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SIMULTANEOUS DETERMINATION OF THIORIDAZINE AND ITS S-OXIDIZED AND N-DEMETHYLATED METABOLITES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON RADIALLY COMPRESSED SILICA

CLINTON D. KILTS*

Clinical Psychopharmacology Laboratory, Department of Psychiatry, Box 3870, Duke University Medical Center, Durham, NC 27710 (U.S.A.)

KENNERLY S. PATRICK

Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514 (U.S.A.)

and

GEORGE R. BREESE and RICHARD B. MAILMAN

Departments of Psychiatry and Pharmacology and the Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514 (U.S.A.)

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SUMMARY

A method for simultaneously quantifying thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone and thioridazine-5-oxide in serum and plasma is described. Following solvent extraction these compounds were separated by high-performance liquid chromatography on radially compressed silica gel and detected by UV absorbance at 254 nm. Chromatography time is less than 7 min. The relative retention of these compounds as a function of the methanol and methylamine content of the mobile phase is discussed. Practical limits of detection, based upon an assayed plasma or serum volume of 1 ml, were 20 ng/ml for thioridazine-5-oxide and 10 ng/ml for the other compounds. The coefficient of variation for all compounds was less than 13%. The method is compared with more conventional high-performance liquid chromatographic and gas chromatographic methodology.

INTRODUCTION

The clinical pharmacokinetics of antipsychotic drugs have become an area

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of intense study in recent years in an attempt to individualize the pharmacotherapy of psychiatric disorders. Of paramount importance to such studies is the development of analytical methods suitable for determining the concentrations of the parent drug as well as any pharmacologically active metabolites in biological fluids. Thioridazine (Mellaril®), a widely used phenothiazine antipsychotic, typifies many of these considerations. The extent to which its metabolites accumulate following thioridazine administration, and their neuropharmacological activity, makes their quantification a potentially important component when attempting to empirically optimize the balance between therapeutic effect and the incidence of side effects.

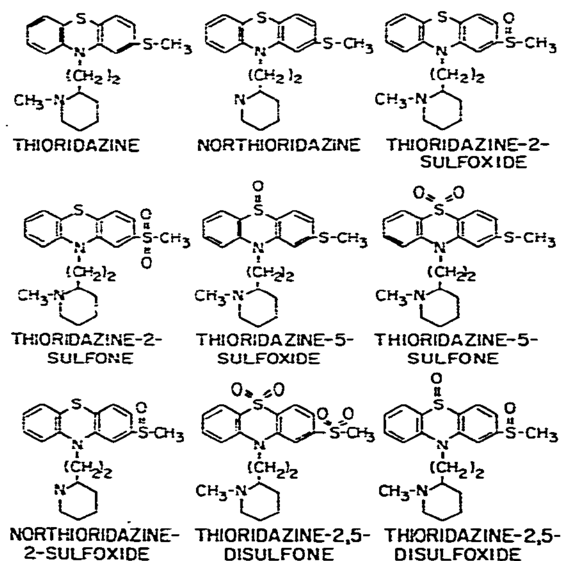


Fig. 1. Structures of thioridazine and its major metabolites.

Thioridazine undergoes extensive transformation in humans with S-oxidation representing the predominant route of metabolism [1, 2]. Unlike other phenothiazines, thioridazine reportedly undergoes little or no metabolic hydroxylation or N-demethylation in humans [3, 4]. The major identified metabolites of thioridazine isolated from human serum and urine are illustrated in Fig. 1. The S-oxidized metabolites differ from thioridazine and one another in a number of important properties: pharmacological activity, both in terms of antipsychotic efficacy [5—8] and side effects [5, 7, 9]; extent of binding to serum [10] or plasma [11] proteins; and time course of accumulation in serum following thioridazine administration [12]. Each of these factors must be considered when attempting to correlate drug concentration with clinical response and necessitates the accurate quantitative determination of thioridazine and its pharmacologically active and major inactive metabolites when performing such studies.

A variety of purification, separation and detection methods have been used to quantify thioridazine and its metabolites. Initial methods involved solvent extraction or ion-exchange chromatography and fluorometric detec-

tion after oxidation of the compounds of interest to fluorophors [13—16]. Such techniques lack selectivity and do not permit the separate quantification of thioridazine and its metabolites. Subsequent efforts also involved a solvent extraction procedure followed by either gas chromatographic (GC) separation with flame ionization detection [2, 3, 17, 18] or high-performance liquid chromatographic (HPLC) separation with fluorometric or ultraviolet (UV) absorbance detection [19—21].

