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Short communication

Quantification of the antipsychotics flupentixol and haloperidol in human serum by high-performance liquid chromatography with ultraviolet detection

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Abstract

A high-performance liquid chromatography (HPLC) method was developed for quantification of both isomers of the thioxanthene neuroleptic flupentixol and of the butyrophenone derivative haloperidol in human serum. After extraction with diethyl ether–*n*-heptane (50:50, v/v), an isocratic normal-phase HPLC system with a Hypersil cyanopropyl silica column (250×4.6 mm, 5 μm particle size) was used with ultraviolet detection at 254 nm and elution with a mixture of 920 ml acetonitrile, 110 ml methanol, 30 ml 0.1 *M* ammonium acetate, and 50 μl triethylamine. The limit of quantitation of 0.5 ng/ml and 0.3 ng/ml for flupentixol and haloperidol, respectively, was sufficient to quantify both compounds in serum after administration of clinically adjusted doses. The suitability of the described method for therapeutic drug monitoring and clinical pharmacokinetic studies was assessed by analysis of more than 100 trough level serum samples.

Keywords: Flupentixol; Haloperidol

1. Introduction

Flupentixol and haloperidol are highly potent drugs used for treatment of schizophrenia and other neuropsychiatric diseases. The oral form of flupentixol, administered as tablets or drops, is an equimolar mixture of the active *cis*-(*Z*)- and the inactive *trans*-(*E*)-isomer, the parenteral preparation for intramuscular injection is the decanoid acid ester of the isolated *cis*-isomer. The structures of these antipsychotics are given in Fig. 1. There is considerable interest in sufficiently sensitive, robust and inexpensive methods for the quantification of drug concentrations of neuroleptics achieved during treatment. Neuroleptics are frequently associated with severe neurological side effects and a serum-concentration guided drug dosage might help to reduce the number of patients suffering from such side effects due to overdosing. On the other hand, also inefficient drug therapy due to noncompliance or due to individually variable low drug absorption or high drug clearance therapy may be prevented using therapeutic drug monitoring.

Only a few of the published methods were designed to measure blood concentrations of the highly

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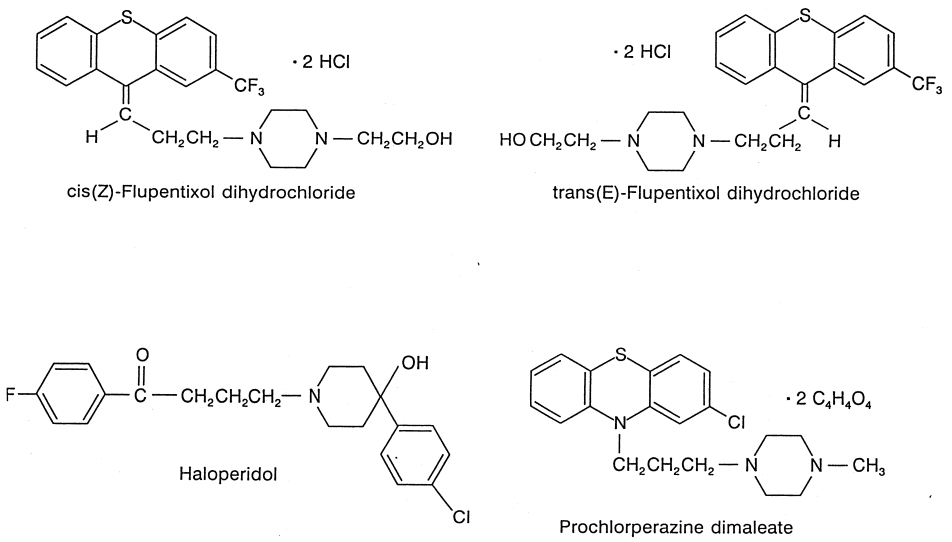

Fig. 1. Structures of *cis*-(*Z*)-flupentixol, *trans*-(*E*)-flupentixol, haloperidol and prochlorperazine (used as internal standard).

potent neuroleptic drug flupentixol with sufficient selectivity and sensitivity. Some published methods were designed for analysis of the pharmaceutical preparations while blood level monitoring has completely different requirements with respect to sensitivity and selectivity to other compounds in the matrix. In order to achieve the required sensitivity, some previously published methods [1,2] used relatively large sample amounts such as 2 ml of serum. Withdrawal of large blood volumes might however cause problems in medical treatment, especially if many samples are required for frequent drug monitoring or pharmacokinetic analysis.

