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Note

Specific high-performance liquid chromatographic method for estimation of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol and a N-dealkyl metabolite

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Clopenthixol, a neuroleptic drug of the thioxanthene group, exis's as two geometric isomers, a *cis*(*Z*)- and a *trans*(*E*)-isomer. It has been shown, that while the *cis*(*Z*)-isomer has a strong neuroleptic activity in pharmacological tests, the *trans*(*E*)-isomer is practically without any effect [1]. To prolong the neuroleptic effect *cis*(*Z*)-clopenthixol was esterified with decanoic acid, dissolved in an oil and administered as an intramuscular depot. Pharmacokinetic studies in animals indicated rapid hydrolysis of the ester after liberation from the depot and showed significant drug levels for considerably longer periods of time after clopenthixol decanoate injection than after orally given clopenthixol [2]. These data correlate well with the long lasting pharmacological effect of clopenthixol decanoate demonstrated in animals [3] and they are in agreement with studies in patients administered clopenthixol decanoate in Viscoleo with intervals of two and four weeks [4, 5].

Analytical methods for determination of clopenthixol in biological material have been developed by Fredricson Overø [4], who used fluorometry after thin-layer separation and oxidation, and Muusze et al. [6], who worked with thin-layer scanning. The former method estimates clopenthixol decanoate and a N-dealkyl metabolite in addition to clopenthixol, but does not distinguish between the isomers. The method by Muusze et al. [6] supplies a separation of the isomers of clopenthixol, but the separation is unsatisfactory and the isomers of the N-dealkyl metabolite are not separated at all. The ester is not estimated by this method. High-performance liquid chromatographic separation of the isomers of clopenthixol has been performed by Li Wan Po and Irwin [7], who used pure solutions of rather high concentrations.

In the present paper a specific high-performance liquid chromatographic method for determination of the concentrations in serum of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol and N-dealkylclopenthixol is presented.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consists of a Rheodyne Model 7120 syringe loading sample injector, a Spherisorb S 5 W (5-μm spherical silica particles from Phase Separations, Queensferry, Great Britiain) column (25 cm × 4.6 mm), a Waters Model 6000A pump, a Waters Model 440 UV-absorbance detector with a measuring wavelength of 254 nm, and a Kipp & Zonen Model BD9 two-channel recorder.

The eluent was *n*-heptane—2-propanol—concentrated ammonia—water (85:15:0.4:0.2, v/v), and the flow-rate 1 ml/min. The chromatograph was operated at room temperature and at a pressure of about 70 bar.

Chemicals

Glass distilled water and organic solvents of analytical grade were used in the analysis. The hexane was from Mallinckrodt (St. Louis, MO, U.S.A.); the other solvents, hydrochloric acid and the NaOH pellets from Merck (Darmstadt, G.F.R.). The isopropylamine was distilled every fortnight.

Reference substances

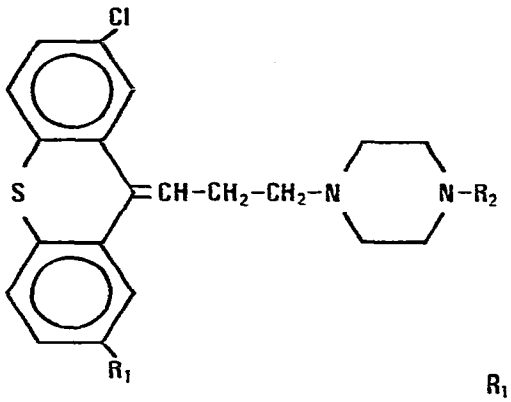
The dihydrochloride of the *cis*(*Z*)- and the *trans*(*E*)-isomers of clopenthixol and the dimaleate of the N-dealkylated clopenthixol consisting of 44% *cis*(*Z*)- and 56% *trans*(*E*)-isomer were used as reference substances. The percentages of *cis*(*Z*)- and *trans*(*E*)-N-dealkylclopenthixol were estimated from the peak areas. It was necessary to make the assumption that the *cis*(*Z*)- and *trans*(*E*)-isomers of N-dealkylclopenthixol have equal molar UV-absorption as the pure isomers cannot be produced. Stock standard solutions were made in ethanol (1 mg/ml) and stored in a refrigerator for 1 month. Dilutions were made every day. The substances were all synthesized in our laboratories.

