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# Sensitive gas–liquid chromatographic method for the assay of the neuroleptic drug *cis*(Z)-flupentixol in human serum or plasma

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**Abstract**

A gas–liquid chromatographic (GLC) assay suitable for the analysis of the *cis*(Z)-stereoisomer of the antipsychotic drug flupentixol in human serum or plasma was developed. The minimal quantifiable concentration was 0.5 ng/ml and the day-to-day coefficient of variation was 11.2% at 1 ng/ml and 8.7% at 10 ng/ml. Following addition of perphenazine as the internal standard (I.S.) and aqueous NaOH, samples (2 ml) are extracted with *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) (solvent), back-extracted to 0.1 *M* HCl and after one washing-step and addition of aqueous NaOH again extracted into 100 μl solvent. After evaporation to dryness, the extract is reconstituted in 20 μl solvent and evaporated to approximative 10 μl. A 4-μl aliquot is injected cool on-column onto the GLC system. A gas chromatograph HP 5890 with on-column injection port, nitrogen–phosphorus detector (NPD), a HP-1 25 m × 0.32 mm I.D., 0.52 μm capillary and hydrogen (3 ml/min, automated pressure control) as the carrier gas was applied. The negative influence of light on the assay was measured and discussed. The suitability of this method for clinical pharmacokinetic studies and therapeutic drug monitoring (TDM) was determined by the analysis of serum samples of 12 schizophrenic patients.

## 1. Introduction

The high-potent antipsychotic drug flupentixol (Fig. 1) is used as the dihydrochloride (drops and tablets) or as decanoic ester dissolved in Viscoleo (depot form for i.m. administration). Due to its thioxanthene chemical structure two stereoisomers exist. The oral form contains both the active principle *cis*(Z)-flupentixol and the much less active *trans*(E)-flupentixol in a 1:1 mixture. The intramuscularly applied depot form consists only of the active *cis*(Z)-isomer.

Because very low doses (oral: 1–4 mg/day, i.m.: 20–40 mg/14 days) are often preferred, because metabolization of the stereoisomers may be different [1] and for the aim of comparison of the oral and i.m. forms, a specific and sensitive method for the assay of *cis*(Z)-flupentixol in human serum is desirable.

A radioimmunoassay (RIA) for *cis*(Z)-flupentixol has been developed [2]. For practical purposes the cross-reactivity found with the *trans*-(E)-isomer (7%), with the main metabolites and also with other antipsychotic drugs can be neg-

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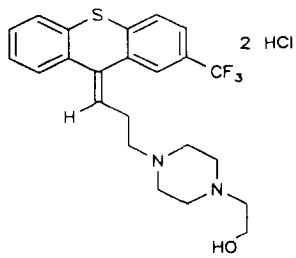


cis(Z)-flupentixol dihydrochloride

Fig. 1. Structure of *cis*(Z)-flupentixol dihydrochloride.

lected. In contrast, an earlier developed GLC method using a packed column did not discriminate between *cis*(Z)- and *trans*(E)-flupentixol [3]. Because of adsorption onto the column material [4] an additional derivatization step was necessary. This problem faced by analysts not using RIAs for the assay of very low concentrations of high-potent antipsychotic drugs has been overcome by the use of HPLC and the application of new series of fused-silica capillary columns in GLC. Comparable to RIA in their analytical performance, HPLC [4] and GLC [1] methods for the assay of *cis*(Z)-flupentixol have been developed. As an advantage, the simultaneous assay of the *trans*(E)-isomer is possible.

The sensitivity of flupentixol to light is well-known. The main photochemical reaction is *cis–trans* isomerization [5]. It is therefor astonishing, that for instance in the above mentioned *cis–trans* discriminating method [1] no hint is given weather or not this light sensitivity has been taken into account during handling and extraction of samples. For other neuroleptics sensitivity to light is also reported [6], but this seems not to be widely acknowledged.

The present method is based on the GLC method described in Ref. [1]. Using cold on-column injection and a 0.32 mm I.D. capillary it was the intention to improve the sensitivity of the assay and the chromatographic resolution of the stereoisomers. Furthermore, the photochemical changes of the analyte during the usual handling of samples and extraction procedure was investigated.

