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Liquid chromatographic analysis of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol in human plasma using a novel solid phase extraction procedure

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Abstract

A simple and highly sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous determination of *cis*(*Z*)-clopenthixol and *trans*(*E*)-clopenthixol in human plasma has been developed. The chromatographic analysis was carried out isocratically on a reversed-phase column (C_8 150×4.6 mm I.D., 5 μm) using a mixture of 25 m*M* phosphate buffer and acetonitrile (65:35 v/v, pH* 3.0) as the mobile phase, and ultraviolet detection at 230 nm. Plasma sample pretreatment was accomplished by means of an original solid-phase extraction (SPE) procedure carried out on cyanopropyl cartridges, with a high extraction yield and good selectivity. Under the optimum conditions, calibration graphs of spiked human plasma samples were obtained over the concentration ranges 1–300 ng ml^{-1} for *cis*(*Z*)-clopenthixol and 1–200 ng ml^{-1} for *trans*(*E*)-clopenthixol. The limit of detection (LOD) was 0.3 ng ml^{-1} for both *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol. The method was successfully applied to the determination of *cis*(*Z*)-clopenthixol and *trans*(*E*)-clopenthixol in plasma samples of schizophrenic patients undergoing therapy with zuclopenthixol.

Keywords: Clopenthixol

1. Introduction

Clopenthixol, 4-(3-[2-chloro-9H-thioxanthene-9-ylidene]-propyl)-1-piperazine ethanol, is an effective antipsychotic agent which has been in clinical use as a mixture of *cis*(*Z*)- and *trans*(*E*)-isomers for the past 20 years. In fact, clopenthixol has an olefinic double bond between the central-ring carbon atom at position 10 and a side chain, and thus exists as two geometric isomers (Fig. 1): *cis*(*Z*)-clopenthixol (Z-CLO, zuclopenthixol) and *trans*(*E*)-clopenthixol (E-CLO) [1]. The *cis* (or α) isomer of clopenthixol is more active against antipsychotic symptoms [2], whereas *trans*(*E*)-clopenthixol seems to be inactive as a neuroleptic (it has antimicrobial activity) [3]. For this reason in the last few years, Z-CLO has substituted on the drug market the older CLO in pharmaceutical formulations. Zuclopenthixol, a D_1, D_2 dopamine receptor antagonist, is usually used for the treatment of schizophrenia, mania and other

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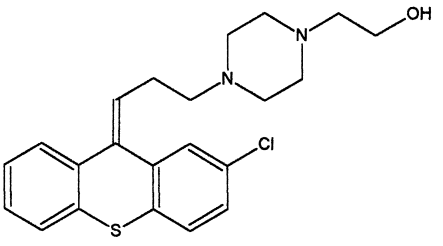
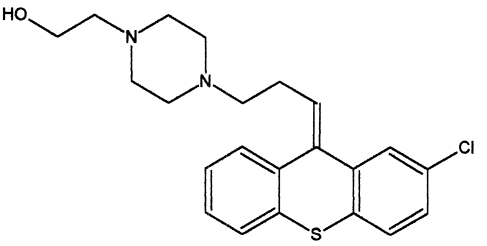
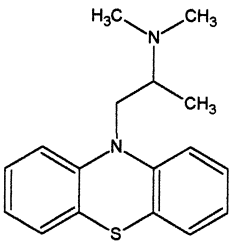


cis(Z)-clopenthixol (Z-CLO)



trans(E)-clopenthixol (E-CLO)



Promethazine (IS)

Fig. 1. Chemical structures of *cis*(*Z*)-clopenthixol, *trans*(*E*)-clopenthixol and promethazine (I.S.).

