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Sensitive method for the simultaneous measurement of fluphenazine decanoate and fluphenazine in plasma by high-performance liquid chromatography with coulometric detection

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Abstract

A highly sensitive and specific high-performance liquid chromatographic method with coulometric detection was developed for the simultaneous assay of fluphenazine decanoate and fluphenazine in plasma. The extraction and sample clean-up procedures are simple, rapid to execute, yet yield chromatograms relatively free of any interference from endogenous plasma constituents, such that the extraordinary sensitivity of the coulometric detector can be exploited fully. This is the first analytical procedure for the simultaneous determination of fluphenazine decanoate and fluphenazine. The detection limits for both fluphenazine decanoate and fluphenazine were 0.1 ng/ml plasma and the limits of quantitation were 0.25 ng/ml plasma. Standard curves from 0.25 to 10 ng/ml were linear with coefficients of variation <10%. The method was applied to measure plasma levels of fluphenazine decanoate and fluphenazine in patients under medication with 25–50 mg biweekly intramuscular (i.m.) injections of fluphenazine decanoate. It was possible to monitor the plasma levels of fluphenazine in all cases. Fluphenazine decanoate was present in measurable concentration in the plasma of 4 out of 5 patients who received biweekly i.m. injections of 50 mg fluphenazine decanoate. In a pilot experiment with a dog, the method was used to follow fluphenazine decanoate and fluphenazine plasma levels up to 13 days, at least, after i.m. single dose (10 mg/kg).

Keywords: Fluphenazine deconate; Fluphenazine

1. Introduction

A difficult task facing the psychiatrist is the determination of the optimal dose of neuroleptic for an individual patient. One problem is the difficulty in switching the patient from oral to depot medication when the patient in the acute phase of a psychosis is stabilized with oral medication [1]. The long absorption half-life of the depot formulation means that it takes several months for fluphenazine to reach steady state [2]. For example, patients treated with 25 mg of fluphenazine decanoate every 2 weeks required 3 months for fluphenazine plasma levels to reach steady state [3], during which time the patient may experience a drop in plasma levels and therefore may be more vulnerable to psychotic exacerbation and relapse [3]. Possible solutions to the problem are: (i) initiate depot therapy with a loading dose followed

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by a reduced dose in subsequent injections and (ii) injection of a mixture of the decanoate ester with a second, less lipophilic ester with a shorter rate-limiting half-life than the decanoate. For example, zuclopenthixol acetate [4] has a short half-life of 36 h in comparison with the decanoate (half-life 7 days) and a more rapid onset of action [5]. Moreover, zuclopenthixol acetate and decanoate esters could be co-injected in the same syringe to produce rapid stabilization at the commencement of therapy with depot therapy. However, the paucity of information about the pharmacokinetic profiles of fluphenazine decanoate and other depot prodrugs means that there are still no rational pharmacokinetic approaches to the conversion from oral to depot therapy [6]. A number of workers have suggested formulae for the conversion [7], although these recommendations were empirical in nature and not based on pharmacokinetic principles. In this sense, knowledge about the pharmacokinetics of fluphenazine decanoate may be very important for the development of rational dosing strategies in patients, and consequently there is a need for the development of sensitive analytical methods for the simultaneous determination of fluphenazine decanoate and fluphenazine in biological fluids.

Recently published methods for the determination of fluphenazine in patient plasma include radioimmunoassay by monoclonal antibodies [8], high-performance liquid chromatography (HPLC) with coulometric detection [9], gas chromatography–mass spectrometry [10,11] and solids probe tandem mass spectrometry [12], but none of them permit the simultaneous determination of fluphenazine decanoate. Confusion on this point occurs because authors sometimes use the terms 'fluphenazine' and 'fluphenazine decanoate' interchangeably in manuscripts describing the pharmacokinetics of the depot neuroleptic. For example, the title of a manuscript by Glazer and co-workers suggests that the steady state profile of 'fluphenazine decanoate' was monitored in patients, although their gas chromatographic procedure monitored fluphenazine, not the prodrug [10]. The present report, however, describes the development of a HPLC procedure based on coulometric detection for the simultaneous measurement of fluphenazine decanoate and fluphenazine in plasma.