## 2. Experimental

### 2.1. Patients

Serum levels of *cis*(Z)-flupentixol have been measured for 12 patients over a time of therapy of 6 weeks (8 male, 4 female, 36.4 ± 5.9 yr, 6 smokers, 6 non-smokers, schizophrenic or schizodepressive patients according to DSM-3R). The patients received all i.m. *cis*(Z)-flupentixol (cF, Fluanxol Depot) every 2 weeks. Four patients additionally received orally *cis*(Z)- and *trans*(E)-flupentixol (c + tF, Fluanxol drops or tablets) but only for 2 or 3 weeks. Six patients received no concomitant medication except for the anticholinergic biperidene. Three patients received supplementary low-potent neuroleptics (promethazine, zotepine), three patients received a low-potent neuroleptic (levomepromazine, chlorprothixene) plus one antidepressant (amitriptyline). Once a week blood samples were drawn in vacutainers. When oral flupentixol was administered sampling was before the morning dose. Serum was protected from light, separated within one hour after blood sampling and stored at −20°C until analysis. All patients gave their written consent to participate in the investigation and the protocol was approved by the Ethical Committee of the Medical Faculty.

### 2.2. Chemicals

*cis*(Z)-Flupentixol (cF) was purchased from RBI (Natick, MA, USA), perphenazine was obtained from Sigma (Deisenhofen, Germany). The organic solvents *n*-hexane p.a., methanol p.a., isoamyl alcohol p.a. (mixture of isomers) and toluene were from Merck (Darmstadt, Germany). For the preparation of aqueous NaOH and HCl water Pestanal from Riedel-de Haën (Seelze, Germany) was used. NaOH p.a., HCl conc. p.a. were obtained from Merck, NaCl was obtained from Laborchemie Apolda (Apolda, Germany). Dichlordimethylsilane was purchased from Chemiewerk Nünchritz (Nünchritz, Germany).

### 2.3. Solutions

Aqueous NaOH (1 *M*) with 6% NaCl was prepared by dissolution of 20 g NaOH and 30 g NaCl in 500 ml water. HCl solution (0.1 *M*) was prepared by diluting 4.2 ml HCl conc. in 500 ml water. The extraction solvent was prepared by mixing 98.5 volumes of *n*-hexane with 1.5 volumes of isoamyl alcohol. For the silanization of glassware a solution of 5% dichlordimethylsilane in toluene was used.

### 2.4. Reference and standard solutions

The reference and standard solutions (1 μg/ml) were prepared as described in Ref. [3] daily by diluting 1:100 with methanol stock solutions of *cis*(Z)-flupentixol and perphenazine in methanol (100 μg/ml). The stock solutions were stored at −20°C.

### 2.5. Glassware

Glassware was silanized with 5% dichlordimethylsilane in toluene monthly. A special cleaning procedure including 30-min sonification in 0.001 *M* HCl was applied. Polypropylene tubes have been tested to give no advantage but are uncomfortable in volume handling because of their opaque consistency. Polystyrene tubes burst when used in the extraction.

### 2.6. Sample preparation

A 3-step liquid–liquid extraction procedure plus one washing step has been developed to obtain a highly concentrated and very pure extract of the drug from body fluid. Serum (2 ml) was mixed in a 10-ml glass tube with 50 μl of standard solution containing 1 μg/ml perphenazine in methanol. A 0.5-ml volume of aqueous NaOH with 6% NaCl and 4 ml *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) were added. The first extraction step was carried out by 20 min shaking. After 5 min centrifugation at 1500 *g* as much as possible (ca. 3.5 ml) of the organic layer were transferred to 1.0 ml 0.1 *M* HCl in a 10-ml glass tube and shaken for 10 min. After 2 min of centrifugation at 1500 *g* the organic layer was discarded. A 1-ml volume of *n*-hexane–isoamyl alcohol was added, the two phases vortex-mixed for 30 s and separated again by 2 to 5 min centrifugation. From the lower phase (0.1 *M* HCl) as much as possible was carefully removed and placed in a 4-ml glass tube. The inner diameter of these tubes was 8 mm instead of 15 mm for the tubes used in the first and second extraction steps. A 150-μl volume of the aqueous NaOH and 100 μl of *n*-hexane–isoamyl alcohol were added and vortex-mixed for 30 s. After 2 min centrifugation as much as possible of the organic layer (ca. 80 μl) was separated to a tapered 4-ml glass tube. The solution is evaporated to dryness for 3 min in a vacuum-evaporator and reconstituted in 20 μl of *n*-hexane–isoamyl alcohol. By standing 10 min at 40°C in a heating block (Techne DP-2P) the solution is evaporated to approximately 8 to 10 μl. A 4-μl aliquot is injected onto the GC system.