The present study reports a method for the rapid, simultaneous determination of plasma or serum concentrations of thioridazine, northioridazine, thioridazine-2-sulfone, thioridazine-2-sulfoxide and thioridazine-5-oxide. The technique utilizes HPLC separation on radially compressed silica gel with 254-nm UV absorbance detection. Chromatography on radially compressed packed columns is compared with more conventional reversed-phase and adsorption HPLC using packed steel columns, and with GC separation with thermionic (nitrogen—phosphorus) selective detection.

EXPERIMENTAL

Chemicals

The 2,2,4-trimethylpentane, methanol, methylene chloride and hexane were HPLC grade (Fisher Scientific, Fairlawn, NJ, U.S.A.). Methylamine was obtained as a 40% solution in water (Aldrich, Milwaukee, WI, U.S.A.). Diethyl ether (peroxide-free) was reagent grade (Mallinckrodt, St. Louis, MO, U.S.A.). Thioridazine hydrochloride, northioridazine, thioridazine-2-sulfoxide hydrochloride, thioridazine-2-sulfone, thioridazine-5-oxide hydrochloride, thioridazine-2,5-disulfoxide, thioridazine-2,5-disulfone and northioridazine-2-sulfoxide were generously supplied by Sandoz Pharmaceuticals (East Hanover, NJ, U.S.A.). The 2-acetylphenothiazine was from Aldrich.

General

All glassware used in sample preparation, except pipettes, was treated with a 10% solution of dimethyldichlorosilane in toluene, rinsed with methanol, and oven dried at 100°C. Pipettes used to transfer solvents were rinsed with methanol immediately prior to their use. These precautions minimized the loss of thioridazine and its metabolites during sample preparation due to adsorption onto glass surfaces. Screw caps for the conical centrifuge tubes were fitted with PTFE liners. Solutions of thioridazine, its metabolites, 2-acetylphenothiazine or other drugs tested were made in methanol at a concentration of 25 ng/μl (calculated as the free base) and stored in the dark at 4°C.

Sample preparation

A 1-ml aliquot of plasma was added to a 15-ml conical centrifuge tube, and the pH was adjusted to approximately 10.5 by adding 400 μl of 2 *M* sodium hydroxide solution. The mixture was extracted with 2.5 ml of diethyl ether—hexane (3:1, v/v) by mixing for 10 min on a mechanical shaker followed by centrifugation at 500 *g* for 5 min. The organic layer was then transferred to another centrifuge tube containing 1 ml of 0.1 *M* hydrochloric

acid. Extraction and phase separation were performed as in the preceding step and the organic phase was aspirated and discarded. The remaining aqueous phase was washed with 2 ml of the diethyl ether—hexane solvent, the tubes vortexed (20 sec) and centrifuged, and the organic layer was discarded. The aqueous phase was made alkaline (pH > 10) by adding 150 μl of 2 *M* sodium hydroxide solution and the mixture was extracted twice with 1.5 ml volumes of the diethyl ether—hexane solvent. The organic phases were pipetted into another centrifuge tube containing 100 ng of 2-acetylphenothiazine and evaporated to dryness under nitrogen with gentle heating using a heat gun. The residue was dissolved in 60 μl of hexane—methanol—methylene chloride (8:1:1), the tubes vortexed (20 sec), and 20—40 μl aliquots injected into the liquid chromatograph. For GC assay the residue was dissolved in 10 μl of cyclohexane and 2—4-μl aliquots were injected.

Instrumentation

A Laboratory Data Control (LDC, Riviera Beach, FL, U.S.A.) ConstaMetric IIG solvent delivery pump was used in conjunction with a Model 1203 UV absorbance detector (LDC) which was operated at 254 nm with a time constant of 0.5 sec. Detector output was displayed on a 10-mV recorder using a chart speed of 0.25 cm/min. A six-port rotary valve (Model 7125, Rheodyne, Berkeley, CA, U.S.A.) with a 50-μl sample loop was used for sample injection. Chromatographic separations were performed using an RCM-100 radial compression separation system with a cartridge (8 mm I.D.) packed with microparticulate (5 μm diameter) silica gel, both from Waters Assoc. (Milford, MA, U.S.A.). A precolumn filter with a 2-μm frit (Rheodyne) effectively minimized the accumulation of particulate matter on the analytical column. Steel HPLC columns (25 cm × 4.6 mm I.D.) packed with either 8-μm silica gel or C_{18}-bonded 5-μm silica (Spherisorb ODS) were from LDC.