Internal standard

A compound, Lu 9-215, structurally related to clopenthixol, but without the double bond in the side chain was used as internal standard (Fig. 1). Solution and dilutions were made in the same way as for the reference substances. The substance was synthesized in our laboratories and is available on request.

Extraction procedure

To a serum sample of 2 ml in a stoppered glass tube were added 25 ng of Lu 9-215, 300 μl ethanol, 100 μl of 7 *N* NaOH solution and 8 ml of hexane containing 0.1% isopropylamine. The samples were shaken for 15 min and then centrifuged at 2400 *g* for 5 min. The hexane phase was transferred to another tube, and 2 ml of 0.1 *N* HCl were added, the samples were shaken for 15 min and centrifuged for 5 min. The hexane phase was discarded, and to the HCl phase were added 200 μl of 7 *N* NaOH and 4 ml of hexane with 0.1% isopropylamine. The samples were again shaken and centrifuged for 15 and 5 min, respectively. Thereafter the hexane phase was transferred to a conical glass tube and was evaporated to dryness under a stream of air at 30°C. The sample was redissolved in 1 ml of hexane, evaporated and redissolved in 100 μl of hexane



		R_1	R_2
Clopenthixol	(CPT)	H	$-(CH_2)_2-OH$
Dealkyl clopenthixol	(CPT-NH)	H	H
Internal Standard	(Lu 9-215)*	CF_3	$-(CH_2)_2-OH$

*Without the double bond in the side chain to the ring structure

Fig. 1. Structures of clopenthixol, N-dealkylclopenthixol and the internal standard.

containing 0.1% isopropylamine. A 70-μl aliquot of the final solution was injected on the chromatograph.

RESULTS AND DISCUSSION

Identification and quantitation

The drug, the metabolites and the internal standard were identified by their retention times.

Standard curves were evaluated by measuring the peak heights relative to that of the internal standard. As the variations in the daily standard curves obtained for *cis*(*Z*)-clopenthixol and *cis*(*Z*)- and *trans*(*E*)-N-dealkylclopenthixol were small, three mean standard curves were made for these compounds on the basis of the data from the individual curves. The standard curves did not differ significantly from linearity ($p > 0.10$). The standard curve used for determination of *cis*(*Z*)-clopenthixol was also used for *trans*(*E*)-clopenthixol as the peak heights of these two isomers were not significantly different for the same amount of drug. The standard curve used for calculation of *cis*(*Z*)- and *trans*(*E*)-clopenthixol is shown in Fig. 2. The line is based on 206 determinations as indicated on the figure. The equation for the line is $y = 0.713\ x + 0.0054$. The corresponding standard curves for *cis*(*Z*)- and *trans*(*E*)-N-dealkylclopenthixol are based on 208 and 198 determinations and the equations are $y = 0.0415\ x - 0.0482$ and $y = 0.0270\ x + 0.0390$, respectively.

Every day standards of *cis*(*Z*)-clopenthixol and *cis*(*Z*)/*trans*(*E*)-N-dealkylclopenthixol added to blank serum samples are run through the procedure in order to check that the standard curves are still valid (standards are within the 95% confidence limits). The reason for not adding *trans*(*E*)-clopenthixol to the samples is to be sure of the degree of isomerization of *cis*(*Z*)- to *trans*(*E*)-clopenthixol during the analytical procedure. The *cis*(*Z*)-clopenthixol standard