psychoses [4]. Some papers have suggested the use of zuclopenthixol for the treatment of chronic neuroleptic-resistant aggressive schizophrenia, psychotic oligophrenic patients [5] and in acute psychosis (schizophreniform and substance-induced psychotic disorders) [6]. Used in the treatment of schizophrenia and other psychotic disorders, *cis*(*Z*)-clopenthixol is usually administered orally as hydrochloride at doses of 20–50 mg/day or intramuscularly as somewhat longer-acting acetate in doses of 50–150 mg every 2–3 days or longer-acting decanoate in doses of 200–400 mg every 2–4 weeks [2,7,8]. The therapeutic blood levels of zuclopenthixol can widely vary within a range of 5–100 ng ml^{-1}, while the toxic levels range from 150 to 300 ng ml^{-1} [9]. Other studies that used various posologies of Z-CLO and different administration routes (e.g. oral and intramuscular), found therapeutic plasma levels ranging from 2 to 41 ng ml^{-1} [8,10–13]. *Trans*(*E*)-clopenthixol is usually present in serum at trace levels when therapeutic doses of Z-CLO are administered, however it has been found in high concentrations (177–275 ng ml^{-1}) [14,15] in post-mortem specimens after overdosage of Z-CLO; however, since its toxicity has not been well documented [14], its role in overdosage toxicity is not yet clear.

Several analytical methods have been published for the determination of zuclopenthixol alone, in particular in human plasma by means of high-performance liquid chromatography with UV detection [16–18] and also in dosage forms by means of flow-injection using tris(2,2′-dipyridyl) ruthenium(II) chemiluminescence [19]. Some methods also analyse simultaneously both the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol by HPLC with UV detection [20], fluorescence detection with post-column derivatisation [21], mass spectrometric detection [14,15] or diode array detection [15].

Some of the methods, reported in the literature, require very expensive instrumentation such as mass spectrometry [14,15], or laborious and time consuming analysis procedures [19]. Moreover, most of the

described methods have the environmental disadvantage of requiring very high percentages of organic solvents in the mobile phase [15–18,20], and a high amount of plasma samples (2 or 3 ml) for the pretreatment procedure of the biological fluids. These pretreatments are usually carried out by means of liquid–liquid extraction [15,20]; some solid-phase extraction procedures have also been reported which were accomplished by means of sophisticated (fully automated) systems [18,21].

In the present paper, a simple and sensitive high-performance liquid chromatographic method for the determination of plasma levels of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol is presented. The proposed method requires only a moderate percentage of acetonitrile in the mobile phase (35%). The novel SPE procedure needs a small amount of plasma sample (500 μl) for one complete analysis and provides a high extraction yield and other advantages, such as good selectivity and precision.

2. Experimental

2.1. Chemicals and solutions

H. Lundbeck A/S (Copenhagen, Denmark) kindly provided Zuclopenthixol (Z-CLO; *cis*(*Z*)-4-(3-[2-choloro-9H-thioxanthene-9-ylidene]-propyl)-1-piperazine ethanol) dihydrochloride and its isomer *trans*-(*E*)-clopenthixol (E-CLO) dihydrochloride, both 99.8% pure. Promethazine used as the internal standard was purchased from Sigma (St Louis, MO, USA).

ortho-Phosphoric acid (85%), sodium hydroxide and triethylamine were of analytical grade, from Carlo Erba (Milan, Italy). Methanol and acetonitrile, HPLC-grade, were obtained from Sigma (St Louis, MO, USA).

Ultrapure water (18.2 *M*Ω cm) was obtained by means of a Millipore Milli-Q apparatus (Milford, MA, USA).

The stock solutions (1 mg/ml) of the analytes were prepared by dissolving 20 mg of free base in 20 ml of methanol. The different standard solutions were prepared by diluting suitable amounts of the stock solution with mobile phase. Stock solutions of the analytes in methanol were stable for at least 3 months when stored at −20 °C, while the standard working solutions were prepared from the stock solutions immediately before the analysis, rapidly and in the dark.

2.2. Apparatus and chromatographic conditions

The chromatographic assays were carried out on a Jasco (Tokyo, Japan) PU-980 isocratic pump (flow-rate: 1.0 ml/min, loop 20 μl), equipped with a Jasco UV-975 spectrophotometric detector set at 230 nm.

Compounds were separated on a Varian (Harbor City, CA, USA) Microsorb MV C_8 reversed-phase column (150×4.6 mm I.D., 5 μm) connected to a 7×5 mm Phenomenex C_8 (Torrance, CA, USA) precolumn.

The mobile phase was composed of acetonitrile and a pH 2.2, 25 m*M* phosphate buffer (35/65, v/v). Triethylamine 0.25% (v/v) was added to the mixture and the pH was brought to 3.0 with phosphoric acid. An isocratic elution was used for the analysis of clopenthixol. Before use, the mobile phase was filtered through a 0.45-μm Varian nylon filter.

