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Short Communication

HPLC procedure for the determination of some potential fluphenazine metabolites in urine

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Introduction

Fluphenazine is a widely prescribed phenothiazine drug administered in an esterified form for the control of acute schizophrenia. The metabolism of radio-labelled [^{35}S] fluphenazine in human subjects has been investigated by Curry *et al.* [1]. However, quantitation of the known metabolites (fluphenazine sulphoxide and 7-hydroxyfluphenazine) together with unchanged fluphenazine accounted for only 44% of the total radioactivity excreted in the urine. Alternative studies with chronically dosed rats have established the existence of an *N*-dealkylated fluphenazine species which accumulates in tissue [2]. Thin-layer chromatography was utilized for this latter purpose. By a similar means the existence of dealkylated flupenthixol (a molecule closely related to fluphenazine) has been demonstrated in dog liver and in rat faeces [3]. A recent combination of HPLC and RIA has indicated that both fluphenazine and the *N*-dealkylated species are present in the serum of patients dosed with oral fluphenazine hydrochloride [4]. The elimination of the dealkylated compound in urine is thus widely expected although no confirmatory reports have been published. An HPLC procedure has therefore been developed in order to detect and quantify the level of this compound in human urine. The chemical structures of some potential fluphenazine metabolites are given in Fig. 1.

Experimental

Materials

Fluphenazine sulphoxide and fluphenazine sulphone were synthesized by oxidation of fluphenazine with hydrogen peroxide, whereas fluphenazine mono-*N*-oxide and di-*N*-

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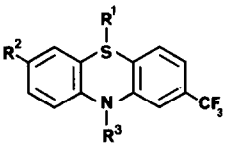

Compound	R^1	R^2	R^3
Fluphenazine	-	H	$-(CH_2)_3-N\ \ N-(CH_2)_2OH$
7-Hydroxyfluphenazine	-	OH	$-(CH_2)_3-N\ \ N-(CH_2)_2OH$
Fluphenazine sulphoxide	O	H	$-(CH_2)_3-N\ \ N-(CH_2)_2OH$
Fluphenazine sulphone	O_2	H	$-(CH_2)_3-N\ \ N-(CH_2)_2OH$
Fluphenazine mono *N*-oxide	-	H	$-(CH_2)_3-N\ \ {}^{+}N-(CH_2)_2OH$ (O^-)
Fluphenazine di *N*-oxide	-	H	$-(CH_2)_3-N^{+}\ \ {}^{+}N-(CH_2)_2OH$ (O^-, O^-)
N[3-(2-trifluoromethyl phenothiazinyl-10)-propyl] piperazine (dealkylated fluphenazine)	-	H	$-(CH_2)_3-N\ \ NH$
N-[3-(2-trifluoromethyl phenothiazinyl-10)-propyl] ethylene diamine (CF_3 – PPED)	-	H	$-(CH_2)_3-NH\ \ NH_2$

Figure 1
Chemical structures of some potential fluphenazine metabolites.

oxide were prepared by oxidation of fluphenazine with *m*-chloroperbenzoic acid. Synthesis of 7-hydroxyfluphenazine [5] and dealkylated fluphenazine [6] was achieved via previously published routes. The identity of the materials was confirmed spectroscopically. A small sample of CF_3 PPED (Fig. 1) was supplied by Dr U. Breyer, University of Tubingen, Federal Republic of Germany.

Reagents

AnalaR grade methanol (BDH, Poole, UK) was used to prepare the mobile phase. All other chemicals were of reagent grade (BDH).

Apparatus

HPLC apparatus was assembled from commercially available components and comprised an Altex 110 reciprocating pump and a Cecil 212 variable-wavelength UV detector. The column (200 × 4.6 mm) of ODS-Hypersil (Shandon Products, Runcorn, UK) was prepared by slurry-packing the material from carbon tetrachloride using a Haskel air-driven fluid pump at a pressure of 4600 psi.

HPLC mobile-phase

A reversed-phase HPLC system for the separation of fluphenazine oxides has been described in a previous publication [7]. However, attempts to chromatograph dealkylated fluphenazine and the compound CF_3-PPED in a similar system were unsuccessful because of severe tailing. Use of a system containing phosphoric acid for the chromatography of a proline derivative has recently been described [8], and application of a similar mobile phase to the current problem resulted in acceptable chromatography. The mobile phase (methanol–1% aqueous potassium chloride containing 0.01% phosphoric acid, 2:1) together with a 200 mm ODS reversed-phase column successfully resolved a series of related compounds and fluphenazine metabolites (Fig. 2).

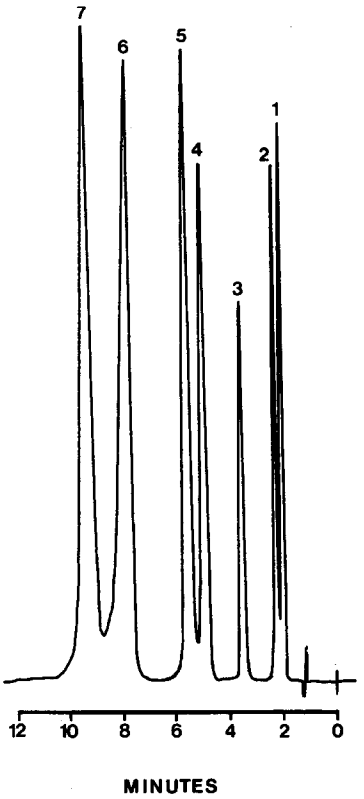

Figure 2
Separation of some potential fluphenazine metabolites. Column, ODS-Hypersil; mobile phase, methanol–1% aqueous potassium chloride containing 0.01% phosphoric acid (2:1); 1 — fluphenazine sulphoxide, 2 — fluphenazone sulphone, 3 — 7-hydroxyfluphenazine, 4 — CF_3 PPED, 5 — fluphenazine di *N*-oxide, 6 — dealkylated fluphenazine, 7 — fluphenazine mono-*N*-oxide.

