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Note

Determination of the serum concentrations of thioridazine and its main metabolites using a solid-phase extraction technique and high-performance liquid chromatography

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Therapeutic drug monitoring of anti-psychotic drugs has begun to be used to optimize the treatment of psychotic patients. The gas chromatographic or high-performance liquid chromatographic (HPLC) methods used up to now to determine plasma or serum drug concentrations all employ extraction procedures that use organic solvents. They are all more or less complicated, time-consuming and costly, besides being poorly suited to routine analysis of large series of samples. Organic solvents may also put the health of laboratory personnel at risk. Improvements in solid extraction systems, and automatic injection systems for HPLC, however, offer a means of making simple, quick and specific analyses of drug concentrations [1].

This paper describes an analytical method for determining the serum concentration of thioridazine, which is one of the most commonly used anti-psychotic drugs; its main metabolites are the thioridazine side-chain sulphoxide, the thioridazine side-chain sulphone and the thioridazine ring sulphoxide. The first two metabolites are known to be anti-psychotically active and to contribute to the total anti-psychotic effect of thioridazine treatment [2–4]. The ring sulphoxide probably lacks anti-psychotic properties but contributes to the side-

effects of thioridazine treatment [4,5]. The method employs commercially available units for solid-phase extraction and an automatic HPLC system.

EXPERIMENTAL

Chemicals

Thioridazine, thioridazine side-chain sulphoxide, thioridazine side-chain sulphone and thioridazine ring sulphoxide, used as standard substances, were obtained from Sandoz (Basel, Switzerland). A standard solution of each was prepared in 99.5% ethanol and stored at 4°C; their concentrations were 1.0, 1.0, 0.25 and 3.0 mM, respectively. Phosphoric acid (0.2 M) was prepared by diluting 0.5 ml of 85% orthophosphoric acid suprapur (Merck, Darmstadt, F.R.G.) to 35 ml with water. The mobile phase buffer (pH 2.2) was prepared by diluting 4.5 ml each of 85% orthophosphoric acid suprapur and triethylamine sequencer grade (Rathburn Chemicals, Walkerburn, U.K.) to 1000 ml with water. The mobile phase was acetonitrile–methanol–mobile phase buffer (1:2:1, v/v). The pH was 4.1 in the mixed mobile phase.

Unless stated otherwise the chemicals used, including water, were of HPLC grade.

Instruments

To extract samples and to make injections, we used the advanced automated sample processor (AASP) system (Varian, Walnut Creek, CA, U.S.A.). It comprises a Prep Station and an automatic HPLC injection system. In the Prep Station the samples are loaded onto sorbent cartridges (here a bonded-silica-type C_{18} sorbent), placed in cassettes and then purified by means of a specific solvent protocol. The injection unit can process up to ten cassettes, each containing ten sorbent cartridges. The HPLC instrument was a Varian Vista 5500 with a Varian 2550 variable-wavelength UV detector and a Varian 4290 automatic integrator system. The analytical column was a reversed-phase Spherisorb ODS-2 Superpac cartridge (5 μm, 250 mm$\times$4 mm I.D., LKB, Bromma, Sweden).

Analytical procedure

The extraction cartridges were conditioned with 1 ml of methanol and 1 ml of water. Then 500 μl of serum were mixed with 500 μl of 0.2 M phosphoric acid. The pH of the mixture was ca. 2.3. After equilibrating for 5 min, 400 μl of this mixture were added to an extraction cartridge in the Prep Station. Then 1 ml of water followed by 1 ml of acetonitrile–water (1:1, v/v) was passed through each cartridge. The cassettes with cartridges were then transferred to the AASP injection unit. It was programmed to elute each cartridge for 30 s with mobile phase onto the analytical column at 15-min intervals. The flow-rate was 1 ml/min at room temperature. The detection wavelength was 254 mm. Up to 65 samples can be analysed overnight.

Standardization and calculation

Standards were made from human serum (obtained from volunteers) to which aliquots of the standard solutions were added; this gave a set of five standards used in each run. The concentration ranges were: for thioridazine, 0.5–4.0 μM; for the side-chain sulphoxide, 0.5–4.0 μM; for the side-chain sulphone, 0.125–1.0 μM; and for the ring sulphoxide, 1.5–12.0 μM. Standard curves of chromatographic peak height as a function of concentration were calculated according to a linear model.