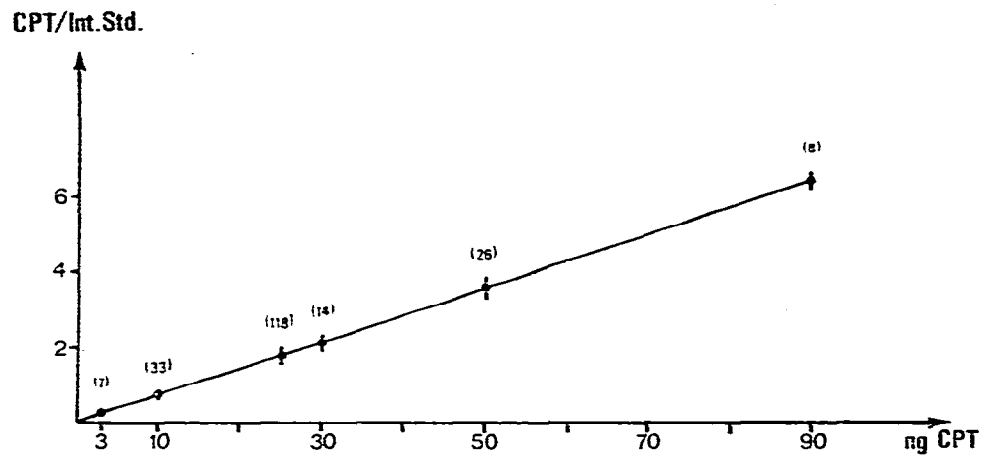


Fig. 2. Standard curve for calculation of the amounts of *cis*(*Z*)- and *trans*(*E*)-clopenthixol (CPT) in a serum sample. Mean ± S.D. is given for each concentration. The figures in the brackets indicate the number of observations.

contains 1—2% of *trans*(*E*)-clopenthixol, and during the extraction procedure this normally increases to about 6%. If the samples are handled in an inexpedient way (heat, light), further isomerization can take place.

Sensitivity and precision

The lower limit of sensitivity was for clopenthixol about 0.5 ng/ml and for N-dealkylclopenthixol about 2.5 ng/ml in a 2-ml sample. Because of this high sensitivity it is possible to follow the drug concentrations in serum for a relatively long time, even after a single dose as low as 10 mg of *cis*(*Z*)-clopenthixol. The method is thus more sensitive than the other methods described for clopenthixol and N-dealkylclopenthixol [4, 6].

Based on the assay of 4—6 identical samples containing 3, 10, 30, and 90 ng of *cis*(*Z*)-clopenthixol and 10, 30, 90, and 270 ng of *cis*(*Z*)/*trans*(*E*)-N-dealkylclopenthixol, coefficients of variance of 3—7 were found. The coefficients of variance were independent of the drug concentration.

Specificity

The assay of clopenthixol and the metabolite is not disturbed by compounds from the serum as seen in Fig. 3A.

Other relevant drugs have been assayed in the chromatographic system described in this paper, to investigate a possible interference with the assay. The tricyclic antidepressants amitriptyline, nortriptyline, and imipramine do not interfere with the assay, the same is true for the antiparkinsonian drugs orfenadin, procyclidin, and biperidin, whereas most of the benzodiazepines interfere with the assay. Estazolam does not interfere with clopenthixol, but is very close to the *cis*(*Z*)-isomer of the metabolite in the chromatogram. Metabolites of the above-mentioned drugs have not been investigated and it therefore cannot be excluded that some of them will interfere with the clopenthixol assay. However, until now no peaks from other compounds have disturbed the assay.