### 2.7. Apparatus

A Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a nitrogen–phosphorus detector (NPD) and an on-column injection port was used for the analysis. Separation was obtained with a 25 m × 0.32 mm I.D., 0.52 μm HP-1 capillary and hydrogen (3.0 ml/min, automated pressure control) was used as the carrier gas. Flow-rates of the detector gases were: air, 100 ml/min (275 kPa); hydrogen, 0.7 ml/min (25 kPa); and the make-up gas nitrogen, 27 ml/min (220 kPa). The detection port was maintained at 300°C. A temperature program was used for the oven [$T_1 = 60$°C, $T_2 = 260$°C, $T_3 = 290$°C; $\text{rate}_1 = 40$°C/min, $\text{rate}_2 = 2$°C/min; $t(T_1) = 0$ min, $t(T_2) = 0$ min, $t(T_3) = 5$ min] and the on-column injector port [$T_1 = 63$°C, $T_2 = 263$°C, $T_3 = 293$°C; $\text{rate}_1 = 40$°C/min, $\text{rate}_2 = 2$°C/min; $t(T_1) = 0$ min, $t(T_2) = 0$ min, $t(T_3) = 5$ min]. The NPD was operated at a baseline of 70–80 pA. An integrator HP 3398 Series II was

used for the calculation of retention times, peak widths and peak areas.

### 2.8. Quantitation

Calibration curves were constructed by plotting the peak-area ratios ($A_{cF}/A_{IS}$) obtained from blank serum spiked with the above mentioned reference solution of *cis*(Z)-flupentixol. Eight equidistant concentrations ranging from 1 to 8 ng/ml have been used.

### 2.9. Accuracy and precision

Accuracy was assessed by determining the concentrations of drug measured in samples (0.5 ng/ml, 1.0 ng/ml, 2.0 ng/ml, 10.0 ng/ml, $n = 6$ each) relative to the known concentration added. Precision was determined utilizing the relative standard deviation (%) of the within-day and between-day ($n = 6$) variations.

### 2.10. Chromatographic separation of stereoisomers

The separation of the two stereoisomers was found to be dependent on the volume injected. This should be mainly caused by the increased floating zone during cold on-column injection. The dependence of the resolution factor $R_s$ on the injected volume was investigated as follows.

To obtain a mixture of the stereoisomers a solution of 10 ng/$\mu$l *cis*(Z)-flupentixol in *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) was bubbled with nitrogen for 5 min and irradiated with UV light (365 nm) for 10 min. In the chromatogram, beside the peak of the *cis*(Z)-stereoisomer ($t_r^{cis}$ = 11.203 min, Fig. 2) a new peak at $t_r$ = 11.388 min appeared. From the known photochemical reaction [5] and the chromatograms shown in Ref. [1] this can only be the *trans*(E)-stereoisomer of flupentixol. The peak separation $R_s$ was calculated using the following formula [7]: $R_s = 1.177 (t_r^{trans} - t_r^{cis})/(w_H^{trans} + w_H^{cis})$, where $t_r$ = retention time and $w_H$ = peak width at half-height.

The solution of 10 ng/$\mu$l [approximative 5 ng/$\mu$l *cis*(Z)- and *trans*(E)-flupentixol each] was diluted stepwise to obtain solutions of 5.0, 3.3, 2.5, 2.0 and 1.65 ng/$\mu$l flupentixol (each containing half the concentrations of the two stereoisomers). Therefore, when increasing volumes of 1 to 6 $\mu$l are injected, the absolute amount of each stereoisomer injected remains 5 ng.