Chromatography

The mobile phase was a mixture of 2,2,4-trimethylpentane—methylene chloride (water saturated)—methanol (8:1:1) containing 0.036% methylamine. The methylamine was added to the methanol prior to mixing. Dissolved gases in the mobile phase were minimized by ultrasonic agitation immediately before use. All separations were performed isocratically at a flowrate of 2.25 ml/min.

Calculations

Blank-corrected standard curves for the quantification of thioridazine and its metabolites were prepared by assaying a series of standard solutions containing varying amounts (0—1000 ng) of thioridazine and its metabolites. The standard solutions were 1-ml aliquots of drug-free plasma which were subjected to the identical preparative steps used to extract unknown plasma samples. The detector response for each compound was determined for each sample and standard. The concentrations of thioridazine and each metabolite in an unknown plasma sample were calculated from their peak heights relative to the external standard, using the slope and intercept of the standard curve.

UV absorbance spectra

The molar absorptivities of the compounds of interest were determined on a Beckman UV 5230 spectrophotometer (Irvine, CA, U.S.A.). The instrument was standardized for wavelength and absorption versus a solution of potassium dichromate. Values for the standard solution were within ± 0.5 nm and ± 0.5% A of published values [22]. All relevant values were read between 0.47—1.4 au except for the A_{254} for thioridazine-2,5-disulfone. All solutions of thioridazine and its metabolites were prepared in methanol at a concentration of 10 μg/ml.

Gas chromatography

A Varian (Palo Alto, CA, U.S.A.) Model 2440 gas chromatograph was used in conjunction with a thermionic (nitrogen—phosphorous) selective detector (Varian). All separations were performed using dimethyldichlorosilane-treated glass columns (2 mm I.D.) packed with varying stationary phases on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.).

Mass spectrometry

Electron impact mass spectra were obtained using a Model 3300 gas chromatograph—mass spectrometer (Finnigan, Sunnyvale, CA, U.S.A.) interfaced with a Finnigan 6100 Data System. Fragmentation was accomplished by electron impact at 70 eV and 500 μA. Spectra were obtained at a scanning rate of 20 a.m.u./sec.

RESULTS AND DISCUSSION

Choice of separation mechanism

Gas chromatography. GC with thermionic (nitrogen—phosphorus) selective detection was initially examined as a means of separating and detecting thioridazine and its metabolites. Previously published GC assays for thioridazine and its S-oxidized and N-demethylated metabolites have utilized flame ionization detection [2, 3, 17, 18], a relatively non-selective and non-sensitive mode of GC detection. This lack of detector sensitivity necessitated the use of large volumes of serum or plasma samples (i.e., 4—5 ml) for accurate determinations. Of these assays, three [3, 17, 18] utilized a stationary phase (OV-17) which incompletely resolved thioridazine and northioridazine as well as thioridazine-2-sulfoxide and thioridazine-2-sulfone. A fourth [2] employed a stationary phase (OV-225) which is not readily compatible with thermionic (nitrogen—phosphorus) selective detection. A further evaluation of the GC retention behavior of thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone and thioridazine-5-oxide on stationary phases of varying polarity and percent loading on the solid support are summarized in Table I. Thermionic (nitrogen—phosphorus) selective detection was promising in that it proved to be two or more orders of magnitude more sensitive than flame ionization detection. However, no combination of stationary phase, column length and column oven temperature (isothermal or programmed) examined resulted in a usable separation of the compounds of interest. Derivatization techniques are often used to resolve phenothiazines from their N-demethylated

TABLE I

GC RETENTION BEHAVIOR OF THIORIDAZINE (THD), NORTHIORIDAZINE (norTHD), THIORIDAZINE-2-SULFOXIDE (THD-2-SO), THIORIDAZINE-2-SULFONE (THD-2-SO_2) AND THIORIDAZINE-5-OXIDE (THD-5-SO) ON COLUMNS OF VARYING POLARITY, LENGTHS AND TEMPERATURES

Chromatography conditions: injector and detector temperatures: 280°C; flow-rates: helium (carrier gas), 35 ml/min; air, 160 ml/min; hydrogen, 3.2 ml/min.