2.3. Plasma sample collection

Plasma samples were taken from schizophrenic patients of the Psychiatric Services, University of Bologna (Italy), undergoing therapy with zuclopenthixol, and put into vials containing EDTA as the anticoagulant. The blood was immediately centrifuged for 20 min at 1400 *g* and the supernatant plasma frozen and maintained at −20 °C until analysis which was usually carried out within 1 month. The samples were protected from light during storage, pretreatment and analysis.

The same procedure was used to separate plasma from the blood of healthy volunteers (''blank'' plasma).

2.4. Extraction procedure

Patient plasma, or blank plasma to which a suitable amount of standard solution was added, was subjected to an accurate solid-phase extraction before HPLC analysis. Aliquots of 500 μl of plasma diluted with 500 μl of water were loaded onto CN cartridges (100 mg, 1 ml) from Analytichem International

(Harbor City, CA, USA). Before loading, the cartridges were activated with 1 ml of methanol five times and conditioned with 1 ml of ultrapure water five times. After loading the samples, the cartridges were washed twice with 1 ml of water and with 1 ml of water–methanol (90:10, v/v) mixture and then elution was carried out with 1 ml of methanol. The methanol extract was evaporated to dryness in a rotary evaporator at 37 °C and the residue redissolved in 100 μl of mobile phase. Twenty microliters of sample were then injected into the HPLC system.

2.5. Method validation

2.5.1. Linearity

Calibration curves were obtained in the 1–300 ng ml^{-1} concentration range for Z-CLO and in the 1–200 ng ml^{-1} concentration range for E-CLO by spiking 500 μl of blank plasma with suitable amounts (e.g. 50 μl) of standard solution of the analytes, followed by the extraction procedure and analytical HPLC procedures described above.

The ratios between the area of analytes and that of the I.S. (prometazine) were plotted against the analyte concentrations added to blank plasma. The calibration curves were obtained by means of the least-squares method.

2.5.2. Precision

The blank plasma was spiked with Z-CLO, E-CLO and I.S. at three different concentrations to give final concentrations of 1–50–150 ng ml^{-1} for Z-CLO and 1–50–100 ng ml^{-1} for E-CLO. After thorough mixing, extraction and HPLC analysis were then performed. The procedure was repeated at least six times within the same day to obtain the repeatability and over different days to obtain the intermediate precision.

2.5.3. Extraction yield

Different samples of spiked blank plasma were extracted as previously described and injected into the HPLC system. The mean extraction yield values of the analytes were calculated by comparing the peak areas of blank plasma spiked with the analytes and subjected to the extraction procedure with the peak areas obtained from the corresponding standard solutions.

The precision and recovery assays were carried out according to USP XXIV [22] guidelines.

2.5.4. Accuracy

The accuracy of the proposed method was evaluated by means of recovery studies. Appropriate amounts of the two analytes (namely 2.5, 50, 100 ng ml^{-1} for Z-CLO and 2.5, 5, 10 ng ml^{-1} for E-CLO) were added to plasma samples containing known amounts of Z-CLO and E-CLO (i.e. previously analysed samples). The spiked samples were subjected to the extraction procedure described above and analysed by HPLC. Recoveries of the analytes added to spiked human plasma were calculated by interpolating the ratio of peak areas on the calibration curves. The procedure was repeated six times in the same day to obtain repeatability values.

3. Results and discussion

3.1. Selection of the optimal chromatographic conditions

The analysis of clopenthixol was carried out using a liquid chromatographic apparatus with a UV detector set at 230 nm. Preliminary assays were carried out on a reversed-phase column (C_8, 150× 4.6 mm, 5 μm) using a 0.1 mol/l ammonium acetate, methanol and acetonitrile (40:30:30, v/v/v) mixture as the mobile phase, according to the chromatographic conditions reported by Rop [14]. Under these conditions, the peak of Z-CLO was detected at 19.5 min. In order to reduce the analysis time, a more lipophilic and more acidic mobile phase was tested; in fact, methanol was substituted with acetonitrile (i.e. a 40:60 mixture of buffer and acetonitrile) and ammonium acetate was substituted with a pH 2.2, 25 m*M* phosphate buffer. Under these conditions, the retention times were shorter; however, the peaks of the two isomers were not fully baseline separated. To enhance the resolution of Z-CLO and E-CLO, the percentage of acetonitrile in the mobile phase was varied within the 20–60% range. From the dependence of the resolution on the acetonitrile percentage, it could be derived that the most favourable acetonitrile percentage was 35%. To find the optimal leading conditions for the HPLC analysis of the