Shu et al. [3], whose intention was to isolate and identify the metabolites of flupentixol, also used a high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection, but their method was not intended to be used as a quantitative method and they did not provided any data on sensitivity and precision. The importance to analyze both isomers of flupentixol was supported by the work of Balant-Gorgia et al. [4], who pointed to the difference in the metabolism of the *cis*- and *trans*-forms. They used a gas chromatography (GC) method with derivatization and nitrogen selective detection with a quantitation limit of 0.5 ng/ml when extracting 2 ml of serum.

By comparison of GC with radioimmunoassay (RIA) [2], the concentrations measured by the RIA method were higher than those detected by GC due to the lack of specificity of the antiserum towards parent compound and some metabolites. There is another method for analysis of flupentixol in pharmaceutical preparations [5]. Such methods cannot generally be transferred to analysis of the very low concentrations found in body fluids.

We developed a rapid one-step extraction procedure. This is economically advantageous and might give more precise results because of the time dependent *cis–trans* isomerization during exposure to daylight [1].

During our validation for possible interfering drugs we found that also several other antipsychotics might be quantified using this method primarily developed for concentration analysis of flupentixol. In particular, the method allows one to quantify haloperidol with a quantification limit of 0.3 ng/ml. There are available several methods for quantification of haloperidol [6–13]. Many of these methods used however electrochemical detection [10–13], a HPLC method which is not optimal for rapid on-demand analyses due to long equilibration times of most electrochemical detectors.

In this respect, methods based on UV detection may be preferable for routine applications such as therapeutic drug monitoring.

2. Experimental

2.1. Apparatus

The HPLC system comprised a Shimadzu LC-10 AS liquid chromatograph, a Shimadzu UV–Vis spectrophotometric detector (SPD-10 AV), adjusted to 254 nm and a Shimadzu SIL-10 A autoinjector with a 100-μl sample loop. The Class-LC-10 program (Shimadzu) was used to record, integrate and store the chromatograms.

2.2. Chemicals, reference compounds and samples

Flupentixol was kindly provided by Bayer (Leverkusen, Germany), *cis*- and *trans*-flupentixol and prochlorperazine, used as the internal standard, were obtained from RBI (distributed by Bio Trend, Cologne, Germany). All organic solvents were of chromatography-grade and purchased from Merck (Darmstadt, Germany). Ammonium acetate was of analytical grade (Merck). Stock solutions of flupentixol, haloperidol and prochlorperazine (1 mg/100 ml methanol) were stored at 4°C and proved to be stable for at least six months as tested by repeated HPLC analyses comparing with freshly dissolved reference compounds. These standard solutions were used to spike drug-free human serum for quality control samples and for the calibrators. Serum samples were separated by centrifugation and were kept frozen (−20°C) until analysis.

2.3. Extraction

Serum (1 ml) was mixed in a 10-ml glass tube with the internal standard prochlorperazine (10 ng, dissolved in methanol) and 50 μl saturated solution of sodium carbonate. Seven ml of diethyl ether–*n*-heptane (50:50, v/v) was added and the tubes were shaken for 30 min. After 10 min centrifugation at 4000 *g*, the organic phase was transferred into a 10-ml glass tube and evaporated to dryness in a Speed-vac concentrator (50°C). The aqueous phase was extracted again and this extract was combined with the first dried extract and evaporated to dryness. After reconstitution in 100 μl of the mobile phase, 75 μl was injected onto the chromatographic system.