Stationary phase	Column length (feet)	Oven temperature (°C)	Retention time (min)				
			THD	norTHD	THD-2-SO	THD-2-SO_2	THD-5-SO
1% OV-17	3	250	3.0	3.1	8.1	9.2	13.7
3% SP-2250 (OV-17)	6	275	6.0	6.2	14.0	15.5	20.7
3% SP-2250	1.5	260	1.2	1.2	2.8	3.2	5.6
1% OV-25	3	250	4.5	4.8	12.0	13.1	18.5
1% OV-1	3	250	2.4	2.5	4.7	4.8	10.4

metabolites [3, 23]. However, the commonly used acid anhydrides have been reported to reduce the S-oxidized metabolites of chlorpromazine, a structurally related phenothiazine, to their corresponding sulfides [23, 24]. Similarly, the reaction of trifluoroacetic anhydride (100°C, 15 min) with thioridazine-2-sulfoxide, thioridazine-5-oxide and northioridazine-2-sulfoxide resulted in the formation of the corresponding sulfides, as well as degradation products. Furthermore, when thioridazine-5-oxide was chromatographed at relatively high column temperatures ($>$ 230°C) a compound with the same retention time as thioridazine was detected and subsequently identified as thioridazine by electron impact mass spectrometry. It would thus appear that thioridazine-5-oxide undergoes a thermal reduction to thioridazine. Based upon the inability to form stable volatile derivatives of thioridazine or its tertiary amine-containing metabolites, their apparent reduction by commonly used derivatizing reagents, their poor GC resolution and the apparent thermal lability of thioridazine-5-oxide, the use of GC techniques as a means of separating these compounds was discontinued.

High-performance liquid chromatography. Due to the low volatility of thioridazine and its metabolites, HPLC was evaluated as an alternative method of resolving these compounds prior to detection. The retention behavior of these compounds using steel columns with either reversed-phase (C_{18}) or adsorption (silica gel) chromatography were compared. Of the two, the microparticulate silica gel exhibited a greater separation selectivity than that obtained using reversed-phase chromatography. The superiority of adsorption chromatography on silica gel, over reversed-phase chromatography, has been demonstrated previously for the separation of a number of tricyclic antidepressants of closely related structures [25–27].

The recent introduction of compressible, polyethylene HPLC columns packed with various adsorbents has offered an apparent solution to the problem of column dead volume, particularly between the packing material and column wall, that is characteristic of steel HPLC columns [28]. The advantages of using adsorbents under radial compression are thought to arise from a higher degree of interaction between the solute and the homogenously packed adsorbent [28]. A further comparison of the retention behavior of thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone and

thioridazine-5-oxide on conventional steel columns packed with silica gel and on radially compressed microparticulate silica gel was made. Radial compression offered significant improvements in resolution efficiency, and the ability to utilize high mobile phase flow-rates which decreased chromatogram time and permitted a more rapid rate of equilibration between the mobile phase and the column packing. Further, the radial compression separation system offers considerably more ease of operation in terms of lack of conventional column end fittings, lack of unidirectionality of mobile phase flow and longer column lifetime. The chromatographic separation using this method was excellent as can be seen from representative chromatograms of these compounds from standard solutions or from extracts of spiked plasma samples (Fig. 2). Furthermore, the chromatography time is less than 7 min.

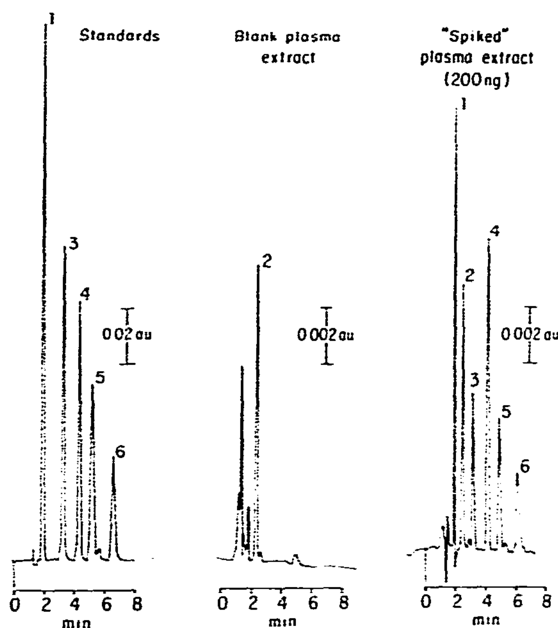


Fig. 2. Chromatograms of 20 μl of a standard mixture containing 25 ng/μl of thioridazine and its metabolites (left) and extracts of blank plasma (center) and plasma to which 200 ng of thioridazine and its metabolites have been added (right). Mobile phase flow-rate was 2.5 ml/min and detector output displayed at a recorder chart speed of 5 mm/min. Peaks: 1 = thioridazine; 2 = 2-acetylphenothiazine; 3 = northioridazine; 4 = thioridazine-2-sulfone; 5 = thioridazine-2-sulfoxide; 6 = thioridazine-5-oxide.