isomers of clopenthixol, the final apparent pH* of the mobile phase was varied between 2.0 and 3.5. A comparison of these analyses showed that the best resolution and the shortest analysis time were achieved at pH 3.0. Moreover, in order to reduce peak tailing and to obtain better peak symmetry, triethylamine was added to the mobile phase at a percentage of 0.25% (v/v), adjusting the final apparent pH to 3.0 with phosphoric acid.

In conclusion, good baseline separation and satisfactory resolution for all analytes were obtained within 8.5 min with a mobile phase composed of 25 m*M* phosphate buffer, containing 35% acetonitrile and 0.25% triethylamine, at pH* 3.0.

In order to find a suitable internal standard, different CNS drugs were investigated (e.g. fluphenazine, chlorpromazine, prometazine, etc.) which have a quite similar molecular structure. Prometazine was chosen as the internal standard because it can be quantitatively extracted better using the SPE procedure described above. Moreover, prometazine has a retention time lower than that of Z-CLO, and this allows analysis within a short time.

3.2. HPLC analysis of standard solutions

The chromatogram of a mixture of Z-CLO, E-CLO and I.S. (prometazine, 125 ng ml^{-1}) is shown in Fig. 2. The analytes are well separated as neat chromatographic peaks; the peak of Z-CLO (50 ng ml^{-1}) has a retention time of 7.4 min, the peak of E-CLO (50 ng ml^{-1}) has a retention time of 7.9 min, while the peak of the I.S. (125 ng ml^{-1}) has a retention time of 6.3 min.

The standard calibration curves were established by plotting the ratio between the area of analytes and that of the I.S. against the analyte concentrations, and were linear in the 1–300 ng ml^{-1} concentration range for Z-CLO and in the 1–200 ng ml^{-1} concentration range for E-CLO. At therapeutic dosages, the plasma levels of E-CLO are very low (a few nanograms per millilitre); however, a large range was tested in order to allow for the analysis of E-CLO also in toxicological and forensic samples where its plasma levels can reach a few hundred nanograms per millilitre [14,15].

Precision data were satisfactory: in fact, the mean percentage relative standard deviation (RSD%) values ranged from 0.8 to 1.9 for repeatability and from 1.2 to 2.1 for intermediate precision. The limit of quantitation (LOQ) was 1 ng ml^{-1}, while the limit of detection (LOD) was 0.3 ng ml^{-1} for both isomers analysed. LOD and LOQ were calculated as the analyte concentrations which give rise to signals equal to three and 10 times the baseline noise, respectively, according to USP guidelines [22].

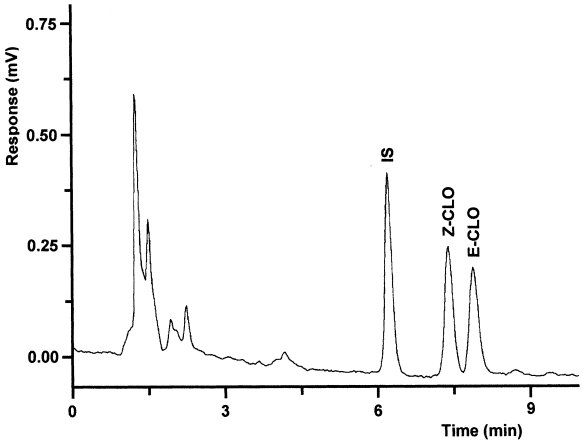


Fig. 2. Chromatogram of a standard solution containing Z-CLO (50 ng ml^{-1}), E-CLO (50 ng ml^{-1}) and I.S. (125 ng ml^{-1}). Chromatographic conditions: stationary phase, Microsorb MV C_8 column (150×4.6 mm I.D., 5 μm); mobile phase composed of 25 m*M* phosphate buffer, containing 35% acetonitrile and 0.25% triethylamine, at pH* 3.0.; flow rate, 1.0 ml min^{-1}; detection: λ=230 nm.