RESULTS

Chromatographic separation and standardization

Fig. 1 shows a chromatogram of the plasma of a patient. The ring sulphoxide peak is split, probably because two isomers partially separated. The ratio of the two ring sulphoxide fractions is ca. 1:1 in patient samples, but the reference substance contains only trace amounts of the slower moving fraction. The standard curves were straight lines for all four substances and approached the zero intercept closely.

Extraction yield

The extraction yield was calculated as the quotient between two peak heights, the first derived from spiked serum samples and the second from directly injected solutions containing the same amounts of thioridazine and its metabolites. The extraction yields were: thioridazine, 75%; side-chain sulphoxide, 75%; side-chain sulphone, 76%; ring sulphoxide 71% ($n = 10$, coefficients of variation, 1.23, 6.85, 4.88 and 6.71%, respectively). Essentially the same figures were obtained when peak areas were measured. When spiked water samples were used instead of serum samples, yields were essentially the same for the
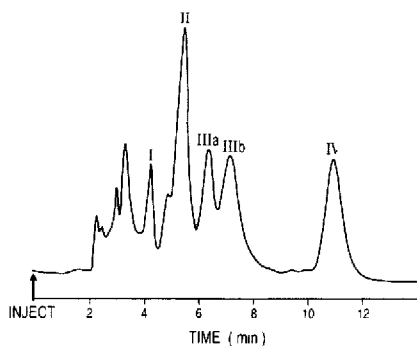

Fig. 1. Chromatogram obtained from a serum sample from a patient treated with 500 mg of thioridazine per day (additional medication: orphenadin 150 mg per day, phenytoin 300 mg per day, levomepromazine 25 mg per day and perphenazine 200 mg per week). Peaks I = thioridazine side-chain sulphone (0.3 μM), II = thioridazine side-chain sulphoxide (1.43 μM); IIIa and IIIb = thioridazine ring sulphoxide (3.62 μM); IV = thioridazine (1.20 μM).
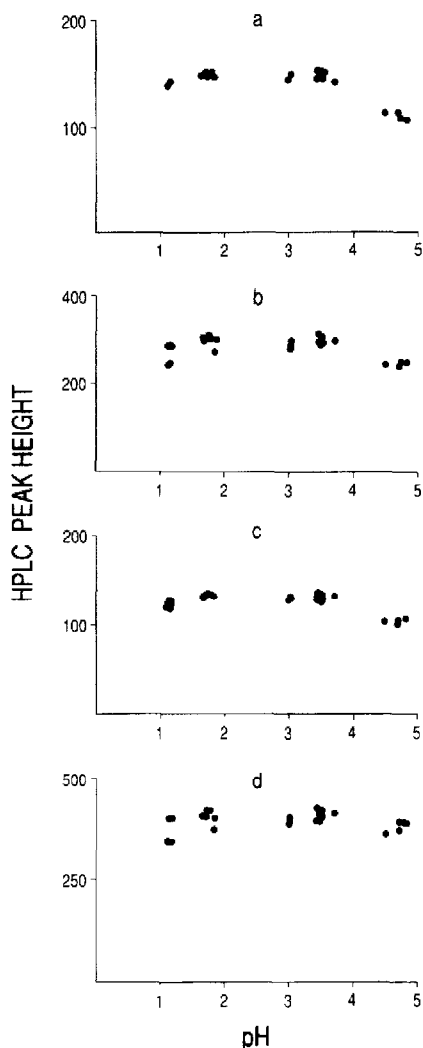
Fig. 2. Relations between pH of a diluted serum sample spiked with thioridazine and its metabolites and HPLC peak heights. (a) Thioridazine, (b) thioridazine side-chain sulphoxide; (c) thioridazine side-chain sulphone; (d) thioridazine ring sulphoxide.

side-chain sulphoxide metabolites but were significantly lower for thioridazine and significantly higher for the ring sulphoxide, 60 and 76%, respectively ($p < 0.001$ and $p < 0.01$). The extraction losses were not caused by incomplete absorption since no thioridazine or metabolites were found by gas chromatography (GC) [6] in the eluted serum samples and washings from the sorbent cartridges.

As Fig. 2 shows, the pH of the serum samples influenced the yield. Hydrochloric, phosphoric and citric acids were used in various concentrations to obtain different pH values.