After a preliminary report of our method was described [29], Skinner et al. [21] published a somewhat similar procedure utilizing HPLC separation on silica gel packed in steel columns and 254-nm absorbance detection. However, these authors report that only between 0 to 60% of commercially available prepacked columns yielded usable chromatographic separations. Further, they do not report the ability to be able to separate or detect several important metabolites of thioridazine such as thioridazine-5-oxide as well as mixed 2,5-disulfones or disulfoxides. The use of radially compressed column

packings apparently circumvents these separation problems and increases the already great flexibility of HPLC.

Choice of HPLC detection mechanism

The two most widely used forms of sample detection of HPLC effluents are UV absorption and fluorometric methods. Thioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone, thioridazine-5-oxide and northioridazine were found to have little or no native fluorescence. While fluorophors of these compounds have been formed by oxidative mechanisms [14—16, 19], only one study [19] has reported the use of such reactions in conjunction with HPLC and a fluorometer. UV absorption detection would appear to be well suited to the needs for detector sensitivity. The molar absorptivities at 254 nm for thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone and thioridazine-5-oxide exceed 18.2 mM^{-1} cm^{-1} (Table II), and thus permit their detection in small volumes of plasma or serum (0.5—1 ml) following therapeutic doses of thioridazine.

TABLE II

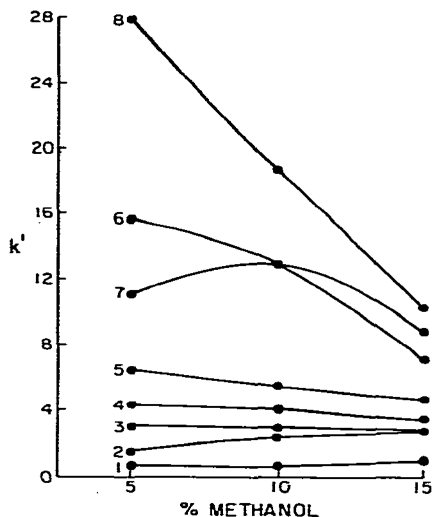
MOLAR ABSORPTIVITIES OF THIORIDAZINE AND ITS METABOLITES

See Experimental section for details.

Compound	ϵ_{254} (mM^{-1} cm^{-1})	λ_{max} (nm)	ϵ_{max} (mM^{-1} cm^{-1})
Thioridazine	31.9	264	40.8
Northioridazine	22.9	264	27.9
Thioridazine-2-sulfone	23.8	266	35.9
Thioridazine-2-sulfoxide	24.0	262	30.5
Thioridazine-5-oxide	18.2	279	44.3
Thioridazine-2,5-disulfoxide	18.1	(broad: 210—275 nm)	
Thioridazine-2,5-disulfone	9.1	234	42.2

Effect of the solvent strength and methylamine content of the mobile phase on solute retention

The relative retention (capacity factor, k') of thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone, thioridazine-5-oxide, northioridazine-2-sulfoxide, thioridazine-2,5-disulfone and thioridazine-2,5-disulfoxide was found to be a function of the methanol and methylamine content of the mobile phase. The order of elution using radially compressed silica gel was inversely related to the polarity of the functional groups of these compounds. Increasing the solvent strength of the mobile phase by increasing the methanol content produced a concentration-dependent decrease in the retention of the S-oxidized metabolites of thioridazine, especially the strongly adsorbed disulfone and disulfoxide (Fig. 3). Conversely, increasing the methanol content of the mobile phase increased the capacity factor for northioridazine. The addition of methylamine to the mobile phase produced a concentration-dependent decrease in the retention of all of the compounds tested



