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# HUMAN METABOLISM OF PHENOTHIAZINES TO SULFOXIDES DETERMINED BY A NEW HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–ELECTROCHEMICAL DETECTION METHOD

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**Abstract**—The metabolism of phenothiazine drugs may contribute to both their therapeutic and toxic actions by production of active metabolites *in vivo*. Idiosyncratic reactions or treatment failure may be a consequence of differing patterns of metabolism in different patients. In this report, a modification of our method for the detection of metabolites of phenothiazines is described, which also permits the simultaneous determination of sulfoxide metabolites in human plasma. Application of this method to human plasma identifies marked individual differences in patterns of phenothiazine metabolism.

Pharmacokinetic parameters such as metabolism may be central to the understanding of idiosyncratic responses to psychoactive drugs. These individual variations can affect patient response to the clinical use of neuroleptic drugs, including the phenothiazines. Drug metabolism can make quantification of neuroleptic blood levels difficult, by the production of interfering metabolites [1]. Metabolism may change the total bioavailability of antipsychotic medicine and also influence the occurrence of adverse effects of the medication. Such adverse effects may be mediated by drug metabolites active at other receptor sites, such as cholinergic or alpha adrenergic receptors [1–4].

We previously developed in this laboratory a new method for the quantification of neuroleptic blood levels with both research and clinical applications [5]. The sensitivity and selectivity compare favorably with other currently available methods [6–10]. High performance liquid chromatography (HPLC) with electrochemical detection (EC) was used to separate, identify, and detect parent drug and metabolites for fluphenazine (FPZ), a commonly used high potency neuroleptic. This method is also compatible with post-column analyses such as radioreceptor assays, which can provide information on the pharmacodynamics of the separated metabolites. Using this method, individual variations in plasma drug levels and drug metabolism in human subjects taking these drugs can be evaluated [5].

The electrochemical method employed detects current produced by the oxidation of the sulfur-containing component of the phenothiazine ring structure at a fixed potential. This method has, then, general applicability to the detection of all phenothiazine-related compounds. However, *in vivo* oxidation at this site may occur in the course of drug metabolism, generating oxidized metabolites. Of these the sulfoxides are the most prominent, but are not detectable by HPLC–EC due to their prior biological oxidation [5, 11].

To resolve this problem, and extend the usefulness of this method to the full range of biological metabolites, a new approach has been employed which permits the detection of fully-oxidized phenothiazine metabolites by electrochemical reduction of the HPLC effluent prior to its passage over the oxidizing electrode of the detector. The method, and its application to the identification of biosynthesis of FPZ sulfoxide in human subjects, are described in this report.

## METHODS

FPZ and metabolites (FPZ-7-hydroxyl, -8-hydroxyl, -sulfoxide, and -*N*-oxide) were provided by E. R. Squibb & Sons, Inc. (Princeton, NJ). Chlorpromazine and di-*n*-butylamine were purchased from the Sigma Chemical Co. (St. Louis, MO). Trifluoperazine was purchased from Wyeth Laboratories (Philadelphia, PA). Isopentyl alcohol (AR) was from Mallinckrodt (St. Louis, MO). Methanol and water were HPLC grade (American Burdick & Jackson, St. Louis, MO). Formic acid (ACS reagent grade) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Sulfoxides of the phenothiazines were prepared by incubation at room temperature with 30% hydrogen peroxide.

Plasma collection was performed as described previously [5], from patients giving informed consent who had been taking FPZ (5 mg b.i.d., p.o.) for at least 2 weeks. Collection was performed after the morning dose. Blood was drawn into Vacutainers containing sodium citrate and was then centrifuged to obtain plasma. The plasma was alkalinized with an equal volume of 0.5 M potassium carbonate and was extracted into 5 vol. of isopentyl alcohol, which was evaporated in a vacuum centrifuge. The extract