3.3. Implementation of a novel extraction procedure

Most extractive procedures developed for the analysis of clopenthixol in human plasma have been based on liquid–liquid extraction, which require a plasma volume of 2 or 3 ml [20] and are time consuming. Only a few methods report the use of solid-phase extraction procedures for the pretreatment of biological fluids containing Z-CLO; these procedures need high volumes of plasma (e.g. 2 ml) and give rather low extraction yield results (about 75%) [18,21]. In order to obtain cleaner plasma samples with a higher extraction yield of the analytes and to use a smaller volume of plasma, a novel SPE procedure was implemented. During the SPE development, cartridges with different sorbent types

(cyanopropyl, cyanopropyl endcapped, octyl, octadecyl, phenyl) were tested for purification of the biological samples. At the beginning, the SPE procedure was the same for all cartridges. As detailed in the Experimental section, the cartridges were activated with methanol and conditioned with water, then the cartridges were loaded with plasma (500 μl, spiked with the analytes) and washed twice with water and the analytes eluted with methanol. The methanol eluate was evaporated and the residue redissolved in 100 μl of mobile phase. Twenty microliters of the extract was injected into the HPLC system and the extraction efficiency was evaluated by comparison with the response from an equivalent standard solution. The extraction yield data obtained with the different cartridges are reported in Table 1. The best results were obtained using CN cartridges, for which the mean extraction yield of the analytes was 98%. After treatment with these cartridges, however, the eluate contained interfering compounds not fully separated from the I.S. and Z-CLO peaks. To overcome this problem, an additional washing step with a water/methanol mixture was introduced. The methanol percentage was varied in the 0–30% range. The best results were obtained when the final washing step was performed with water and 10% methanol; in fact, when methanol was used at a higher percentage, a decrease in the extraction yield was observed.

Table 1
Performance of different sorbents for SPE

Cartridge	Analyte	Extraction yield (%)
Cyanopropyl (CN)	Z-CLO	98
	E-CLO	94
	IS	101
Cyanopropyl endcapped (CN-N)	Z-CLO	90
	E-CLO	91
	IS	89
Octyl (C_8)	Z-CLO	92
	E-CLO	86
	IS	90
Octadecyl (C_{18})	Z-CLO	83
	E-CLO	79
	IS	84
Phenyl (PH)	Z-CLO	78
	E-CLO	72
	IS	81

3.4. HPLC of blank plasma

Both blank and spiked blank plasma samples were analysed and subjected to the extraction procedure described above. As is apparent from Table 1 and Fig. 3, the results demonstrated that this novel extraction procedure gave a good extraction yield and eliminated matrix interference. Fig. 3a shows the chromatogram of a blank plasma spiked with the I.S. (125 ng ml^{-1}) and subjected to the SPE procedure, while Fig. 3b shows the chromatogram of the same blank plasma sample spiked with 125 ng ml^{-1} of I.S., 30 ng ml^{-1} of Z-CLO and 30 ng ml^{-1} E-CLO. Moreover, the chromatogram of a blank plasma sample spiked with the analytes at a concentration equal to the LOQ (i.e. 1 ng ml^{-1}) and the I.S. is reported in Fig. 3c. As can be seen, the analytes are detected in all cases as symmetrical chromatographic peaks at a retention time of 6.3 min for the I.S., 7.5 min for Z-CLO and 7.9 min for E-CLO, and no interference is present at the retention times corresponding to the analyte peaks.

Calibration curves were obtained for spiked blank plasma samples in the 1–300 ng ml^{-1} concentration range for Z-CLO and in the 1–200 ng ml^{-1} concentration range for E-CLO. The regression equation for Z-CLO (obtained by means of the least-squares method) was $y=0.0628\ (\pm 0.0445)+0.0140\ (\pm 0.0008)x$, where y is the ratio between the area of Z-CLO and that of the I.S., and x is the concentration of Z-CLO, expressed as ng ml^{-1}. The linear correlation coefficient was $r=0.9968$. The regression equation for E-CLO (obtained by means of the least-squares method) was $y=-0.0009\ (\pm 0.0021)+0.0138\ (\pm 0.0001)x$, where y is the ratio between the area of E-CLO and that of the I.S., and x is the concentration of E-CLO, expressed as ng ml^{-1}. The linear correlation coefficient was $r=0.9999$. The LOQ was 1 ng ml^{-1} and the LOD value was 0.3 ng ml^{-1} for both analytes. These values were calculated according to USP XXIV [22].