Precision

The within-run precisions, calculated from duplicate values from patient samples with concentrations within the therapeutic range, were (coefficients of variation): thioridazine, 3.7%; side-chain sulphoxide, 4.2%; side-chain sulphone, 4.6%; ring sulphoxide, 4.5% ($n = 32$).

In ten separate analyses, the between-run precisions were calculated from values for a spiked serum sample with added thioridazine ($1.0 \mu M$), side-chain sulphone ($0.25 \mu M$) and ring sulphoxide ($3.0 \mu M$); these values were 6.4, 7.9, 8.3 and 5.1% respectively.

Accuracy: comparison with a previous GC method

Samples of serum drawn routinely from 70 thioridazine-treated patients were analysed both with the method under discussion and with a GC method [7] in current use in our laboratory. A comparison of the two sets of results is presented in Fig. 3. Most of the patients were additionally medicated, mainly with one or more other neuroleptic drugs, tricyclic anti-depressants, anti-epileptic drugs and anti-Parkinson drugs.

Influence by other drugs

The retention times and absorbances of a number of commonly used psychoactive drugs were determined (Table I).
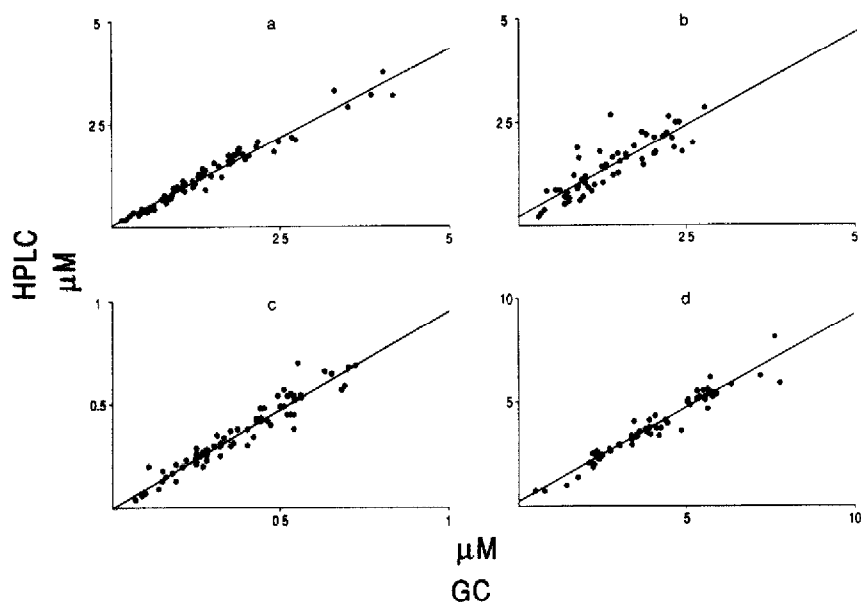

Fig. 3. Comparison between serum concentrations obtained with the present HPLC method and a GC method. (a) Thioridazine; (b) side-chain sulphoxide; (c) side-chain sulphone; (d) ring sulphoxide. Regression lines and r values: (a) $y = 0.86x + 0.03$, $r = 0.98$; (b) $y = 0.89x + 0.21$, $r = 0.86$; (c) $y = 0.96x + 0.004$, $r = 0.96$; (d) $y = 0.90x + 0.21$, $r = 0.97$.

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TABLE I

RETENTION TIMES AND ABSORBANCE AT 254 nm OF SOME PSYCHOACTIVE DRUGS

	Retention time (min)	Absorbance (A.U./nmol)
Carbamazepine[a]	–	–
Phenytoin[a]	–	–
Clonazepam	2.41	0.077
Nitrazepam	2.44	0.206
Flunitrazepam	3.09	0.158
Lorazepam	3.23	0.086
Oxazepam	3.30	0.110
Alprazolam	3.68	0.082
Haloperidol	4.06	0.048
Thioridazine side-chain sulphone	4.12	1 921
Diazepam	4.71	0.053
Thioridazine side-chain sulphoxide	4.98	1.098
Zuclopentixol	5.23	0.024
Protriptyline	5.43	0.009
Desmethylimipramine	5.65	0.047
Thioridazine ring sulphoxide I	5.73	0.475
Nortriptyline	6.04	0.012
Maprotiline	6.00	0.002
Prometazine	6.25	0.041
Imipramine	6.31	0.018
Thioridazine ring sulphoxide II	6.40	–[b]
Amitriptyline	6.73	0.009
Levomepromazine	6.79	0.069
Trimipramine	7.03	0.019
Desmethylclomipramine	7.43	0.038
Chlorpromazine	8.01	0.055
Clomipramine	8.25	0.034
Perphenazine	8.29	0.021
Fluphenazine	8.77	0.004
Thioridazine	10.95	0.768
Prochlorperazine	11.41	0.158