2.4. Chromatography

The mobile phase was prepared by mixing 920 ml acetonitrile, 110 ml methanol, 30 ml 0.1 *M* ammonium acetate, and 50 μl triethylamine. Separation was performed on a Hypersil CPS column (cyanopropylsilica) with 5 μm particle size and 120 Å pore size (250×4.6 mm) with a guard column containing the same packing material (Hypersil CPS, 10×4.6 mm) and kept at 40°C (using a Shimadzu oven CTO-10 A). Flow-rate was 1 ml/min and the eluent was monitored at 254 nm.

2.5. Methods for data analysis, statistical methods

Concentrations of the calibrators ranged from 0 to 10 ng/ml for both isomers of flupentixol (seven calibrators: 0, 0.5, 2.5, 5, 7.5, 10 ng/ml) and from 0 to 20 ng/ml for haloperidol (eight calibrators: 0, 0.31, 0.63, 1.25, 2.5, 5, 10, 20). These concentrations covered the range of most previously published serum levels under routine treatment [1,10,14–17]. All samples were analysed in 'true' duplicates, i.e., every patient sample was spiked with internal standard, extracted and chromatographed two times, the arithmetic mean of both values was used for further calculations. The concentrations of the control samples (serum spiked with the drugs) were 1.0 and 7.5 ng/ml for the isomers of flupentixol and 1.25 and 7.5 ng/ml for haloperidol. With these samples, the intra- and inter-assay variances were estimated. All concentrations were calculated from peak height ratios of the substances to be analyzed over the internal standard.

3. Results

The retention times of *cis*- and *trans*-flupentixol were 9.368 and 10.6 min, respectively with baseline separation as illustrated in the Figs. 2 and 3. The retention time of haloperidol was 9.965 min. The retention times of the internal standard and several other antipsychotics are given Table 1.

The limit of detection (LOD) is determined as concentration corresponding to signal-to-noise-ratio of 3:1. Measured with the standard solutions of the

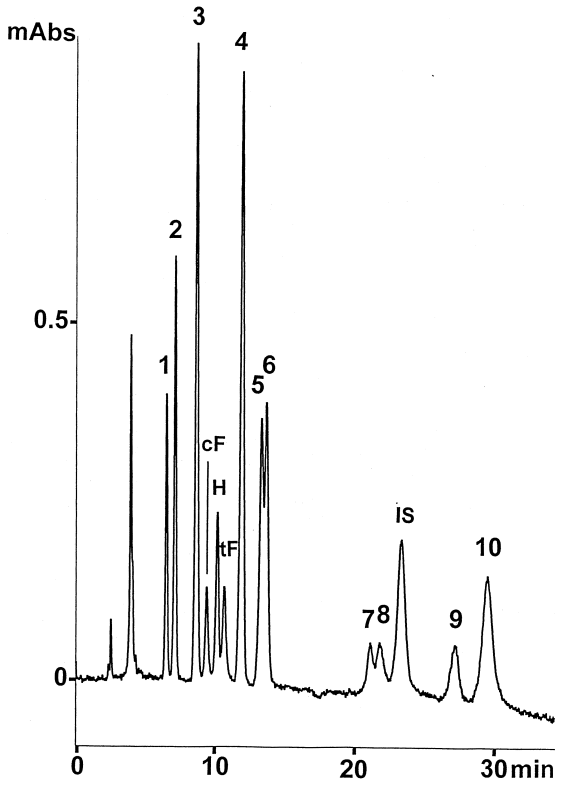

Fig. 2. Chromatographic separation of several antipsychotic drugs and some of their metabolites. For all compounds, except for flupentixol, an amount of 2 ng was injected, for flupentixol 1 ng per isomer was injected. 1=Benperidol, 2=haloperidol metabolite III [which is 3-(4-fluorobenzoyl)propionic acid], 3=clozapine, cF=*cis*-(*Z*)-flupentixol, H=haloperidol, tF=*trans*-(*E*)-flupentixol, 4=levomepromazine, 5=chlorprothixene, 6=olanzapine, 7=risperidone, 8=fluphenazinesulfoxide, I.S.=prochlorperazine (serving as internal standard), 9=melperone, 10=perazine.