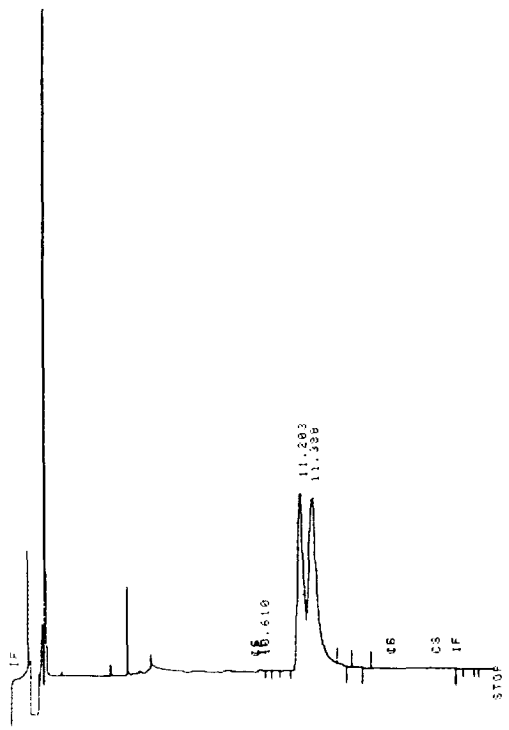


Fig. 2. Chromatographic separation of *cis*(Z)- and *trans*(E)-flupentixol. 5 ng each, 4-$\mu$l aliquot injected, chartspeed changed from 0.5 to 1.5 cm/min in the region of the peaks of interest (indicated by CS in the chromatogram).

## 3. Results and discussion

As in Ref. [1] no baseline separation of the stereoisomer peaks was found (Fig. 2), even though an $R_s$ of ca. 1 has been calculated when 1–2 $\mu$l are injected. The disagreement of the shape of the chromatograms with the theoretical value $R_s$ is caused by a slight tailing of the peaks. The resolution decreased as the injected volume was increased (Table 1). Because, with respect to an optimal sensitivity of the method, we want to inject as much as possible of the final extract,

Table 1
Dependence of the resolution of *cis*(Z)- and *trans*(E)-flupentixol on the injected volume

| Injected volume (μl) | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $R_s$ | 1.10 | 1.07 | 1.02 | 0.85 | 0.65 | 0.59 |

4 μl can be regarded as the optimal injection volume ($R_s = 0.85$). Concerning the resolution of the stereoisomers no general advantage compared with the method in Ref. [1] ($R_s = 0.75$), which uses a megabore capillary and helium as the carrier gas, was achieved. When using helium in the present method also no improvement of the resolution was found. A retention gap usually increases the resolution of chromatograms with on-column injection. However, in the case of flupentixol a considerable decay of peak areas was found when using a retention gap (HP, 5 m or 1 m × 0.32 mm I.D.). Obviously, deactivation of the inner surface of the retention gap seems to be insufficient for the analyte.

The recoveries of *cis*(Z)-flupentixol and I.S. were determined by comparison with the direct injection of the compounds onto the chromatographic system. The recoveries of the examined extraction procedure were 45% for *cis*(Z)-flupentixol and I.S.. No improvement of the recovery was found when the extraction times were increased. Also the use of a larger volume of extraction solvent in the first extraction step, a double first extraction step or the use of an increased volume of the final extraction solvent (e.g. 500 μl instead of 100 μl) gave no general improvement. In silanized glassware the handling of such a small volume of 100 μl was not less comfortable than e.g. a 500-μl volume. The loss of volume may be increased (about 20%). However, we found that the recovery tends to decrease when increasing volumes have to be evaporated. In Ref. [1] a recovery of 75% is reported. This increased recovery of the otherwise very similar extraction procedure should be the result of the application of the more polar solvent *n*-heptane–2-propanol (90:10, v/v). However, the handling of volumes is rather difficult when using this solvent. In two attempts examining this method a 55% recovery has been obtained. Although 75% should be attainable, *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) was favored because of the easier volume handling. Furthermore, the extracts obtained with *n*-heptane–2-propanol (90:10, v/v) were considerable less pure. An exorbitant peak from $t_r \sim 5$ to 9 min was found in the chromatograms, possibly due to co-extracted endogenous material. After injection of several extracts the performance of the NPD considerably decreased. This problem was also apparent, when a simple one-step extraction with *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) was tested.