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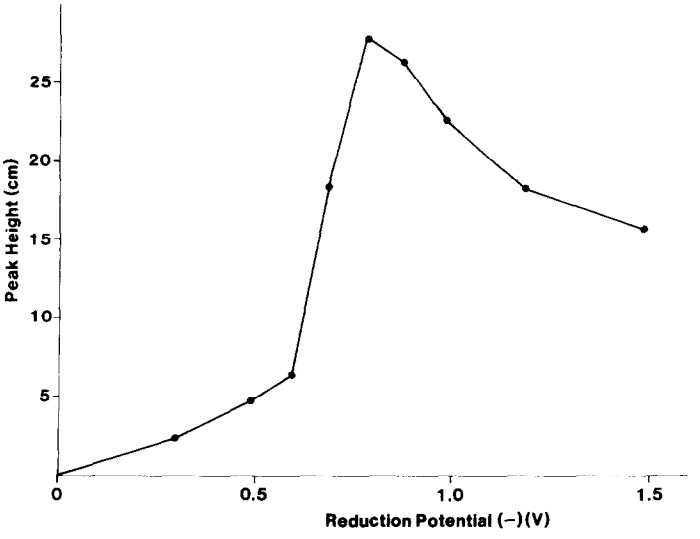


Fig. 1. Electrode response at oxidizing electrode (+0.85 V) with reduction potentials from 0 to −1.5 V.

was reconstituted in the HPLC mobile phase for chromatography.

Fluphenazine, a high potency phenothiazine with a piperazine side chain, was used for the development of this method. This drug requires sensitive methods for identification of usual blood levels, is highly metabolized to both active and inactive metabolites, and, because of its piperazine side chain, is among the most difficult phenothiazines to chromatograph well. The HPLC–EC method for the identification of FPZ and most of its metabolites has been described previously [5]. Briefly, FPZ and