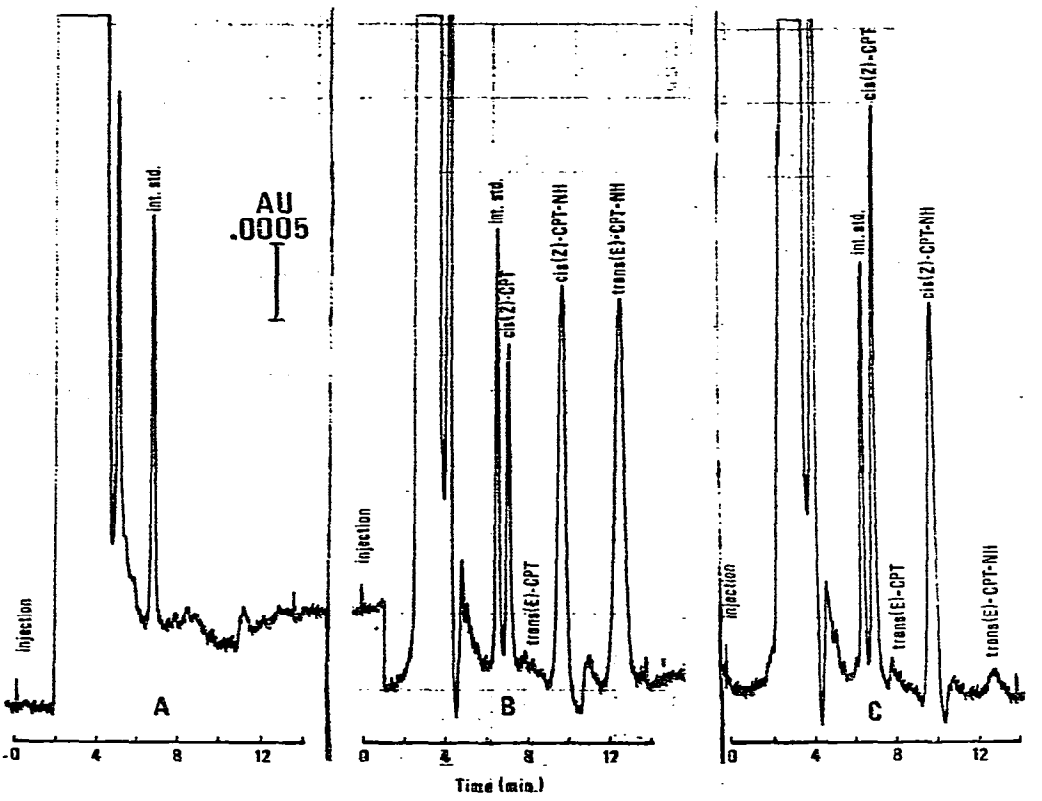


Fig. 3. Chromatograms of (A) blank serum with 25 ng internal standard; (B) blank serum with 25 ng internal standard, 10 ng *cis*(*Z*)-clopenthixol [*cis*(*Z*)-CPT], 22 ng *cis*(*Z*)-N-dealkylclopenthixol [*cis*(*Z*)-CPT-NH], and 28 ng *trans*(*E*)-N-dealkylclopenthixol [*trans*(*E*)-CPT-NH]; (C) serum sample from a patient given daily dosages of *cis*(*Z*)-clopenthixol, 25 ng of internal standard were added to the sample containing 19.8 ng *cis*(*Z*)-clopenthixol and 24.9 ng *cis*(*Z*)-N-dealkylclopenthixol, traces of *trans*(*E*)-clopenthixol and *trans*(*E*)-N-dealkylclopenthixol were also present.

Serum samples

The method has until now only been used for serum samples from rat, dog, and man. In Fig. 3 are shown examples of chromatograms of extracts of human serum. Fig. 3A shows a blank serum sample to which are added 25 ng of internal standard; the same is seen in Fig. 3B, but with the addition of *cis*(*Z*)-clopenthixol (10 ng) and *cis*(*Z*)- and *trans*(*E*)-N-dealkylclopenthixol (22 and 28 ng, respectively). Fig. 3C shows a serum sample from a patient with added internal standard (25 ng). The amounts of *cis*(*Z*)-clopenthixol and *cis*(*Z*)-N-dealkylclopenthixol are 19.8 and 24.9 ng, respectively. The corresponding *trans* isomers are present in amounts below the limit of detection.

Fig. 4 shows the levels of *cis*(*Z*)- and *trans*(*E*)-clopenthixol in a human volunteer given a single dose of 30 mg Sordinol®, i.e. about 10 mg *cis*(*Z*)- and 20 mg *trans*(*E*)-clopenthixol. The peak levels are obtained 3 and 4 h after administration. Thereafter the curves decline slowly and the drug is still measurable after 48 h. The biological half-lives are almost one day. The isomers of the dealkylated metabolite were only seen as traces.