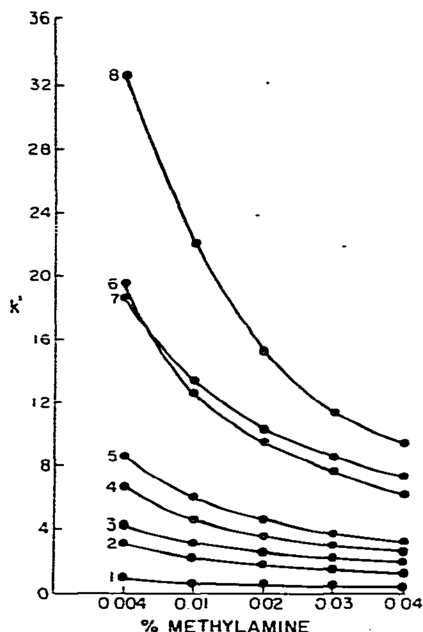


Fig. 3. Effect of methanol content of the mobile phase on the retention of thioridazine and its metabolites. Mobile phase: 2,2,4-trimethylpentane—methylene chloride—methanol (7.5—8.5:1:0.5—1.5) and 0.02% methylamine. The water content of the mobile phase was constant. The capacity factor (k') was calculated by $k' = (t_R - t_{uu})/t_{uu}$ where t_R and t_{uu} are the retention times of the compound of interest and an unretarded compound (chlorobenzene), respectively. Curves: 1 = thioridazine; 2 = northioridazine; 3 = thioridazine-2-sulfone; 4 = thioridazine-2-sulfoxide; 5 = thioridazine-5-oxide; 6 = thioridazine-2,5-disulfone; 7 = northioridazine-2-sulfoxide; 8 = thioridazine-2,5-disulfoxide.

Fig. 4. Effect of methylamine content of the mobile phase on the retention of thioridazine and its metabolites. Mobile phase: 2,2,4-trimethylpentane—methylene chloride—methanol (8:1:1). The water content of the mobile phase was constant. See Fig. 3 for derivation of k' and numbering of curves.

(Fig. 4) and eliminated the peak tailing noted for the later eluting compounds. These effects most probably result from the ability of methylamine to compete with these basic compounds for the acidic silanol groups on the silica surface, and to suppress phenothiazine protonation by increasing the pH of the mobile phase. Caude et al. [30] have similarly noted the need to include a solvent modifier (ethylamine) for the chromatographic separation of a mixture of phenothiazine derivatives on silica gel.

Solvent extraction procedure

Small amounts of isoamyl alcohol in a nonpolar solvent such as heptane are generally used as an extraction solvent for thioridazine and its metabolites [2, 3, 14, 16, 17]. The isoamyl alcohol is thought to prevent the adsorption of these basic compounds to glass surfaces and to minimize the formation of emulsions. However, addition of 1—4% isoamyl alcohol to hexane produced lower extraction recoveries, greater losses during evaporation and increased evaporation time when compared to the combined use of the diethyl ether—hexane solvent, pipettes rinsed with methanol and glassware pretreated

with dimethyldichlorosilane. The rinsing of pipettes with methanol also greatly facilitated the quantitative transfer of the low viscosity extraction solvent. It is essential that the diethyl ether be free of peroxides as their presence results in significant losses of thioridazine, northioridazine and thioridazine-2-sulfone upon evaporation of the extraction solvent to dryness. Therefore the diethyl ether should be periodically tested for peroxides and if present, the diethyl ether should be redistilled over cuprous chloride. The substitution of methyl *tert*-butyl ether (HPLC grade, Fisher) for diethyl ether in the extraction solvent yielded similar extraction recoveries yet greatly decreased evaporation losses, probably due to the much slower rate of peroxide formation of the former solvent. The absolute and relative recoveries from spiked plasma samples are shown in Table III. The relative recoveries of thioridazine and its metabolites were greater than their absolute recoveries at both concentrations examined. Furthermore, the absolute and relative recoveries of the compounds of interest were slightly greater at the higher concentration. The coefficients of variation (C.V.) of the calculated recoveries were 10% or less and did not consistently vary with concentrations.

TABLE III

ANALYTICAL RECOVERIES AND EXTRACTION PRECISION

n = 5 for both concentrations.

Drug	Concentration (ng/ml)	Recovery*			
		Relative	C.V.	Absolute	C.V.
Thioridazine	75	94.7	9.9	75.0	8.9
	500	102.3	2.5	78.0	5.9
Northioridazine	75	83.0	9.0	65.4	5.4
	500	101.7	7.6	73.9	8.1
Thioridazine-2-sulfone	75	92.2	7.7	78.0	2.4
	500	99.1	4.8	91.3	5.5
Thioridazine-2-sulfoxide	75	87.9	4.3	81.6	7.7
	500	103.0	9.7	94.6	6.3
Thioridazine-5-oxide	75	90.2	8.1	72.3	10.0
	500	101.3	8.6	79.2	4.7

*Recoveries were determined from 1-ml aliquots of human serum to which the indicated concentrations of the compounds were added and the samples extracted as in Experimental. The observed detector response was compared to that obtained for either equal volumes of water supplemented to the same concentrations and subjected to an identical extraction procedure (relative recovery) or the same amount of each compound evaporated to dryness, reconstituted and directly injected (absolute recovery). 2-Acetyl phenothiazine (100 ng) was added to all tubes prior to solvent evaporation.