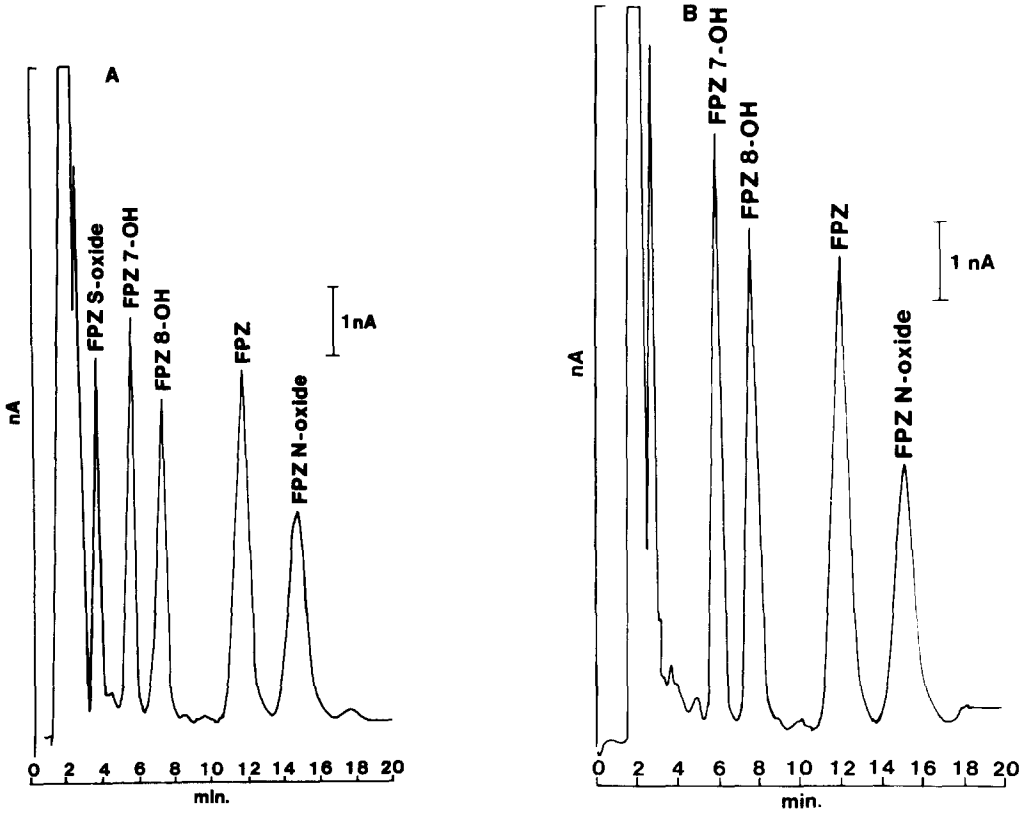


Fig. 2. Chromatograms of drug-free plasma spiked with synthetic FPZ and metabolites, extracted and filtered as described in the text, and eluted with 0.15 M formic acid and 0.01 M dibutylamine in 50% methanol, pH 3.2, at 2.0 ml/min. Oxidation electrode at +0.85 V. Reduction electrode at (A) −0.8 V, or (B) 0 V.