Intra-day and inter-day precision assays were carried out to evaluate the precision of the method (extraction and chromatography). The extraction yield obtained at different concentrations of Z-CLO and its isomer, and the precision data are shown in Table 2. The results show that the mean extraction yield values ranged from 94 to 98%. Relative

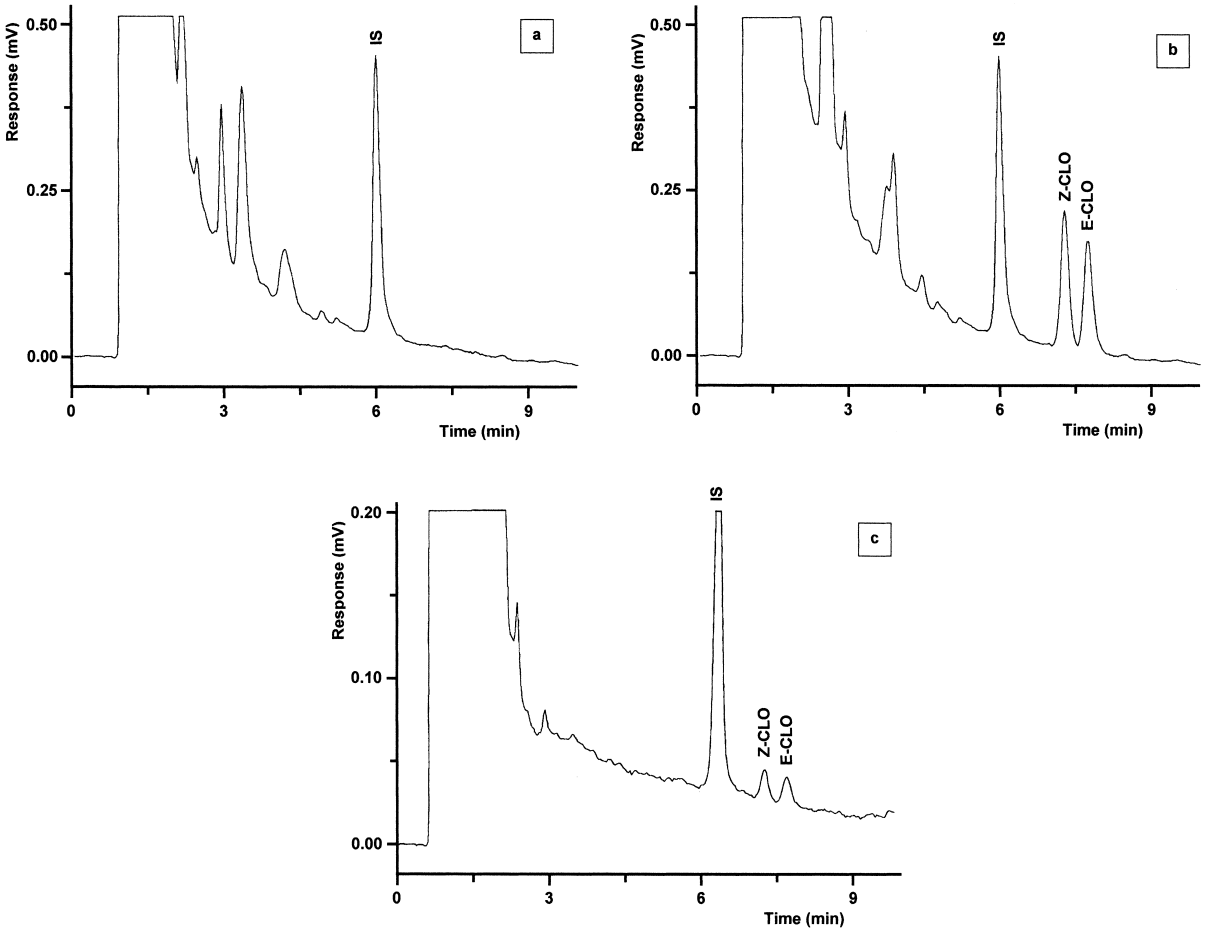


Fig. 3. Chromatograms of (a) a blank plasma sample spiked with the I.S. (125 ng ml^{-1}); (b) the same blank plasma sample spiked with Z-CLO (30 ng ml^{-1}), E-CLO (30 ng ml^{-1}) and I.S. (125 ng ml^{-1}) and (c) the same blank plasma sample spiked with Z-CLO and E-CLO at the LOQ concentration (i.e. 1 ng ml^{-1}) and I.S. (125 ng ml^{-1}). Chromatographic conditions as in Fig. 2.