While internal standardization would be expected to improve the accuracy and precision of the method, no single compound has been found which effectively controls for losses of all of these compounds of widely differing polarity during sample preparation. However, 2-dimethylaminoethyl phenothiazine-10-carboxylate hydrochloride (k' = 0.69), which precipitates upon condensing 2-dimethylaminoethanol (Aldrich) with phenothiazine-10-car-

bonyl chloride in refluxing dry toluene, has been initially promising as an internal standard. Routinely, the addition of 100 ng of 2-acetyl phenothiazine ($k' = 0.82$) to the samples prior to solvent evaporation effectively controls for losses during sample evaporation and variabilities in reconstitution, as well as differences in volume injected and within-run variations in column and detector performance. The use of 2-acetylphenothiazine in this manner as an external standard appreciably increases the precision and accuracy of the method.

A back-extraction step into hydrochloric acid greatly decreased the chromatographic interference from plasma constituents. The addition of 2.5 ml of methylene chloride in the initial and final solvent extraction steps was necessary to extract the most polar metabolites, i.e., thioridazine-2,5-disulfoxide and thioridazine-2,5-disulfone. The diethyl ether—hexane solvent extracted these compounds poorly. The choice of solvents used to dissolve the residue obtained upon evaporation of the extraction solvent was critical. While thioridazine and its metabolites were more soluble in relatively polar solvents (e.g., methanol), and therefore could be highly concentrated prior to injection into the HPLC, the injection of such solvents resulted in a loss of resolution and significant broadening of the peaks of the chromatogram. A mixture of hexane—methylene chloride—methanol (8:1:1) was selected as the sample solvent based upon a combination of sample solubility and compatibility with the mobile phase.

Compound identification

Several approaches were taken to insure the accurate identification of the peaks of the chromatograms. First, the calculated capacity factor for each reference standard was determined and compared to chromatograms of plasma extracts from human patients who had previously received thioridazine. Secondly, the nondestructive nature of UV absorbance detection permitted the collection of the detector effluent at the predetermined retention time of each individual compound. The solvent was evaporated to dryness and the samples reconstituted in 10 μl methanol and directly introduced into the analyzer of the mass spectrometer via the solid probe. Electron impact mass spectra were characterized by parent ions of low relative abundance, yet verified the correct assignment of chemical structure to the chromatographic peaks.

Interfering substances

Using packed steel columns or radially compressed columns containing 10-μm silica, extracts of drug-free plasma were found to contain a compound of unknown identity which coeluted with thioridazine. However, this interfering substance was present in relatively low, consistent amounts and its influence was effectively eliminated by blank-correction of the standard curves. The greater separation efficiency of radially compressed 5-μm silica effectively resolved this unknown plasma component from thioridazine and therefore this packing material is recommended for routine use. The possibility of interconversion of thioridazine and its metabolites during the course of sample preparation [31] and the resulting cross interference was also exam-

ined. The extraction, chromatographic separation and detection of 250 ng of a given metabolite or the parent compound added to separate 1-ml aliquots of drug-free plasma demonstrated the lack of interference by any one compound in the quantification of another.

Seven drugs which are often concurrently administered to pediatric and adult patient populations receiving treatment with thioridazine were also examined for their possible interference with the quantification of thioridazine or its metabolites using this assay procedure (Table IV). None of the drugs tested interfered in the assay of thioridazine or its metabolites. Of the additional antipsychotics tested, chlorpromazine and thiothixene were found to interfere in the determination of thioridazine and thioridazine-2-sulfone, respectively. Furthermore, as the majority of psychoactive drugs are extensively biotransformed to a multitude of appreciably accumulated metabolites of unknown potential for interference, a careful examination of the recent drug history of a given patient prior to sample collection and use of this methodology seems warranted.