Fig. 3 shows chromatograms of blank plasma, plasma spiked with 0.5 ng/ml and 10.0 ng/ml *cis*(Z)-flupentixol (each with I.S.) and of a patient plasma calculated to contain 4.9 ng/ml *cis*(Z)-flupentixol. The peak of the analyte occurs at $t_r^{cis} = 11.10$–$11.45$ min and the peak of the I.S. at $t_r = 15.20$–$15.55$ min. Blank plasma samples did not show *cis*(Z)-flupentixol or I.S. peaks. Just a very small peak occasionally appeared as a shoulder of *cis*(Z)-flupentixol, but this did not influence the calculation of peak areas. Calibration curves were typically described by the formula $y = 0.0388x - 0.0009$, where $y$ is the peak-area ratio $A_{cF}/A_{I.S.}$ and $x$ is the concentration of *cis*(Z)-flupentixol. The calculated peak-area ratios and the added concentrations displayed a good linear relationship between 0.5 and 10.0 ng/ml with a correlation coefficient $r = 0.997$. The assay of *cis*(Z)-flupentixol was accurate and precise as summarized in Table 2. Precision, as determined by the relative standard deviation of the within-day and between-day variations of samples of 0.5, 1.0, 2.0 and 10.0 ng/ml, was consistently less than 10–15%. Accuracy, indicated as the concentration of drug measured in samples of 0.5, 1.0, 2.0 and 10.0 ng/ml relative to the known concentrations, was in the range of 97–115%. The lower limit of detection was 0.15 ng/ml under the described conditions at a signal-to-noise ratio of 3. Due to the accuracy and precision found at 0.5 ng/ml, this value can be regarded as the minimal quantifiable concentration. Compared with the method described in Ref. [1], this is a 3-fold increase in the sensitivity for *cis*(Z)-flupentixol at an equal