Table 2
Extraction yield and precision data

Analyte	Amount added (ng ml^{-1})	% Mean extraction yield	Repeatability RSD%[a]	Intermediate precision RSD%[a]
Z-CLO	1	94	3.1	4.0
	50	97	2.0	2.8
	150	98	1.0	3.2
E-CLO	1	94	3.6	4.8
	50	95	1.2	4.5
	100	96	0.9	3.5

[a] $n=6$.

standard deviations were lower than 3.6 for repeatability (RSD% intra-day) and lower than 4.8 for the intermediate precision (RSD% inter-day). The results obtained show that the proposed method is suitable for clinical monitoring of zuclopenthixol, in terms of LOD, LOQ, and precision.

3.5. Application to human plasma samples

Having thus validated the method, it was applied to the analysis of some plasma samples from schizophrenic patients undergoing therapy with zuclopenthixol hydrochloride. The chromatogram of a plasma sample taken from a schizophrenic patient undergoing therapy with Z-CLO (30 mg day^{-1}) is reported in Fig. 4. The peaks corresponding to Z-CLO and E-CLO are well separated and no interference was detected. Another peak (at retention time 5.2 min) is also present in the chromatogram, which was not detectable in the chromatogram of spiked blank plasma (Fig. 3b), and it is probably due to one of the coadministered drugs. In fact, the patient was subjected to simultaneous therapy with clorazepam and zolpidem as well as zuclopenthixol.

The determination of the analytes was carried out by interpolating the ratio between the area of the analyte and that of the I.S. on the calibration curves obtained with spiked blank plasma. The levels of the analytes found in this sample are 20 ng ml^{-1} for Z-CLO and 1.5 ng ml^{-1} for E-CLO and are compatible with those reported in the literature [11].

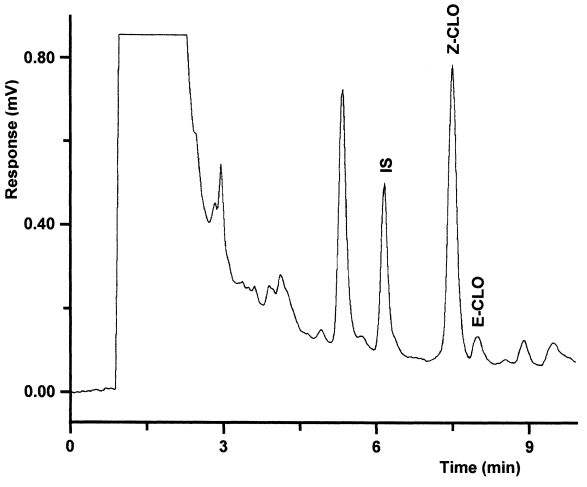


Fig. 4. Chromatogram of a plasma sample from a patient treated with 30 mg day^{-1} of zuclopenthixol. Chromatographic conditions as in Fig. 2.

Table 3
Accuracy of the method in spiked real human plasma

Analyte	Amount added (ng ml^{-1})	% Mean recovery	Repeatability RSD%[a]
Z-CLO	2.5	98	2.8
	50	101	2.3
	100	102	1.8
E-CLO	2.5	100	3.0
	5	93	2.7
	10	94	2.2

[a] $n=6$.

It should be noted that the method is also sufficiently sensitive for the determination of the E-CLO plasma levels that are usually very low in real plasma samples.

The accuracy of the proposed method, evaluated by means of recovery studies, was very high, as reported in Table 3. Recovery values of Z-CLO, from plasma samples, spiked at three levels (2.5, 50, 100 ng ml^{-1}) ranged from 98 to 102%, while recovery values of E-CLO from plasma samples, spiked at three levels (2.5, 5, 10 ng ml^{-1}) ranged from 93 to 100%. The method therefore showed satisfactory accuracy.