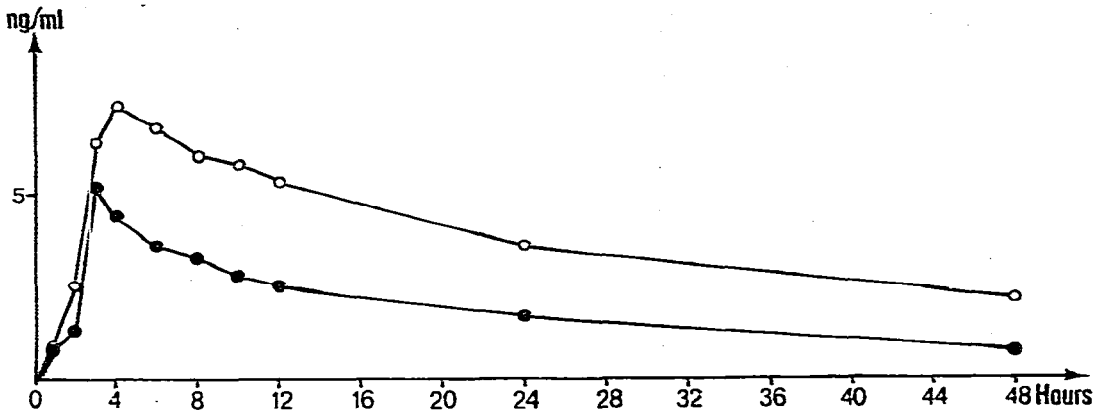


Fig. 4. Concentrations of *cis*(*Z*)-clopenthixol (•) and *trans*(*E*)-clopenthixol (○) in serum from a human volunteer given a single dose of 30 mg Sordinol®.

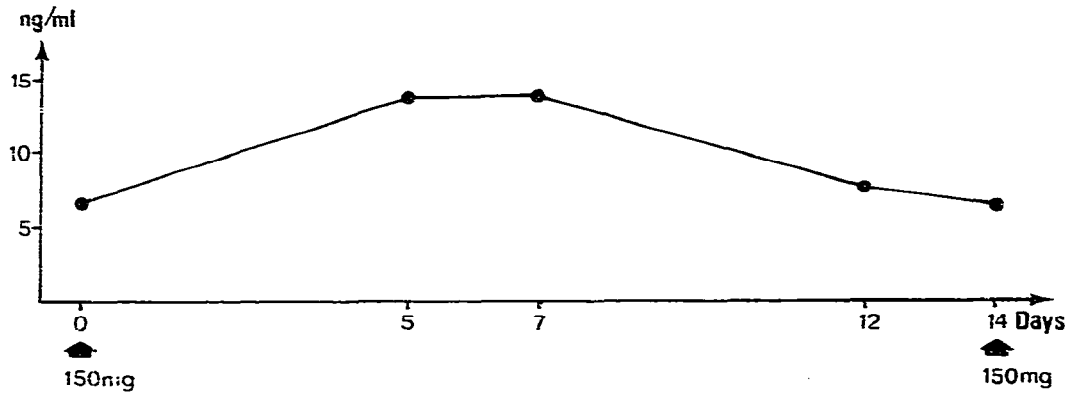


Fig. 5. Concentrations of *cis*(*Z*)-clopenthixol in serum from a patient given *cis*(*Z*)-clopenthixol decanoate intramuscularly in Viscoleo, 150 mg fortnightly.

Another example of the application of the method is seen in Fig. 5 showing the concentration of *cis*(*Z*)-clopenthixol after intramuscular injection of *cis*(*Z*)-clopenthixol decanoate in Viscoleo to a patient in fortnightly doses of 150 mg. It is seen that the curve shows a maximum between days 5 and 7 and that a maximum/minimum fluctuation of about 2 occurs. Although *cis*(*Z*)-clopenthixol decanoate and not *cis*(*Z*)-clopenthixol has been administered to the patient, only *cis*(*Z*)-clopenthixol has been measured as this seems to be the compound responsible for the clinical effect. This assumption is based on animal studies [2] which showed rapid hydrolysis of the ester, clopenthixol being the dominating compound in the tissues, and on earlier investigations in patients [4, 5] in whom the ester was hardly detectable in serum. In addition to *cis*(*Z*)-clopenthixol *trans*(*E*)-clopenthixol was observed in trace amounts. *cis*(*Z*)-N-Dealkylclopenthixol was seen in a concentration of about half of the *cis*(*Z*)-clopenthixol concentration, while the *trans*(*E*)-N-dealkylclopenthixol was seen in trace amounts.

The present paper describes a method for the estimation of the two isomers of clopenthixol and the two isomers of the clopenthixol metabolite, N-dealkyl-

clopenthixol. Of these compounds *cis*(*Z*)-clopenthixol is by far the most important compound, as it is the compound exerting the pharmacological and clinical activity also after ester administration. Measurement of *trans*(*E*)-clopenthixol is of less importance since the compound is almost without pharmacological activity [1], but it gives an indication of the degree to which the *cis*(*Z*)-isomer is transformed to the *trans*(*E*)-isomer. Also the measurement of the isomers of N-dealkylclopenthixol is of less importance as the compounds have very low pharmacological activity [8]. However, the measurement of the metabolite in serum may give an indication as to the metabolic capacity of the animal or the human from whom the serum sample originates.

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