A GLC–Nitrogen Phosphorous Detector Assay for Trifluoperazine in Plasma

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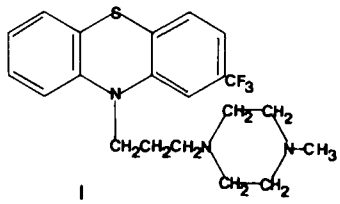
Abstract □ A GLC–nitrogen phosphorous detector (NPD) method for the quantitative determination of trifluoperazine in plasma is described. It depends on an organic extraction of trifluoperazine and the internal standard prochlorperazine from basified plasma. Following the extraction, the organic solvent is evaporated to dryness and the residue is reconstituted in a small volume of methyl alcohol. GLC analysis of aliquots of the methanolic solution using a NPD permitted the determination of 0.5 ng/ml of trifluoperazine in plasma. Standard curves for trifluoperazine over a concentration range of 0.5 to 15 ng/ml of plasma were linear with an overall variation coefficient of 5.3%. In isolated samples obtained from a normal healthy volunteer, application of this method to plasma concentration determinations after oral administration of a 5-mg trifluoperazine tablet, is demonstrated and compared with the concentrations obtained by GLC–mass spectrometry and radioimmunoassay procedures.

Keyphrases □ Trifluoperazine—GLC–nitrogen phosphorous detector analysis in human plasma □ Prochlorperazine—GLC–nitrogen phosphorous detector analysis of trifluoperazine in human plasma □ GLC–Nitrogen phosphorous detector—analysis of prochlorperazine in human plasma

Since the introduction of the major tranquilizers in the early 1950's, the phenothiazines with the piperazine ring system have been widely used. Over the past 25 years, several publications (1–5) have been concerned with the quantitation of chlorpromazine in biological fluids; however, the analysis of piperazine containing phenothiazines has been more difficult. Being therapeutically more potent, these antipsychotic drugs are administered in much smaller doses than chlorpromazine. Like chlorpromazine, these agents undergo extensive metabolism leading to numerous metabolites, several of which are pharmacologically active. These drugs are also subject to extensive first-pass (gut wall and/or hepatic) biotransformation. Other properties of these drugs include large interindividual variations in plasma levels following identical doses to volunteers or patients, extensive binding to multiple sites, large volumes of distribution, and their extreme instability in all stages of handling during analysis (4–7). Published reports indicate that the plasma concentrations of these piperazinyl phenothiazines in patients are much lower than those encountered with chlorpromazine. Thus, sensitivity requirements for these drugs are more stringent than those for the assay of chlorpromazine (8–10).

The techniques which may have the sensitivity for the quantitation of chlorpromazine and other antipsychotics include GLC–nitrogen phosphorous detector (NPD), GLC–electron capture detector (ECD), GLC–mass spectrometry (MS), HPLC–UV, HPLC–spectrofluorometric detector (SPF), and other procedures such as radioimmuno- and radioreceptor assays. For the orally administered antipsychotic agent, trifluoperazine, only the radioimmunoassay and GLC–MS methods of Midha *et al.* (11, 12) have demonstrated the necessary sensitivity to follow plasma concentration–time profiles following the



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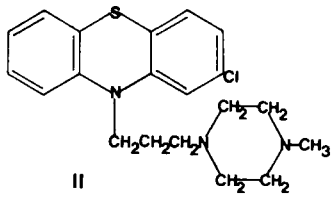

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administration of single 5-mg doses of trifluoperazine in healthy human volunteers. These procedures are capable of quantitating plasma trifluoperazine concentrations in specimens collected as late as 24 hr postadministration of trifluoperazine.

A new GLC method for trifluoperazine (I) based on an organic extraction of basified plasma and quantitation using a more conventional NPD system with prochlorperazine (II) (the 7-chloro analog of trifluoperazine) as internal standard is described. Trifluoperazine in the concentration range 0.5–15 ng/ml of plasma can be quantitated in this method with an overall variation coefficient of 5.3%.

EXPERIMENTAL

Materials—Trifluoperazine dihydrochloride[1], *N*-desmethyltrifluoperazine dimaleate[1], trifluoperazine sulfoxide[1], 7-hydroxytrifluoperazine[1], and prochlorperazine dimaleate[2] were generously donated.

Heparinized evacuated tubes[3] were commercially obtained. All solvents used were HPLC grade, and all other chemicals were commercial analytical grade, used without further purification.