[a]No peaks were observed after injection of 20 nmol of carbamazepine and 60 nmol of phenytoin.
[b]No reference substance was available for this assumed isomer.

At an early stage we noticed that the values for thioridazine side-chain sulphone for some patients who were also medicated with levomepromazine seemed much too high. In samples from two patients treated with high doses of levomepromazine (600 mg per day), a small peak appeared with the same retention time as thioridazine side-chain sulphone. This was probably caused by a metabolite of levomepromazine.

To test for possible interference by some commonly used psychoactive drugs, serum samples from patients treated with amitriptyline (1 patient, 150 mg per

day), imipramine (1 patient, 150 mg per day), clomipramine (3 patient, 150 mg per day), lofepramine (1 patient, 245 mg per day), maprotiline (1 patient, 200 mg per day), trimipramine (1 patient, 150 mg per day), chlorpromazine (1 patient, 50 mg per day), levomepromazine (2 patients, 50 mg per day), carbamazepine (2 patients, 50 mg per day) and propranolol (1 patient, 80 mg per day) were spiked with thioridazine and its metabolites to give the same centrations as in a control serum (thioridazine 1.06 μM, side-chain sulphoxide 1.27 μM, side-chain sulphone 0.34 μM, ring sulphoxide 3.81 μM). The samples were analysed in duplicate and compared with the control serum. The results indicated that the side-chain sulphoxide value was influenced by amitriptyline and trimipramine metabolites (ca. 10% increase), the side-chain sulphone value was influenced by amitriptyline, clomipramine and trimipramine metabolites (ca. 10% increase) and by some levomepromazine metabolites (ca. 100% increase), and the ring sulphoxide value was influenced by some clomipramine metabolite (15–30% increase). No influence on the thioridazine value was observed.

DISCUSSION

The present method allows the serum concentrations of thioridazine and its main metabolites to be quickly and specifically assessed. It is suitable for routine clinical analysis for therapeutic drug monitoring.

The extraction yields in the solid-phase extraction were ca. 75%. The extraction losses were probably caused by incomplete desorption from the extraction cartridges. Thioridazine and its metabolites are strongly bound to protein in native serum ca. 98% for metabolites and ca. 99.8% for thioridazine) [7], but this binding is weaker at lower pH. At the pH used in the present extraction procedure, the binding to protein is less likely to influence the extraction because the extraction yields (except for the ring sulphoxide) were not lower than those derived from a water solution.

Because linear standard curves were obtained, it is probable that the capacity of the sorbent column was not exceeded despite the wide range of the amounts of drugs in the standards. HPLC analysis results for samples that contained a variety of other drugs agreed well with the results of GC analysis [8].

The values of thioridazine concentration obtained with this method are slightly lower than those obtained with a GC method, and so far we cannot explain this difference. It is reasonable to suppose that systematic errors can arise in the GC method (on account of its harsher conditions for extraction) as well as in chromatography; for instance, small amounts of other metabolites can be converted into the ones that are measured.

In the samples from patients, the HPLC peak with the retention time of the ring sulphoxide reference was immediately followed by another peak. We have assumed the second peak to be an isomer of the ring sulphoxide because the

ratio of the two peaks is essentially the same in all samples and because their sum equals the expected value for the whole ring sulphoxide fraction. HPLC separation of the isomers of thioridazine ring sulphoxide has also been described earlier [9].

As psychiatric patients are commonly treated with more than one psychoactive drug, this may create interference problems when analysing drug concentrations in body fluids. However, the listed retention times and molar extinction values of a number of commonly used psychoactive drugs suggest that these problems will be only minor. We tested the interference of the metabolites of some drugs and found indications that the metabolites of several tricyclic antidepressants may influence the thioridazine metabolite values to a minor degree and that levomepromazine metabolites will strongly interfere with the thioridazine side-chain sulphone values.

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