TABLE IV

RELATIVE RETENTION (k') OF THIORIDAZINE, ITS MAJOR METABOLITES, OTHER ANTIPSYCHOTICS, AND DRUGS OFTEN ADMINISTERED CONCURRENTLY WITH THIORIDAZINE

Drug	k'	Drug	k'
Imipramine	0.16	Phenobarbital	9.01
Thioridazine	0.41	Thioridazine-2,5-disulfoxide	9.49
Diazepam	0.72	Diphenhydramine	nd*
Northioridazine	1.19	Methylphenidate	nd
Benztropine	1.66		
Thioridazine-2-sulfone	1.86	*Other antipsychotics*	
Thioridazine-2-sulfoxide	2.31	Trifluopromazine	0.24
Thioridazine-5-oxide	3.06	Chlorpromazine	0.43
Phenytoin	4.60	Thiothixene	1.81
Northioridazine-2-sulfoxide	5.71	Haloperidol	2.12
Thioridazine-2,5-disulfone	7.43	Fluphenazine	2.83

*nd = No detector responses.

Accuracy, precision and linearity

The accuracy and within-run precision of the present method were determined by assaying 1-ml aliquots of plasma containing either 75 or 500 ng amounts of thioridazine, northioridazine, thioridazine-2-sulfoxide, thioridazine-2-sulfone and thioridazine-5-oxide (Table V). The deviation of the amount found from the known amount added, and the coefficient of variation, were independent of concentration. Typical blank-corrected standard curves, based upon peak height ratios relative to 2-acetylphenothiazine, were linearly related to concentration with correlation coefficients for each compound being consistently greater than 0.99.

TABLE V

WITHIN-RUN PRECISION AND ACCURACY

n = 9 for both concentrations.

Drug	Concentration (ng/ml)		
	Added	Found	
		$\bar{X}$ ± S.D.	C.V. (%)
Thioridazine	75	74.0 ± 7.9	10.7
	500	512.6 ± 56.2	11.0
Northioridazine	75	72.7 ± 7.3	10.0
	500	484.5 ± 49.1	10.1
Thioridazine-2-sulfone	75	72.6 ± 6.6	9.0
	500	474.1 ± 39.7	8.4
Thioridazine-2-sulfoxide	75	75.7 ± 9.6	12.7
	500	490.9 ± 38.1	7.8
Thioridazine-5-oxide	75	68.5 ± 7.8	11.4
	500	490.4 ± 44.0	9.0

Sensitivity

The combination of low detector noise following injections of plasma extracts, high extraction efficiencies, and the high molar absorptivities (ϵ_{254}) of thioridazine and its metabolites permit their determination in human plasma at concentrations well below those reported following therapeutic doses of thioridazine. Detection limits were 10 ng/ml for thioridazine, northioridazine, thioridazine-2-sulfone and thioridazine-2-sulfoxide and 20 ng/ml for thioridazine-5-oxide, based upon an assayed plasma volume of 1 ml. Limits were defined by a minimum signal-to-noise ratio of 5 and coefficients of variation for replicate determinations (n = 8) of 15% or less. Concentrations below these values generally yielded coefficients of variation greater than 20% and these values were therefore taken as a lower practical limit of detection. The detection limit for thioridazine-5-oxide may be improved, if desired, by the use of UV absorbance detection at 280 nm, a value closer to the absorption maxima for this compound.

Patient data and summary

The present method permits the accurate quantification of thioridazine and its major metabolites at therapeutic plasma concentrations and in samples obtained for pharmacokinetic analyses. Table VI lists the concentrations of thioridazine and its metabolites in plasma from an adult male schizophrenic patient 1, 2, 4 and 24 h following an initial oral dose (200 mg) of thioridazine and 11 days later (sample obtained prior to morning dose). The present method has been in use in this laboratory for the past eighteen months and is routinely used to assay 20—25 plasma samples, plus a 6 or 7 point standard curve, in a normal working day. Furthermore, the more polar metabolites of thioridazine which are predominantly eliminated in urine [2] (e.g., thioridazine-2,5-disulfoxide, thioridazine-2,5-disulfone and northioridazine-2-sulfoxide) are readily separated and detected by this procedure (Figs. 3 and 4).

TABLE VI

PLASMA CONCENTRATIONS OF THIORIDAZINE AND ITS METABOLITES AT VARIOUS TIMES FOLLOWING THE INITIATION OF A DOSAGE REGIMEN OF 200 mg THIORIDAZINE PER DAY

Times given indicate the interval between drug administration and blood sample collection. The blood sample collected after 11 days was drawn immediately prior to the administration of the morning dose.

Time (h)	Plasma concentration (ng/ml)				
	THD*	norTHD	THD-2-SO_2	THD-2-SO	THD-5-SO
1	7	nd**	nd	nd	nd
2	258	nd	37	415	54
4	358	nd	92	875	173
24	31	nd	78	320	189
11 days	187	nd	98	438	538

*See Table I for definition of abbreviations.
**nd = Non-detectable.

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