The assay was done in subdued light. Standard solutions of trifluoperazine, trifluoperazine metabolites, and prochlorperazine were prepared by dilution in ethanol and/or double distilled water. Appropriate dilutions of the standard solutions were made in outdated human plasma[4].

Plasma Level Study—A normal, healthy male volunteer (57 kg) fasted overnight was given a single 5-mg commercial trifluoperazine tablet orally[5]. Blood samples (10 ml) were withdrawn from the cubital vein into heparinized evacuated tubes, centrifuged, and separated plasma was stored at −4° for a maximum period of 7 days. During blood sample collection, care was taken to avoid contact of the blood with the rubber stopper of the evacuated tube.

Extraction of Trifluoperazine—One milliliter of aqueous internal

[1] Smith Kline and French Canada, Ltd., Mississauga, Ontario, Canada and Dr. A. A. Manian, National Institute of Mental Health, Rockville, Md.
[2] Rhône-Poulenc Pharma Inc., Montreal, Quebec, Canada.
[3] Venoject, Kimble-Terumo, Inc., Elkton, Md.
[4] Canadian Red Cross.
[5] Stelazine, Smith Kline and French Laboratories, Philadelphia, Pa.

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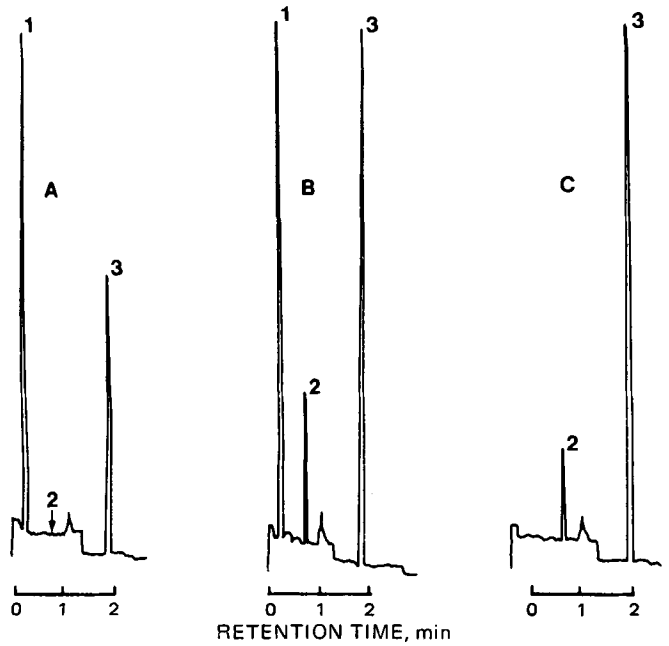

Figure 1—*GLC–NPD chromatogram of: (A), extracted blank human plasma; (B), extracted human plasma spiked with 2.5 ng/ml of trifluoperazine; (C), extracted plasma obtained from human volunteer 2.0 hr following the administration of 5 mg of trifluoperazine. (1, caffeine; 2, trifluoperazine; 3, prochlorperazine.)*

standard solution of prochlorperazine (100 ng/ml) and 0.5 ml of 10 *N* NaOH were added to a 2-ml plasma sample (spiked or from dosed volunteer) in a 10-ml polytef-lined screw-capped test tube. The sample was gently mixed[6] and 5 ml of *n*-pentane–isopropyl alcohol (50:1) was added. The tube was tightly capped and the drug and the internal standard were extracted from plasma by first mixing[7] for 15 min and then centrifuging[8] for 15 min at room temperature at 1720×*g*. Following centrifugation, ~4 ml of the upper organic layer was transferred with the aid of a disposable Pasteur pipet to a fresh tube, taking extreme care not to contact the organic–aqueous interface. The organic extract was evaporated[9] almost to dryness at 55°. The sides of the tube were washed down with ~1 ml of the extracting solvent and the contents re-evaporated[9] to dryness. The residue was then reconstituted in the same tube with 25 μl of methyl alcohol by mixing[6] and aliquots of 2–4 μl were injected into the gas chromatograph.

GLC—The gas chromatograph[10] was equipped with a nitrogen phosphorous detector. The column was a coiled glass tube[11], 1.8 m long × 2-mm i.d., packed with 3% phenyl methyl silicon[12] coated on acid-washed, dimethylchlorosilane-treated 100–120 mesh high-performance, flux-calcined diatomite support[13].