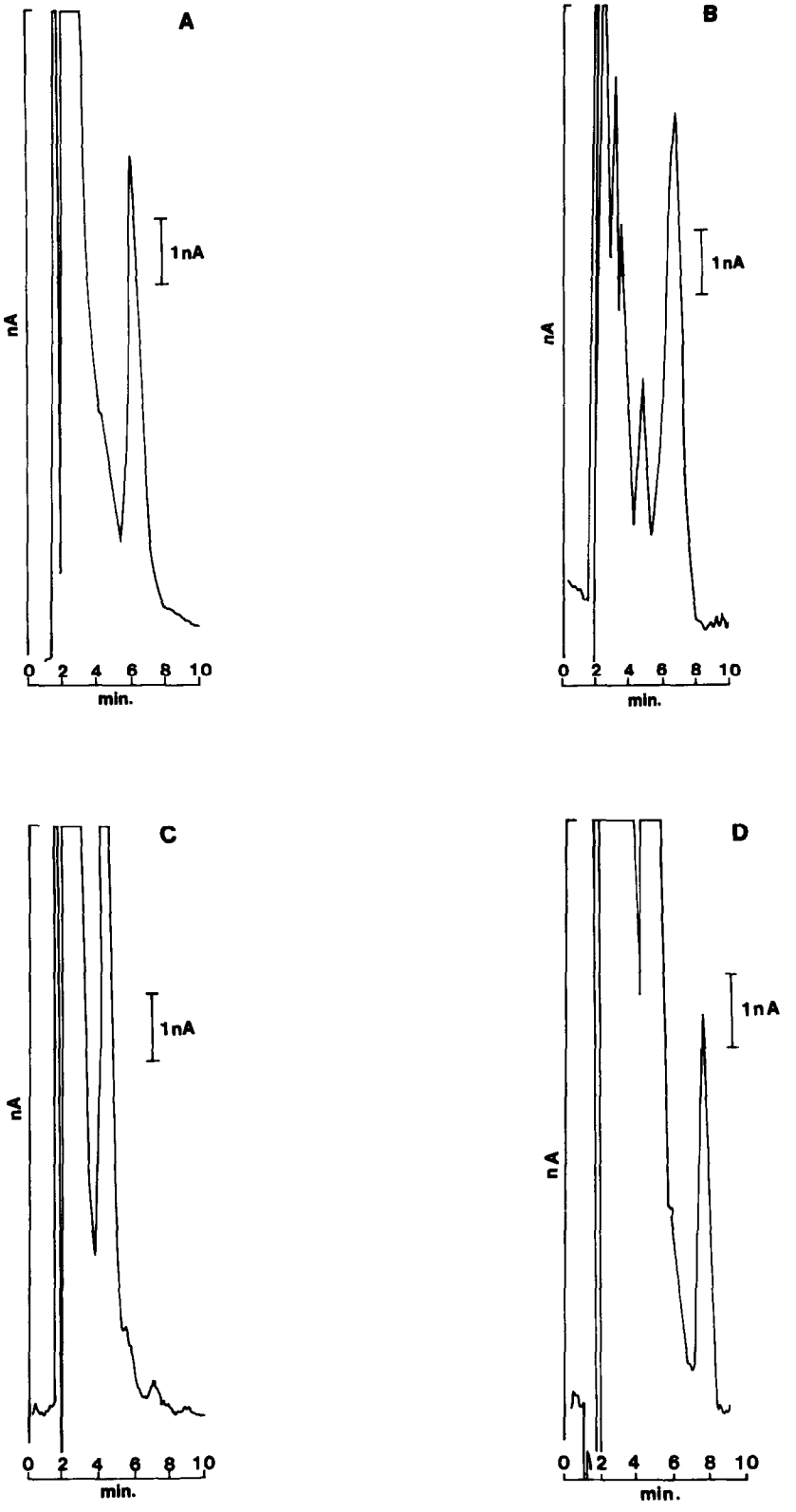


Fig. 3. Plasma extracts of patients currently prescribed FPZ (5 mg b.i.d., p.o.). Conditions were as in Fig. 2, except that the methanol concentration in the mobile phase had been reduced to 40% to demonstrate more clearly the sulfoxide peaks. Reduction was set at −0.8 V. FPZ sulfoxide peaks are seen in two patients (A and B), but not in others (C and D). The large peak in (D) at $R_t$ = 7.4 min can be identified by retention time and by its continued presence with the reducing electrode off as FPZ 7-hydroxyl. The sulfoxide peaks disappeared when reduction was set to 0 V (not shown).

metabolites were resolved on a C18 reverse phase column using a mobile phase of 0.15 M formic acid and 0.01 M dibutylamine in 50% methanol, pH 3.2, at a flow rate of 2.0 ml/min. All the components of the mobile phase form azetropes which are readily removed by lyophilization, and so are compatible with post-column analyses such as radioimmune and radioreceptor assays. The longest-retained metabolite, FPZ *N*-oxide, elutes before 15 min. Detection is performed at a glassy carbon electrode at a potential of +0.85 V. The sulfoxide metabolite of FPZ could not be detected by oxidative EC; however, a retention time of 3.6 min was established with UV detection.

In order to detect FPZ sulfoxide, lyophilized isopentyl alcohol extracts of patient plasma were redissolved in mobile phase and filtered through a 1.0 micron cellulose filter prior to injection onto the HPLC column. The mobile phase was sparged with nitrogen during degassing, and plastic tubing in the HPLC apparatus was replaced with metal. The detection method was modified by the use of an upstream reducing electrode set at −0.8 V, to reduce FPZ sulfoxide prior to the oxidizing detection electrode. Figure 1 indicates the oxidation electrode response to FPZ sulfoxide at different reducing potentials. Maximal response was obtained at −0.8 V.

In Fig. 2, FPZ and metabolite standards added to and then extracted from drug-free plasma were chromatographed both with (Fig. 2A) and without (Fig 2B) reduction. The FPZ sulfoxide peak disappeared after the reducing electrode was set to 0 V. Plasma did not appear to contain interfering substances, nor did it affect the retention time of synthetic FPZ and metabolites added to it prior to extraction (not shown).

## RESULTS

For studies of a broad range of metabolites it was preferable to use a mobile phase containing 50% methanol, with extraction and filtration steps to minimize the injection artifact (Fig. 2). We also used 40% methanol in the mobile phase for sulfoxide studies (Fig. 3), as highly polar molecules such as oxidized metabolites are poorly retained on reverse phase HPLC columns, and are better resolved from the solvent front by a longer retention time.

Using an elution buffer containing 40% methanol, FPZ sulfoxide was identified in the plasma of several patients currently taking FPZ (5 mg b.i.d., p.o.) (Fig. 3, A and B), but was not found in the plasma of other patients taking the same dose (Fig. 3, C and D). Idiosyncrasy in drug metabolism appears pronounced with the phenothiazines, and especially their sulfoxide metabolites. Several different patterns of metabolism have been identified in different patients.

The chromatographic characteristics of other phenothiazines and their sulfoxides, e.g. chlorpromazine and trifluoperazine, were examined with this method. The sulfoxides were made by incubation at room temperature with 30% hydrogen peroxide. Retention times were: chlorpromazine, 6.8 min; sulfoxide, 2.5 min; trifluoperazine, 9.4 min; and sulfoxide, 3.5 min (not shown).