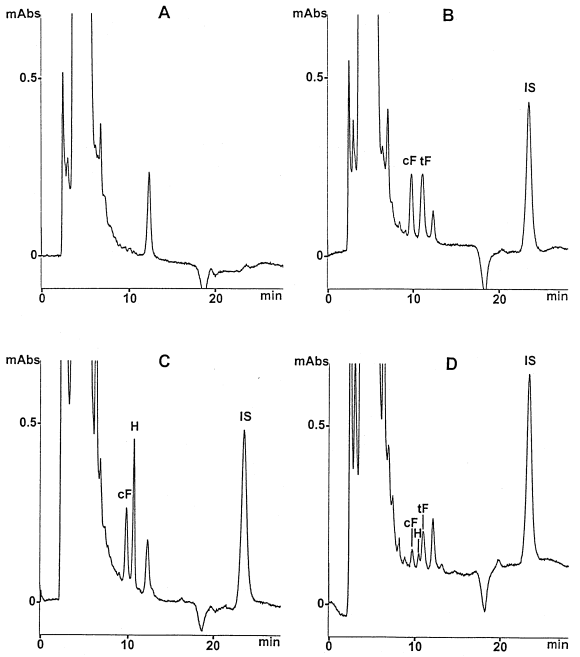

Fig. 3. (A) Shows a chromatogram of blank serum, (B) shows serum spiked with *cis*-flupentixol (cF) and *trans*-flupentixol (tF), 5 ng/ml respectively, and the internal standard prochlorperazine (I.S.; 10 ng/ml). (C) Shows a serum extract from a patient who had received *cis*-flupentixol (Fluanxol Injektionslösung, 100 mg intramuscularly four weeks before withdrawal of the blood sample) and haloperidol (H; 4 mg orally, 14 h before). Concentrations of this sample were 4.72 ng/ml for *cis*-flupentixol and 8.31 ng/ml for haloperidol. (D) Shows a sample from a subject who had received oral racemic flupentixol (Fluanxol, 10 mg/day, last dose given 12 h before) who also had received haloperidol until seven days ago with a dose of 6 mg/day. The concentrations were 1.07 ng/ml for *cis*-flupentixol, 2.00 ng/ml for *trans*-flupentixol and 0.38 ng/ml for haloperidol.

flupentixol isomers, LOD was 0.15 ng for both isomers and 0.1 ng for haloperidol. This corresponded to minimally detectable serum concentrations of 0.3 and 0.2 ng/ml for the flupentixol isomers and haloperidol, respectively, when taking substance loss during extraction and automatic injection into account. Extraction recovery was recorded by comparison with the direct injection of the standard solutions dissolved in methanol onto the chromatographic system. The extraction recovery and inter- as well as intra-assay coefficients of variation (C.V.s) are given in Table 2.

The limit of quantification (LOQ), which is defined as the lowest concentration measured with a C.V. smaller than 20 % was 0.5 ng/ml for the isomers of flupentixol and 0.3 ng/ml for haloperidol. All calibration curves were linear within the measured range with correlation coefficients of peak height ratio versus flupentixol concentrations of at least 0.999.

4. Discussion

Flupentixol and haloperidol are antipsychotic drugs frequently used in psychiatry and their quantification in human body fluids might allow to optimize the individual drug dosage. Nowadays only