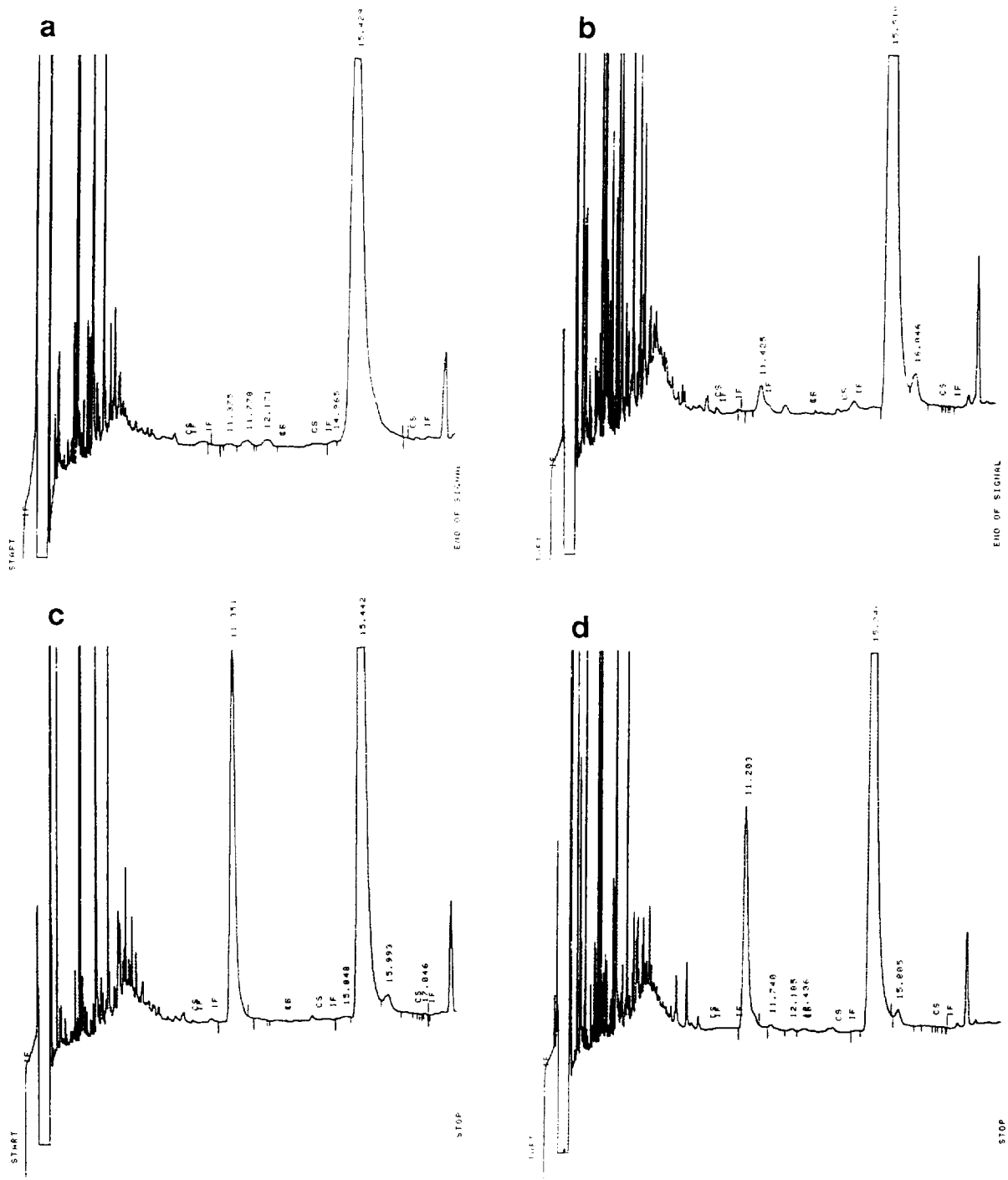


Fig. 3. Chromatograms of extracts of (a) blank plasma, (b) plasma spiked with 0.5 ng/ml and (c) 10.0 ng/ml *cis*(Z)-flupentixol (each with I.S.), and (d) of a patient plasma calculated to contain 4.9 ng/ml *cis*(Z)-flupentixol (4th week of the patient as shown in Fig. 5a, maximum level one week after 100 mg i.m. injection), chartspeed changed from 0.5 to 1.5 cm/min in the region of the peaks of interest (indicated by CS in the chromatograms).

Table 2
Accuracy and precision of the assay

| Concentration added (ng/ml) | Measured concentration (mean ± S.D.) (ng/ml) | | Accuracy (%) | | Precision (R.S.D., %) | |
|---|---|---|---|---|---|---|
| | within-day | between-day | within-day | between-day | within-day | between-day |
| 0.5 | 0.57 ± 0.08 | | 115 | | 13.5 | |
| 1.0 | 1.03 ± 0.09 | 1.04 ± 0.12 | 103 | 104 | 8.7 | 11.5 |
| 2.0 | 1.94 ± 0.16 | | 97 | | 8.2 | |
| 10.0 | 10.8 ± 1.09 | 11.0 ± 1.00 | 108 | 110 | 10.1 | 9.1 |

performance of the chromatographic separation of the stereoisomers. Mainly the different applied injection technique should have caused this improvement.

In the clinical praxis, multiple concomitant medication is usually given when high-potent neuroleptics such as flupentixol are administered. This includes benzodiazepines (e.g. diazepam, nitrazepam, chlordiazepoxid), anticholinergics (e.g. biperidene), low-potent neuroleptics (e.g. chlorpromazine, chlorprothixene, promethazine, perazine) and antidepressants (e.g. amitriptyline, trimipramine, clomipramine, trazodone, dibenzepine). Also metabolites of these drugs, which may occur proportionally to the drug concentrations, have to be taken into consideration (e.g. desmethyl metabolites of antidepressants, 10-hydroxyamitriptyline and -nortriptyline, reduced haloperidol). Other high-potent neuroleptics (e.g. haloperidol, *cis*(Z)-clopentixol, fluphenazine) are usually not co-administered together with flupentixol, but interferences are possible, when changing the depot medication, e.g. from haloperidol decanoate to flupentixol decanoate. Therefore, it has to be ascertained that these compounds give no interferences with *cis*(Z)-flupentixol in the chromatograms. The drugs and metabolites tested for interference with *cis*(Z)-flupentixol and the I.S. perphenazine are shown in Table 3. From the relative retention times, it is obvious that, except for fluphenazine, no drug or metabolite interferes with *cis*(Z)-flupentixol. Thus, in therapeutic drug monitoring (TDM) errors can only occur, when depot medication is switched from fluphenazine decanoate to *cis*(Z)-flupentixol decanoate, because the half-life of fluphenazine decanoate is about 14 days. Therefore, the present method for the assay of *cis*(Z)-flupentixol can only be applied if it is confirmed by the