2. Experimental

2.1. Materials

Reagent grade hydrochloric acid, sodium hydroxide, ammonium acetate, HPLC-grade cyclohexane, *n*-pentane, ethyl acetate and acetonitrile were obtained from BDH Chemicals. Fluphenazine dihydrochloride was purchased from Sigma-Aldrich Canada. Fluphenazine decanoate was obtained from Squibb Canada. Distilled water was passed through a Milli-Q water purification system prior to use.

2.2. Preparation of standard curves

Stock solutions of fluphenazine decanoate, fluphenazine and perphenazine (internal standard) as their respective free bases were prepared monthly in absolute methanol. Standards in the range of 0.25 –10 ng/ml were prepared daily by diluting methanolic stock solutions with drug-free plasma and stored at −20°C until analysis.

2.3. Sample preparation procedure

The internal standard solution (100 μl) containing perphenazine (10 ng) was added to either 1.0 ml of human plasma or 0.5 ml of dog plasma in a disposable borosilicate glass tube. The sample was mixed for a few seconds (Vortex mixer), a mixture of acetone–methanol (1:1) was added (3.0 ml), and sonicated (10 min). Cyclohexane containing 5% acetyl acetate (6.0 ml) was added, the tube capped with a polythene stopper, and the sample was mixed (10 min) (IKA Vibrax shaker). The tube was then allowed to stand for 5 min at room temperature, after which the upper organic layer was transferred by Pasteur pipette to a clean 15-ml disposable borosilicate glass tube containing 1.2 ml of 0.3 *M* HCl. The sample was mixed as before (10 min) and allowed to stand for 2 min, after which the organic layer was aspirated to waste. *n*-Pentane (5 ml) was added to the tube and the tube shaken as before (4 min) and allowed to stand for 2 min, after which the organic layer aspirated as before. Sodium hydroxide (0.8 ml of a 0.5 *M* solution) was added to the tube and the

sample mixed for a few seconds after which 6 ml of *n*-pentane containing ethyl acetate (5%) was added. After mixing (10 min), the organic layer was transferred to a 10-ml disposable borosilicate tube. The sample was evaporated to dryness at 65°C under a stream of nitrogen (Thermolyne Dri-Bath). The residue was dissolved in 100 μl of acetonitrile by mixing for 5 s (Vortex mixer) and 90 μl was injected onto the HPLC system.

2.4. Chromatographic equipment and conditions

Chromatographic analysis was carried out with a Waters Model 590 pump connected to a Rheodyne 7215 valve loop (200 μl) injector. The column was stainless steel (15×4.6 mm I.D.) packed with 3 μm C_8 packing materials. An ESA Coulochem detector Model 5100A was fitted with a Model 5020 guard cell and a Model 5010 high-sensitivity cell. The guard cell was operated at +1.00 V and the high-sensitive cell voltages were +0.50 and +0.75 V for cell 1 and cell 2, respectively. The detector response was recorded with a HP 3392A integrator (Hewlett-Packard). The data was analyzed from a calibration curve by linear regression analysis of the peak-height ratio (quotient of the peak height of an analyte and the peak height of the internal standard) vs. the concentration. The mobile phase (acetonitrile–methanol–0.2 *M* ammonium acetate, 80:10:10) was degassed prior to use. The flow-rate was 1.2 ml/min.

2.5. Plasma level studies

To examine the applicability of the method to measure fluphenazine decanoate and fluphenazine in plasma, a beagle dog (7.0 kg) was dosed intramuscularly with 10 mg/kg of fluphenazine decanoate dissolved in sesame oil (25 mg/ml). Serial blood samples were collected at 0, 1, 4, 6, 8 and 13 days. Plasma samples from patients under medication with fluphenazine decanoate (25–50 mg biweekly) were harvested, and on day 7 after the previous injection. All blood samples were centrifuged immediately after collection and the separated plasma was stored at −20°C until analysis.