Table 1
Retention times of various antipsychotics

Antipsychotic drug or metabolite	Retention time (min)	Retention time relative to *cis*-flupentixol	Retention time relative to *trans*-flupentixol
Pimozide	6.26	0.67	0.59
Benperidol	6.43	0.69	0.61
Haloperidol metabolite III	7.19	0.77	0.68
FF-*N*-Mustard	7.84	0.84	0.74
Clozapine	8.62	0.92	0.82
Fluspirilene	8.85	0.94	0.84
***cis*-Flupentixol**	**9.37**	**1.00**	**0.89**
Bromperidol	9.91	1.06	0.94
Haloperidol	**9.97**	**1.06**	**0.94**
Fluphenazine	10.44	1.11	0.99
***trans*-Flupentixol**	**10.57**	**1.13**	**1.00**
Zuclopenthixol	10.83	1.16	1.02
Levomepromazine	11.72	1.25	1.11
trans-Clopenthixol	11.81	1.26	1.12
Promethazine	11.85	1.27	1.12
Reduced haloperidol	12.12	1.29	1.15
Perphenazine	12.14	1.30	1.15
Chlorprothixene	13.07	1.40	1.24
Olanzapine	13.44	1.43	1.27
Risperidone	20.42	2.18	1.93
Fluphenazinsulfoxide	21.31	2.27	2.02
Prochlorperazine (I.S.)	**22.67**	**2.42**	**2.15**
Pipamperone	23.35	2.49	2.21
Thioridazine	25.77	2.75	2.44
Melperone	26.14	2.79	2.47
Perazine	28.53	3.05	2.70
Normethylperazine	101.54	10.84	9.61
Haloperidol metabolite I	No signal		
9-OH-Risperidone	No signal		

Table 2
Precision, accuracy and extraction recovery of the HPLC method

	cis-Flupentixol		*trans*-Flupentixol		Haloperidol	
Intra-assay						
True concentration (ng/ml)	1.00	7.50	1.00	7.50	1.25	7.50
Detected mean (ng/ml)	1.21	8.61	1.01	8.55	1.33	7.24
C.V. (%)	8.0	7.1	13.4	7.8	4.4	4.5
Trueness (%)	121.0	114.8	101.0	114.0	106.1	96.6
n	10	10	10	10	10	10
Inter-assay						
True concentration (ng/ml)	1.00	7.50	1.00	7.50	1.25	7.50
Detected mean (ng/ml)	0.99	7.52	0.90	7.44	1.28	7.46
C.V. (%)	13.7	10.2	7.3	9.1	8.4	9.9
Trueness (%)	98.7	100.3	89.8	99.2	102.6	99.5
n	11	11	11	11	5	5
Extraction recovery (%)	65	62	62	64	74	69

relatively low doses of these very potent drugs are administered and therefore analytical methods should enable highly sensitive quantification. It was the intention of this work to quantify low serum concentrations of the antipsychotics *cis*-(*Z*)-flupentixol and *trans*-(*E*)-flupentixol by HPLC. As shown in Fig. 3B, a baseline separation of the flupentixol isomers was achieved and an almost complete baseline separation between *trans*-flupentixol and haloperidol was obtained. It should be noted that haloperidol and flupentixol are only rarely administered at the same time and chromatograms shown in Figs. 3C and D were selected here to illustrate the separation of the substances for which the method was validated. In some instances, when using new columns, the separation between *trans*-flupentixol and haloperidol had to be adjusted by adding up to 45 ml methanol to compensate minor column to column variations. Blank serum samples did not show any interfering peaks at the retention times of the analysed drugs and the internal standard (Fig. 3A).

With this HPLC method, we were able to successfully analyze 100 trough serum concentrations of samples from patients who received oral daily flupentixol doses between 2.5 mg and 40 mg or intramuscularly administered flupentixol doses between 10 mg and 60 mg. From these samples, the concentration of the therapeutically active *cis*-flupentixol was above 10 ng/ml only in one sample and in seven samples the serum concentration was below the detection limit of 0.5 ng/ml. We also quantified 70 samples for trough concentrations of haloperidol (oral daily doses between 1 mg and 60 mg).

The availability of a robust isocratic HPLC method with UV detection allowing analysis of several neuroleptics may further help to elucidate the value of therapeutic drug monitoring in psychiatry. The method may also be used in pharmacokinetic studies. Most drugs which are clinically co-administered with flupentixol or haloperidol are well separated (cf. Table 1). Of course, interference with other drugs or their metabolites cannot be completely excluded.