Table 3
Relative retention times of drugs and metabolites

| Drug or metabolite | Relative retention time |
|---|---|
| *cis*(Z)-Flupentixol | 1.00 |
| Fluphenazine | 1.00 |
| *cis*(Z)-Clopentixol | 1.34 |
| Haloperidol | 0.93 |
| Reduced haloperidol | 0.96 |
| Pipamperone | 0.99 ($R_s = 0.57$) |
| Perphenazine | 1.36 |
| Chlorprothixene | 0.65 + 0.66 |
| Promethazine | 0.54 |
| Perazine | 0.80 |
| Zotepine | 0.66 |
| Metophenazat | no signal |
| Clozapine | 0.82 |
| Carbamazepine | 0.59 |
| Clomipramine | 0.60 |
| Desmethyclomipramine | 0.61 |
| Amitriptyline | 0.52 |
| Nortriptyline | 0.53 |
| 10-Hydroxyamitriptyline | 0.61 |
| 10-Hydroxynortriptyline | 0.62 |
| Doxepin | 0.54 |
| Dibenzepine | 0.61 |
| Trazodone | 1.33 |
| Biperidene | < 0.5 |
| Oxazepam | 0.61 |
| Lorazepam | 0.63 |
| Diazepam | 0.64 |
| Nitrazepam | 0.80 |
| Chlordiazepoxid | 0.64 + 0.81 |

clinician, that no fluphenazine decanoate was administered during the last 6 weeks prior to the assay of *cis*(Z)-flupentixol. However, it is assumed that such a change in treatment will occur only in few cases compared with otherwise demands for the TDM of *cis*(Z)-flupentixol. Although using similar chromatographic conditions, this problem is not acknowledged in Refs. [1,3]. The substances examined in Table 3 were often found as concomitant medication in the local university psychiatric clinic. However, the application of other supplementary medication is also possible.

The (negative) influence of light on the assay has been investigated by exposing samples (2 ml of serum in 10-ml glass tubes, 10 ng/ml cF) to diffuse daylight and artificial laboratory light (a room without window) for 0, 1, 2 and 3 h. The samples were shaken every hour for 1 min. Although this set-up is insufficient for exact photokinetic investigations, it should be instructive in the evaluation of possible errors of the assay. In this context it may be important to emphasize, that a common laboratory has daylight conditions. As shown in Fig. 4 already after one hour the concentration of *cis*(Z)-flupentixol in samples standing in daylight has diminished to 69% of the initial concentration. Simultaneously, the peak of the photo-isomerization product *trans*(E)-flupentixol in the chromatograms increases. After 3 h the photo-stationary state with 50% each of *cis*(Z)- and *trans*(E)-flupentixol was nearly achieved. In contrast, no product formation or decrease of *cis*(Z)-flupentixol was detected in samples which were exposed to artificial light. Therefore, in agreement with the absorption spectra of *cis*(Z)-flupentixol, only near UV- and low-wavelength visible light, which do not occur in the artificial light, can cause the aforementioned reaction. An analogous result was found with regard to acidic solutions. In 0.1 *M* HCl the content of *cis*(Z)-flupentixol was decreased to 75% after 30 min exposure to daylight, but no reaction occurred with artificial light. These findings, which are in accordance with those described in Ref. [6], emphasize the necessity to take into consideration possible errors in the assay of neuroleptic drugs (except

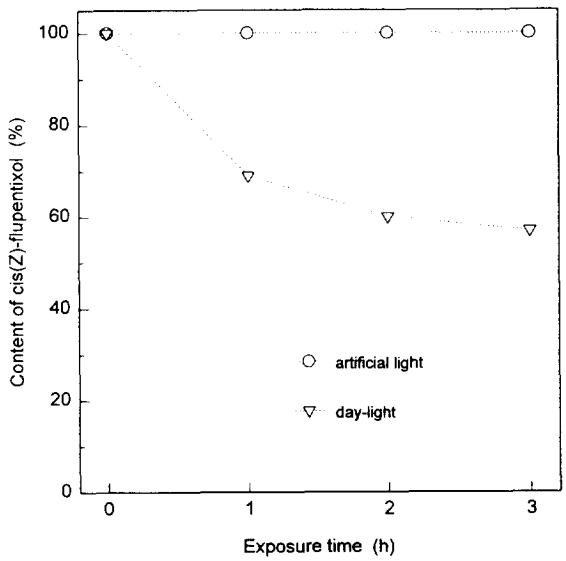


Fig. 4. Photochemical decay of *cis*(Z)-flupentixol in human serum (10 ng/ml) under daylight (a common laboratory with windows) and artificial laboratory light (a room without windows) conditions.

for butyrophenones as haloperidol), which may be caused by such a very reactive "chemical" as light. In Ref. [8] subdued light is recommended for the assay of the phenothiazine trifluoperazine. The present results suggest, that the wavelength of light is more important than the light intensity. In other words, subdued daylight may only decrease the extent of the error but may not completely eliminate such an error.