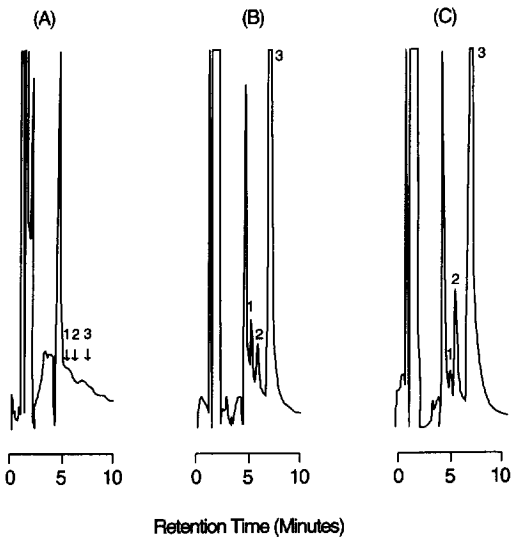

Fig. 1. Sample chromatograms from extracts of (A) blank plasma, (B) blank plasma spiked with 1.0 ng/ml of fluphenazine decanoate and fluphenazine and (C) plasma collected from a patient receiving 50 mg dose of fluphenazine decanoate ester biweekly and containing 0.44 ng/ml of fluphenazine decanoate and 1.91 ng/ml of fluphenazine. Fluphenazine decanoate (1), fluphenazine (2) and perphenazine (3) are labeled.

3. Results and discussion

Fig. 1 shows sample chromatograms of extracts of blank plasma (Fig. 1A), blank plasma spiked with fluphenazine decanoate, fluphenazine and internal standard perphenazine (Fig. 1B) and a plasma sample from a patient who received a 50-mg dose of fluphenazine decanoate biweekly (Fig. 1C). The chromatograms appeared clean and relatively free of interference from any endogenous substances. The calibration curves were linear over the concentration range of 0.25–10 ng/ml plasma spiked with fluphenazine or fluphenazine decanoate. The linear regression line that for fluphenazine decanoate had a mean *y*-intercept of 0.024±0.0013 (S.E.), slope of 0.194±0.0057 (S.E.) and r^2 of 0.995±0.0012 (S.E.), and that for fluphenazine had a mean *y*-intercept of 0.044±0.0032 (S.E.), slope of 0.213±0.0075 (S.E.) and r^2 of 0.996±0.0011 (S.E.) (n=5). The linear regression equations were $y=0.194x+0.0242$ (r^2= 0.995) for fluphenazine decanoate and $y=0.213x+0.044$ (r^2=0.996) for fluphenazine.

Table 1
Coefficients of variation in intra-assay (within-run), inter-assay (between-run) and quality control analyses

Added (ng/ml)	Fluphenazine decanoate		Fluphenazine	
	Measured (ng/ml)	C.V. (%)	Measured (ng/ml)	C.V. (%)
Intra-assay variation (n=6)				
0.25	0.26	9.16	0.26	9.12
2.50	2.66	9.23	2.58	6.94
7.50	7.77	2.86	7.59	5.64
Inter-assay variation (n=6)				
0.25	0.26	10.07	0.25	10.16
2.50	0.25	6.49	2.59	8.27
7.50	7.68	3.27	7.58	2.98
Quality control samples (n=6)				
0.40	0.415	8.25	0.40	5.87
4.00	4.28	4.38	3.95	9.37
9.00	10.12	12.70	9.16	10.75

Intra-assay (within-run) and inter-assay (between-run) variations for fluphenazine decanoate and fluphenazine are shown in Table 1. Note that within-day coefficients of variation (C.V.%) are <10% and inter-assay variations were <11%, despite the low concentrations involved. The quality control (QC) samples were analyzed as unknowns (analyst blind) at the same time as the samples for standard curves and test samples. The results show that the assay was reliable under practical conditions. The coefficients of variation did not exceed 14% at any concentration for either fluphenazine decanoate or fluphenazine. Absolute recoveries were determined by comparing the peak heights of the extracted samples with those obtained from direct injection of the pure standards in acetonitrile (Table 2). The stability of spiked samples (0.50 ng/ml of fluphenazine decanoate or fluphenazine) was assessed after 1, 7, 14 and 30 days in storage at −20°C. The overall mean (C.V.%) was 0.498 ng/ml (12.56%) for fluphenazine decanoate and 0.484 ng/ml (10.12%) for fluphenazine. The results demonstrated that samples stored at −20°C for up to one month were stable, and in four analytical runs over 30 days no C.V.% exceeded 15%. The specificity of the assay was investigated by injecting 20 ng of some known metabolites of fluphenazine decanoate dissolved in 50 μl acetonitrile into the column. 7-Hydroxyfluphenazine (t_R 3.9 min), fluphenazine sulfoxide (t_R 8.14 min) and N-deshydroxyethylfluphenazine (no electrochemical response) did not interfere with the chromatography of either fluphenazine (t_R 6.10 min) or fluphenazine decanoate (t_R 5.28 min).

