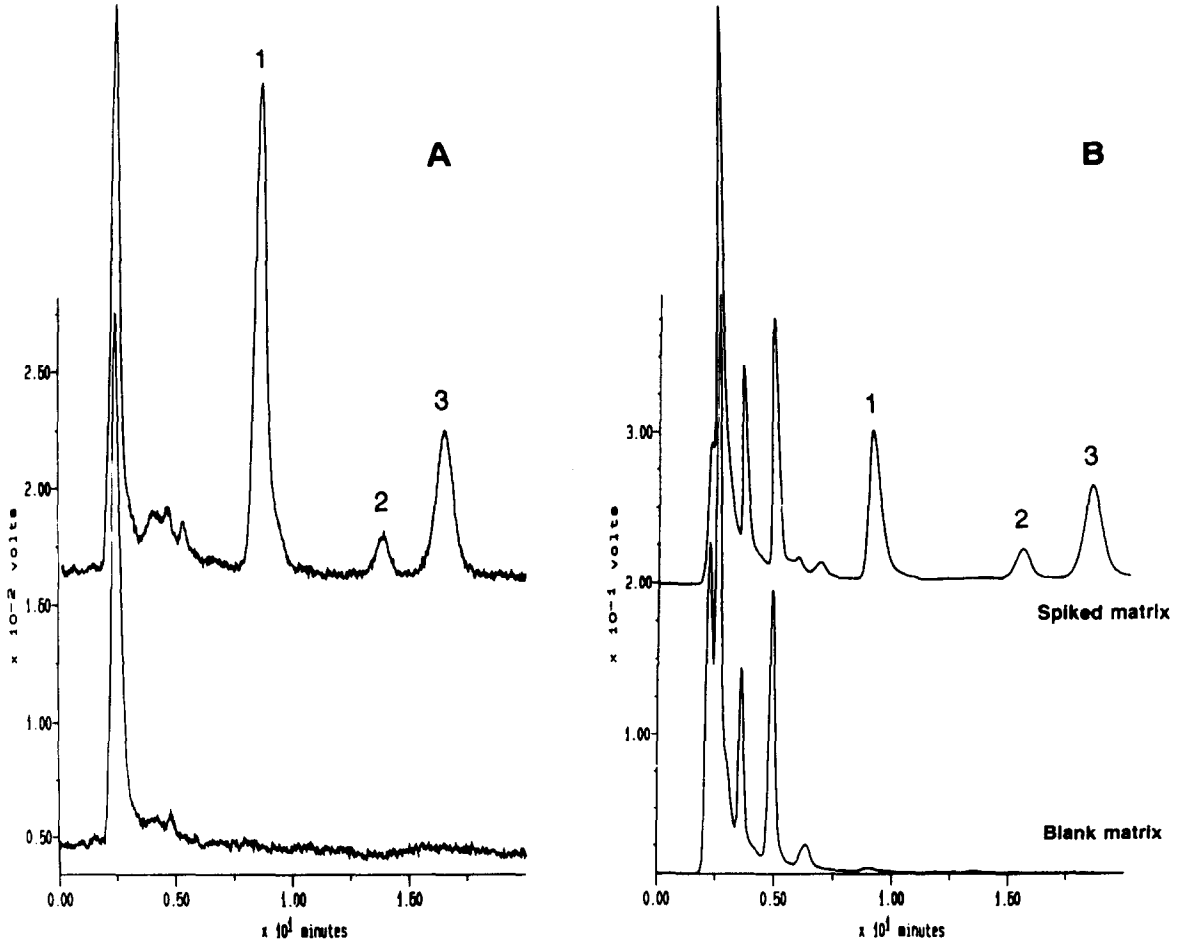

Fig. 5. Chromatograms of (A) plasma, and (B) urine, spiked with zuclopenthixol and its N-dealkylated metabolite. Plasma concentration per ml: 5 ng *cis*-(Z)-clopenthixol, 1 ng *cis*-(Z)-N-dealkyl-clopenthixol and 4 ng *trans*-(E)-N-dealkyl-clopenthixol. Urine concentration per ml: 50 ng *cis*-(Z)-clopenthixol, 10 ng *cis*-(Z)-N-dealkyl-clopenthixol and 40 ng *trans*-(E)-N-dealkyl-clopenthixol. Reference blank plasma and blank urine are shown in the lower chromatograms. Peak identity as in Fig. 4.

Table 1
Precision (C.V.) and recoveries of the assay of *cis*-(Z)-clopenthixol (CPT) and the sum of the *cis*- and *trans*-N-dealkylated metabolites in spiked plasma and urine ($n = 6$)

Matrix	Compound	Amount added (ng/ml)	Recovery (%)	C.V. (%)
Plasma	CPT	0.10	62	16.6
		1.0	73	10.9
		10	79	7.2
	Dealkyl CPT	0.10	–[a]	
		1.0	85	12.5
		10.0	89	6.8
Urine	CPT	5.0	62	12.4
		50.0	72	8.7
	Dealkyl CPT	5.0	82	5.8
		50.0	85	7.2

[a] Below the level of quantitation (LOQ).

quantities of CPT and the N-dealkylated metabolite, at a signal-to-noise ratio of 3, were found to be 6 pg and 20 pg, respectively.

4. Conclusions

The combination of HPLC with on-line, post-column photochemical reaction and fluorescence detection provides a highly selective and sensitive quantitative assay for determination of zuclopenthixol and its N-dealkylated metabolite in biological fluids. Although the method has not yet been applied for routine determinations, there should be no hindrance to that.

The *cis*- and *trans* isomers of the drug and its main metabolite are well separated.

Acknowledgement

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References

[1] P.V. Petersen, N. Lassen, T. Holm, R. Kopf and I.M. Nielsen, *Arzneim. Forsch.*, 8 (1958) 395.
[2] A.R. Khan, *Acta Pharmacol. Toxicol.*, 27 (1969) 202.
[3] A. Jørgensen, V. Hansen, U.D. Larsen and A.R. Khan, *Acta Pharmacol. Toxicol.*, 27 (1969) 301.
[4] T. Aaes-Jørgensen, F.F. Overø, K.P. Bøgesø and A. Jørgensen, *Acta Pharmacol. Toxicol.*, 41 (1977) 103.
[5] T. Aaes-Jørgensen, *J. Chromatogr.*, 183 (1980) 239.
[6] A.C. Tas, J. van der Greef, M.C. ten Noever de Brauw, T.A. Plomp, R.A.A. Maes, M. Hohn and U. Rapp, *J. Anal. Toxicol.*, 10 (1986) 46.
[7] S.H. Hansen, P. Helboe and M. Thomsen, *J. Chromatogr.*, 409 (1987) 71.
[8] S. Tammilehto, R. Pere and M.-L. Hämäläinen, *Acta Pharm. Suec.*, 23 (1986) 289.