A typical time course of the serum level of *cis*(Z)-flupentixol in a patient (hebephrenic, DSM-3R 295.62) receiving fluanxol decanoate (10%, 100 mg/14 days) is shown in Fig. 5a. A maximum appears always one week after the i.m. injection. Although on a stable dose, considerable differences in this peak level may occur. Before the next injection a minimum value was always found (2.4–3.6 ng/ml). This minimum serum level is the basis of investigations of the correlation between serum level and effect. For the present patient the serum level can be regarded as being above the threshold concentration of flupentixol for a satisfactory

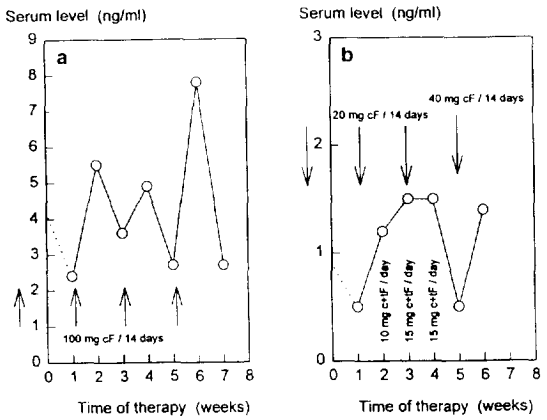


Fig. 5. Time course over 6 weeks of the *cis*(Z)-flupentixol serum level of (a) a hebephrenic patient receiving only i.m. flupentixol, (b) a paranoid psychotic patient receiving concomitant oral flupentixol for 3 weeks; dosage of i.m. (cF) and oral (c + tF) flupentixol at times indicated by arrows.

antipsychotic effect of approximately 2 ng/ml (ca. 1.0 ng/ml *cis*(Z)-flupentixol) [9]. In a withdrawal study during maintenance therapy of 24 schizophrenic outpatients a mean minimal effective serum level of *cis*(Z)-flupentixol of 2.9 ng/ml was found, however with a wide range from 0.5 ng/ml to 14.8 ng/ml [10]. However, in the present study none of the 12 patients had serum levels of *cis*(Z)-flupentixol $>10$ ng/ml. This includes patients receiving 150 mg/14 days i.m. or 20 mg/day orally. An additional patient (160 kg body weight), who received 100 mg/7 days i.m., had serum levels between 5 and 6 ng/ml over several weeks. Therefore, 14.8 ng/ml during maintenance therapy with i.m. flupentixol should be a very rare case.

Fig. 5b shows the serum levels of a female patient (paranoid psychosis, DSM-3R 295.35), who received supplementary oral flupentixol from the 2nd to the 4th week. With i.m. flupentixol alone the minimum serum level was just 0.5 ng/ml. When 10 mg/day or 15 mg/day flupentixol were co-administered orally (i.e. 5 and 7.5 mg/day *cis*(Z)-flupentixol, respectively) the serum levels increased to 1.2 and 1.5 ng/ml, respectively. In the chromatograms the peak of the *trans*(E)-stereoisomer appeared, but was not quantified. The typical shape of the i.m. administration curve with bi-weekly minimum and maximum serum levels (Fig. 5a) is interrupted by the oral administration, as is visible for instance in the 3rd week (no bi-weekly minimum level).

Of the 12 patients receiving i.m. flupentixol bi-weekly (including the 4 patients on temporary oral co-medication) seven received the 2% form ($0.21 \pm 0.07$ mg/kg 14 days, 0.11–0.26 mg/kg) and the other 5 the 10% form ($1.10 \pm 0.6$ mg/kg 14 days, 0.57–1.95 mg/kg). The mean minimum serum level of the first group (2% form, $0.73 \pm 0.61$ ng/ml) was significantly lower than the level of the second group (10% form, $2.06 \pm 0.58$ ng/ml) ($p = 0.0037$). Two patients of the first group even had no detectable minimum serum levels, i.e. $<0.15$ ng/ml. In accordance with the results of Ref. [9] these were assumed to be subtherapeutic serum levels for *cis*(Z)-flupentixol. Therefore, patients with 2% i.m. flupentixol/14 days should be candidates for TDM to avoid subtherapeutic serum levels. This proves right especially for outpatients on maintenance therapy. The GLC method presented here should be a useful tool for the measurement of very low concentrations of *cis*(Z)-flupentixol in human serum and plasma.

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