Table 2
Absolute recoveries of fluphenazine decanoate, fluphenazine and perphenazine

Added (ng/ml)	*n*	Mean recovery (%)	C.V. (%)
Fluphenazine decanoate			
0.50	6	24.58	7.53
5.00	6	28.62	11.20
Fluphenazine			
0.50	6	30.36	6.84
5.00	6	32.67	10.56
Perphenazine			
10.0	10	30.38	10.56

The present analytical method for the simultaneous determination of fluphenazine decanoate and fluphenazine proved to be simple, rapid (assay time <10 min) and reliable. The inclusion in the extraction procedure of a back-extraction step led to low but reproducible recovery and gave chromatograms that were relatively free from interference due to endogenous plasma constituents. The signal-to-noise ratio was thereby optimized, allowing the extraordinary sensitivity of the coulometric detector to be fully exploited. Since fluphenazine decanoate and fluphenazine are highly protein bound drugs [13], deproteinization is required before the extraction. Several agents such as trichloracetic acid or organic solvents such as acetonitrile, methanol or acetone were investigated. The addition of acetone and/or methanol and subsequent sonication proved to be very efficient for the dissociation of the drug–protein complex. A well known problem connected to the deproteinization is that the analytes, especially hydrophobic drugs such as fluphenazine decanoate, are occluded with the protein precipitate. In our experience, basification of plasma samples in the initial extraction, tended to improve the recovery of fluphenazine, but made fluphenazine decanoate unextractable, probably because of occlusion of the prodrug in the denatured protein. In the present method, the effects of occlusion were minimized by slowly adding the plasma to the methanol–acetone mixture, rather than the other way around. This simple strategy led to an improved recovery. The

reproducible measurement of low concentrations of the analytes was facilitated by the use of 5% ethyl acetate in cyclohexane and *n*-pentane, since it prevented the drugs from becoming lost due to adsorption onto glass surfaces during the extraction and clean-up procedures.

Table 3 shows plasma concentrations of fluphenazine and fluphenazine decanoate in 11 patients who received intramuscular injections of fluphenazine decanoate ester (25 to 50 mg biweekly). The method was sensitive enough to monitor fluphenazine levels in all cases. Fluphenazine decanoate was detected in 5 patients who were maintained on relatively higher doses of fluphenazine decanoate. It is possible, however, that the decanoate may have been present in the plasma of patients on lower doses at concentrations below the detection limit of the assay. The pharmacokinetic and clinical implications of this discovery remain to be explored.

Fig. 2 shows plasma concentration vs. time profiles for a dog given a single intramuscular dose (10 mg/kg) of fluphenazine decanoate in sesame oil (25 mg/ml). In this pilot experiment, the method had adequate sensitivity to monitor both fluphenazine decanoate and fluphenazine plasma levels up to 13 days at least after dosing. These data show that fluphenazine decanoate was slowly released into the circulation system from the depot site. Recent data suggests that the lymphatic system is involved in the presystemic absorption of the prodrug (to be published) and in the formation/presystemic absorption of fluphenazine [14]. To the best of our knowledge however, this is the first evidence of a long acting neuroleptic ester found in plasma without the aid of radioisotopes. In an earlier study, both [^{14}C]haloperidol decanoate and [^{14}C]haloperidol were found in rat plasma after i.m. single dose (50 mg/kg) of [^{14}C]haloperidol decanoate [15].