Sample preparation

A 10 ml sample of urine was rendered alkaline with 1 M sodium hydroxide and excess potassium chloride added. The drug and metabolites were extracted into diethyl ether, which was subsequently separated and evaporated to dryness with a stream of nitrogen. The residue was dissolved in HPLC mobile phase (1 ml) and 50 μl aliquots injected on-column.

Results and Discussion

The extraction procedure described proved to be satisfactory for the recovery of fluphenazine (100.2% recovery, SD ± 2.2%), dealkylated fluphenazine (95.9%

recovery, SD ± 2.0%), fluphenazine sulphone (97.3% recovery, SD ± 2.4%), fluphenazine mono-*N*-oxide (92.1% recovery, SD ± 2.1%) and 7-hydroxyfluphenazine (98.0% recovery, SD ± 1.4%) from urine spiked at a level of 1 μg/ml. However, poor recovery of compound CF_3-PPED (34%) was experienced: not enough of this compound was available for further investigation.

The chromatograms obtained using the acidic mobile phase allow quantitation of all the compounds examined except fluphenazine sulphoxide and sulphone which are eluted close to the solvent front together with endogenous material from the urine. The procedure thus offers an alternative to the gas chromatographic method [9] for determining 7-hydroxyfluphenazine. For quantitation of sulphoxide and sulphone a previously reported HPLC system was used [7].

A urine sample obtained from a patient undergoing high-dose Modecate therapy (600 mg $week^{-1}$), when examined by the described analytical procedure (Fig. 3), showed no obvious peak representative of dealkylated fluphenazine (detection limit approximately 0.1 μg ml^{-1} urine). By contrast the level of 7-hydroxyfluphenazine in the same urine sample was determined as 1.9 μg ml^{-1}, after hydrolysing with β-glucuronidase (37°C, 16 h) to free the conjugated metabolite. Similarly, the level of fluphenazine sulphoxide was established at 1.6 μg ml^{-1}. In addition, fluphenazine mono-*N*-oxide was not evident

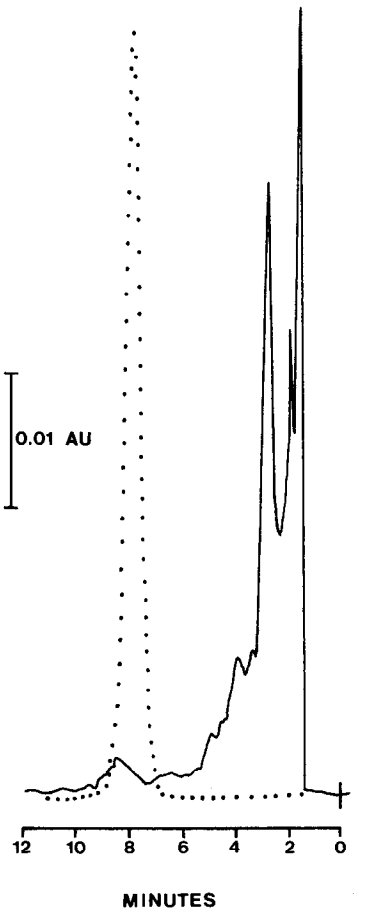

Figure 3
——— HPLC examination of urine extract; . . . . dealkylated fluphenazine, equivalent to 2 μg ml^{-1} urine.

in the chromatogram obtained during quantitation of 7-hydroxyfluphenazine (i.e. following hydrolysis). These initial results indicate that neither dealkylated fluphenazine nor fluphenazine mono-*N*-oxide occur as major metabolites in human urine.

References

[1] S. H. Curry, R. Whelpton, P. J. de Schepper, S. Vranckx and A. A. Schiff, *Br. J. Clin. Pharmacol.* **7**, 325 (1979).
[2] H. J. Gaertner, G. Liomin, D. Villumsen, R. Bertele and U. Breyer, *Drug Metab. Dispos.* **3**, 437 (1975).
[3] A. Jorgensen, K. F. Overo and V. Hansen, *Acta Pharmacol. Toxicol.* **29**, 339 (1971).
[4] S. Goldstein and H. Van Vunakis, *J. Pharmacol. Exp. Ther.* **217**, 36 (1981).
[5] E. A. Nodiff and M. Hausman, *J. Org. Chem.* **31**, 625 (1966).
[6] H. J. Gaertner, U. Breyer and G. Liomin, *Biochem. Pharmacol.* **23**, 303 (1974).
[7] W. F. Heyes, J. R. Salmon and W. Marlow, *J. Chromatogr.* **194**, 416 (1980).
[8] S. Perlman and J. Kirschbaum, *J. Chromatogr.* **206**, 311 (1981).
[9] R. Whelpton and S. H. Curry, *J. Pharm. Pharmacol.* **28**, 869 (1976).

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