## DISCUSSION

Individual variability in pharmacokinetic parameters can affect not only blood levels of therapeutically administered drugs, but also their metabolism to biologically active and inactive forms. In the case of the neuroleptic drugs, not only may metabolites have therapeutic efficacy, but both parent drug and its metabolites may have activities at other receptor sites which may contribute to the appearance of side effects or idiosyncratic reactions [1–4, 12]. Drug metabolism may be a significant factor in both the phenomenon of drug non-responders as well as that of non-compliance due to side effects.

The method described in this and in our earlier publication [5] permits direct quantification of blood levels of neuroleptics and metabolites, with sensitivity which makes them useful even with a high potency (low dose) neuroleptic, e.g. FPZ. As such it is valuable in the clinical monitoring of therapeutic drug levels, especially when combined with a radioreceptor assay, with which it was designed to be compatible. The interfacing of the HPLC method with radioreceptor assays for several receptor sites of clinical and research interest is the subject of a forthcoming manuscript (Hoffman *et al.*, in preparation).

The presence of FPZ sulfoxide in the plasma of patients taking FPZ is consonant with other reports in the literature indicating biosynthesis of this metabolite [2, 10, 13–15]. There are, however, marked differences among published reports regarding identification of phenothiazine metabolites formed *in vivo* [2–5, 7, 10, 13–15]. This may reflect not only methodological differences but also actual variations among individuals and populations in metabolism of neuroleptic drugs. Data from subjects shown in this report (Fig. 3) and in our earlier report [5] demonstrate a wide variation in the metabolites formed and in the blood levels attained by an individual. This broad variability in pharmacokinetics may underlie the idiosyncratic nature of patient reactions to this class of drugs, including lack of therapeutic response and the different neurological disorders which may arise after even brief drug use.

The method described in this report has applicability not only to the neuroleptic drugs such as the phenothiazines but may also be considered with any drug for which a sulfoxide is formed. For the phenothiazines tested (FPZ, chlorpromazine and trifluoperazine), the oxidation appears to be reversible at a reduction potential which permits reasonable sensitivity for electrochemical detection. Such methodology provides a specific and quantitative technique for determination of phenothiazines and their metabolites.

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## REFERENCES

1. M. H. Lewis, E. Widerlov, D. L. Knight, C. D. Kilts and R. B. Mailman, *J. Pharmac. exp. Ther.* **225**, 539 (1983).

2. P-A. Hals, H. Hall and S. G. Dahl, *Eur. J. Pharmac.* **125**, 373 (1986).
3. S. G. Dahl, *Ther. Drug Monit.* **4**, 33 (1982).
4. S. G. Dahl, E. Hough and P-A. Hals, *Biochem. Pharmac.* **35**, 1263 (1986).
5. D. W. Hoffman, R. D. Edkins, S. D. Shillcutt and A. Salama, *J. Chromat.* **414**, 504 (1987).
6. U. R. Tjaden, J. Lankelma, H. B. Poppe and R. G. Muusze, *J. Chromat.* **125**, 275 (1976).
7. S. A. Goldstein and H. Van Vunakis, *J. Pharmac. exp. Ther.* **217**, 36 (1981).
8. G. N. Ko, E. R. Korpi and M. Linnoila, *J. clin. Psychopharmac.* **5**, 253 (1985).
9. L. E. Tune and J. T. Coyle, *Ther. Drug Monit.* **4**, 59 (1982).
10. W. F. Heyes and M. L. Robinson, *J. pharm. biomed. Anal.* **3**, 477 (1985).
11. M. A. Brooks and G. DiDonato, *J. Chromat.* **337**, 351 (1985).
12. E. Richelson and A. Nelson, *Eur. J. Pharmac.* **103**, 197 (1984).
13. U. Breyer, H. J. Gaertner and A. Prox. *Biochem. Pharmac.* **23**, 313 (1974).
14. U. Breyer, A. Prox, R. Bertele and H. J. Gaertner, *J. pharmac. Sci.* **63**, 1842 (1974).
15. S. H. Curry, R. Whelpton, P. J. deSchepper, S. Vranckx and A. A. Schiff, *Br. J. clin. Pharmac.* **7**, 325 (1979).