Table 3
Plasma concentrations of fluphenazine decanoate and fluphenazine in patients medicated with biweekly intramuscular fluphenazine decanoate in sesame oil

Subject	Dose	Fluphenazine decanoate (ng/ml)	Fluphenazine (ng/ml)
1	25	ND	1.48
2	30	ND	0.74
3	30	ND	1.56
4	37.5	ND	1.58
5	40	ND	1.42
6	40	0.30	1.92
7	50	ND	1.61
8	50	0.27	0.87
9	50	0.44	1.91
10	50	0.33	1.74
11	50	0.52	2.59

ND: Not detected.

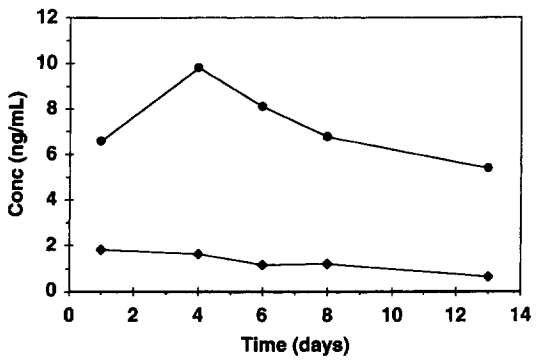

Fig. 2. Plasma concentrations vs. time profiles of fluphenazine decanoate (♦) and fluphenazine (●) after the intramuscular administration of a single dose of fluphenazine decanoate (10 mg/kg) to a beagle dog.

The plasma concentrations of fluphenazine decanoate are extremely low and at present there are no pharmacokinetic data on this important pro-drug. The present method provides an opportunity to investigate the pharmacokinetic relationship between fluphenazine and its pro-drug in dogs following single doses of the pro-drug and at steady state in animal models or in patients who are maintained on higher intramuscular doses of depot formulation of the prodrug.

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References

[1] S.R. Marder, J.W. Hubbard, T. Van Putten and K.K. Midha, Psychopharmacology, 98 (1989) 433.

[2] S.R. Marder, E.M. Hawes, T. Van Putten, J.W. Hubbard, G. McKay, J. Minz, P.R.A. May and K.K. Midha, Psychopharmacology, 88 (1986) 480.
[3] S.R. Marder, K.K. Midha, T. Van Putten, M. Aravagiri, E.M. Hawes, J.W. Hubbard, G. McKay and J. Minz, Br. J. Psychiatry, 158 (1991) 658.
[4] T. Aaes-Jorgensen, Progress in Neuropsychopharmacology and biological psychiatry, 13 (1989) 77.
[5] A. Amdisen, T. Aaes-Jorgensen, N. Thomsen, V. Madsen and M. Nielsen, Psychopharmacology, 90 (1986) 412.
[6] M.W. Jann, L. Ereshefsky and S.R. Saklad, Clin. Pharmacokinet., 10 (1985) 315.
[7] J. Kane, J. Clin. Psychopharmacol., 6 (1986) 20.
[8] G. Mckay, T. Steeves, J.K. Cooper, E.M. Hawes and K.K. Midha, J. Pharm. Sci., 79 (1990) 240.
[9] J. Cooper, E. Hawes, J. Hubbard, G. McKay and K. Midha, Therap. Drug Monitor., 11 (1989) 354.
[10] W.M. Glazer, L.T. Friedhof, S.R. Marder and W.A. Brown, Schizophrenia Res., 8 (1992) 111.
[11] M. Jemal, E. Ivashkiv, D. Both, R. Koski and A.I. Cohen, Biomedical and Environmental Mass Spectrometry, 14 (1987) 699.
[12] S. Hadad, R. Edom, G. Mckay, E.M. Hawes and K.K. Midha, J. Mass Spectrometry, 30 (1995) 849.
[13] K. Freedberg, R. Innis, I. Creese and S. Snyder, Life Sci., 24 (1979) 2467.
[14] Y. Huang, J.W. Hubbard and K.K. Midha, Eur. J. Pharm. Sci., 3 (1995) 15.
[15] Y. Matsunaga, K. Nambu, Y. Oh-e, H. Miyazaki and M. Hashimoto, Eur. J. Drug Metab. Pharmacokinet., 12 (1987) 175.