Nevertheless, in pharmacokinetic analyses and in therapeutic drug monitoring an isocratic UV detection HPLC method may be sufficiently specific and has the advantage of being less expensive than more specific methods like mass spectrometry. Furthermore, the number of co-administered drugs is limited in most psychiatric patients to other sedative, antipsychotic or anticholinergic drugs and most of them have been excluded as interfering substances (Table 1).

A major haloperidol metabolite, the reduced haloperidol, could not be detected with satisfactory sensitivity at 254 nm but might be detectable at 220 nm possibly by using dual-wavelength detection [6]; this metabolite did however not interfere with the concentration analysis as given in Table 1. There are also metabolites of *cis*-flupentixol in human plasma including hydroxylated metabolites and *cis*-flupentixol-sulfoxide [3] as a major metabolite. These metabolites were not available in this study but these more hydrophilic metabolites are very unlikely to have the same retention times as the parent substance.

While there are only very few published HPLC methods for therapeutic drug-monitoring of flupentixol, there are several methods for quantification of haloperidol using electrochemical detection and a few using UV detection. We compared the extraction procedures for haloperidol described by Park et al. [7], Nilson [8], and by El-Sayed et al. [9] with our extraction procedure. All three methods resulted in more contaminating peaks in the corresponding chromatograms without giving higher extraction recoveries compared with the diethyl ether–*n*-heptane extraction.

In summary, a robust and routinely applicable HLPC method for quantification of *cis*-flupentixol and *trans*-flupentixol is described and the validation data are given. The same method was also proven to be useful for therapeutic drug monitoring of haloperidol and it may also be used to quantify many other neuroleptics after additional validation.

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References

[1] S. Ulrich, J. Chromatogr. B 668 (1995) 31–40.
[2] A.E. Balant-Gorgia, L.P. Balant, Ch. Genet, R. Eisele, Ther. Drug Monit. 7 (1985) 229–235.

[3] Y.-Z. Shu, J.W. Hubbard, G. McKay, E.M. Hawes, K.K. Midha, Drug Metab. Dispos. 19 (1991) 154–162.
[4] A.E. Balant-Gorgia, L.P. Balant, M. Gex-Fabry, Ch. Genet, Eur. J. Drug. Metabol. Pharmacokin. 12 (1987) 123–128.
[5] A. Li Wan Po, W.J. Irwin, J. Pharm. Pharmacol. 31 (1979) 512–516.
[6] J. Fang, J.W. Gorrod, J. Chromatogr. 614 (1993) 267–273.
[7] K.H. Park, M.H. Lee, M.G. Lee, J. Chromatogr. 572 (1991) 259–267.
[8] L.B. Nielsson, J. Chromatogr. 431 (1988) 113–122.
[9] Y.M. El-Sayed, S.H. Khidr, E.M. Niazy, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 125–1334.
[10] M. Aravagiri, S.R. Marder, T. van Putten, B.D. Marshall, J. Chromatogr. B 656 (1994) 373–381.
[11] K.K. Midha, J.K. Cooper, E.M. Hawes, J.W. Hubbard, E.D. Korchinski, G. McKay, Ther. Drug Monit. 10 (1988) 177–183.
[12] D.W. Hoffman, R.D. Edkins, Ther. Drug Monit. 16 (1994) 504–508.
[13] E.R. Korpi, B.H. Phelps, H. Granger, W.-H. Chang, M. Linnoila, J.L. Meek, R.J. Wyatt, Clin. Chem. 29 (1983) 624–628.
[14] A. Jørgensen, Eur. J. Clin. Pharmacol. 18 (1980) 355–360.
[15] A. Jørgensen, J. Andersen, N. Bjørndal, S.J. Dencker, L. Lundin, U. Malm, Psychopharmacology 77 (1982) 58–65.
[16] J.K. Saikia, A. Jørgensen, Psychopharmacology 80 (1983) 371–373.
[17] A.E. Balant-Gorgia, R. Eisele, J.M. Aeschlimann, L.P. Balant, G. Garrone, Ther. Drug Monit. 7 (1985) 411–414.