4. Conclusion

The developed liquid chromatographic method with UV detection is highly precise and accurate and shows good sensitivity and selectivity, thus it is suitable for reliable determination of the levels of clopenthixol isomers (*cis*(*Z*)-clopenthixol and *trans*-(*E*)-clopenthixol) in human plasma.

When compared to the studies reported in literature, this HPLC–UV method has the advantage of using simpler chromatographic conditions and equipment. In fact, most methods reported in the literature require sophisticated instrumentation such as mass spectrometry [14,15] or employ laborious and time-consuming procedures [19]. Moreover, the original SPE procedure developed for the plasma sample pretreatment ensures high extraction yields and

requires small amounts of plasma (500 μl) for a complete analysis.

In conclusion, when compared to the most sophisticated methods reported in the literature, the proposed method has the same good sensitivity and, in addition, has good precision and accuracy, and is thus useful for clinical monitoring of patients under therapy with zuclopenthixol.

Furthermore, this method allows for the dosage of Z- and E-CLO in plasma samples from intoxicated patients.

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References

[1] M. Bloch, E. Gur, A.Y. Shalev, Psychopharmacology 109 (1992) 377.
[2] A. Gravem, A. Bugge, Acta Psychiatr. Scand. 64 (1981) 5.
[3] J.A. Kurtzhals, B.J. Andersen, J.E. Kristiansen, S. Jepsen, APMIS 96 (1988) 357.
[4] S.C. Sweetman (Ed.), Martindale, The Complete Drug Reference, 33rd, Pharmaceutical Press, London, 2002, p. 714.
[5] A. Grinshpoon, M. Moskowitz, A. Valevski, A. Kreizman, L. Palei, M. Mark, A. Weiman, Eur. Psychiatry 13 (1998) 273.
[6] S. Brook, M. Berk, S. Selemani, J. Kolloori, I. Nzo, Hum. Psychopharmacol. 13 (1998) 17.
[7] T. Solgaard, K. Kirstrup, T. Aaes-Jørgensen, J. Gerlach, Pharmacopsychiatry 27 (1994) 119.
[8] A. Amdisen, T. Aaes-Jørgensen, N.J. Thomsen, V.T. Madsen, M.S. Nielsen, Psychopharmacology 90 (1986) 412.
[9] M. Schulz, A. Schmoldt, Pharmazie 75 (1997) 895.
[10] T. Aaes-Jørgensen, Acta Psychiatr. Scand. 64 (1981) 64.
[11] T. Aaes-Jørgensen, A. Gravem, A. Jørgensen, Acta Psychiatr. Scand. 64 (1981) 70.
[12] T. Aaes-Jørgensen, L. Kirk, E. Petersen, P. Danneskiold-Samsøe, A. Jørgensen, Psychopharmacology 81 (1983) 68.
[13] T. Aaes-Jørgensen, K.F. Overø, Acta Psychiatr. Scand. 61 (1980) 41.
[14] P.O. Rop, J. Anal. Toxicol. 25 (2001) 348.
[15] A. Tracqui, P. Kintz, V. Cirimele, F. Berthault, P. Mangin, B. Ludes, J. Anal. Toxicol. 21 (1997) 314.
[16] A. Viala, N. Hou, A. Durand, B. Ba, T. Aaes-Jørgensen, A. Jørgensen, J. Pharm. Belg. 38 (1983) 299.
[17] A. Viala, N. Hou, A. Durand, B. Ba, H. Dufour, N.D. Agostino, C. Berda, A. Jørgensen, Psychopharmacology 83 (1984) 147.
[18] H.R. Angelo, A. Petersen, Ther. Drug Monit. 23 (2001) 157.
[19] F.A. Aly, S.A. al-Tamimi, A.A. Alwarthan, Anal. Sci. 17 (2001) 1257.
[20] T. Aaes-Jørgensen, J. Chromatogr. 183 (1980) 239.
[21] B.B. Hansen, S.H. Hansen, J. Chromatogr. 658 (1994) 319.
[22] United States Pharmacopeia, 24th ed. United States Pharmacopeial Convention, Rockville, MD, 2000, pp. 2150–2151.