The column was conditioned at 335° for 12–24 hr with a low helium flow. For plasma analysis, the injection port, detector block, and column oven temperatures were 300, 300, and 310°, respectively. Helium was used as a carrier gas at a flow rate of 30 ml/min. Hydrogen and air flow rates and NPD bead voltages were optimized for maximum response at electrometer settings of 3 or 4.

Calculations—Peak height ratios were calculated by dividing the height of the peak due to trifluoperazine (0.89 min) by that of prochlorperazine, the internal standard (1.90 min). Calibration curves were constructed by plotting the spiked control plasma peak height ratios against the concentrations of trifluoperazine (nanograms per milliliter of plasma).

RESULTS AND DISCUSSION

Under the chromatographic conditions described previously, trifluoperazine and the internal standard prochlorperazine gave sharp and symmetrical peaks with retention times of 0.89 and 1.90 min, respectively. Figure 1A shows a typical chromatogram obtained by processing control blank plasma which contained 50 ng/ml of the internal standard, prochlorperazine. No extraneous peak at the retention time of trifluoperazine was observed. A chromatogram obtained when the method was applied to spiked human plasma containing 2.5 ng/ml of trifluoperazine and 50 ng/ml of the internal standard is shown in Fig. 1B. In the blank and spiked plasma (Fig. 1A, 1B) a sharp peak which had an identical retention time to caffeine was obtained. Caffeine was extracted in the procedure used, and because of the presence of four nitrogen atoms it gave a good response to the NPD.

Figure 1C shows a chromatogram of the plasma sample (2 ml) from blood withdrawn from a male volunteer (57 kg) at 2.0 hr after oral administration of a 5-mg commercial tablet. This sample was estimated to contain 1.45 ng/ml of trifluoperazine. There was no peak due to caffeine, since the subject was asked to withdraw from caffeine-containing beverages and food. A chromatographic analysis time of ~2 min was required for each sample.

The use of a mixed organic solvent system of *n*-pentane and isopropyl alcohol provided quantitative extraction of the drug and the internal standard in one step from basified plasma. The overall recoveries of trifluoperazine and prochlorperazine were of the order of 102.3 ± 3.6 and 90.7 ● 2.8%, respectively, as reported earlier (12). The use of isopropyl alcohol not only provides the necessary polarity needed for the quantitative extraction of the drug and the internal standard, it also retards the adsorption of these basic drugs onto the glass surface during extraction. It was also found useful to keep the concentration of the internal standard high, as the prochlorperazine competes with the drug for the adsorption sites on the glassware used for extraction. This type of observation has been made earlier with the structurally similar drug chlorpromazine (13), where it was found necessary to use very high concentrations of the internal standard mesoridazine.

The important metabolites of trifluoperazine, which are extracted under the conditions described previously, did not interfere in this procedure. These metabolites elute with retention times different from that of the drug and the internal standard. Figure 2 shows the chromatographic separation of these metabolites, *i.e.*, *N*-desmethyltrifluoperazine, 7-hydroxytrifluoperazine, and trifluoperazine sulfoxide, from trifluoperazine and the internal standard prochlorperazine. Trifluoperazine sulfone also did not interfere, as it was found to elute after the trifluoperazine sulfoxide.

After repeated injections into the gas chromatograph, an uneven response from the NPD was observed, which led to reduced sensitivity as well as the ratios of peak heights of the drug and the internal standard becoming irreproducible. This generally required readjustment of the NPD bead voltage or installation of a new bead.

An interesting problem of interference with respect to trifluoperazine was observed in the present GLC–NPD assay. If the plasma samples were prepared from blood collected in the heparinized brand of evacuated tubes[3], an interfering peak with nearly the same retention time as trifluoperazine was observed in the chromatograms. The interference, often observed as a double peak was small but significant. This was overcome by employing a different brand of evacuated tubes[14]. Some additional experimentation revealed that the interfering material was alkaline and aqueous soluble; however, positive chemical identification attempts by mass spectrometry were not successful. It should be noted that although the plasticizer effect (14) with trifluoperazine is not as significant as with other phenothiazines, care must be exercised during the collection of blood by evacuated tubes to see that the blood does not come in contact with the rubber stopper.

Table I—GLC–NPD Estimation of Trifluoperazine Added to Plasma[a]

Plasma Trifluoperazine, ng/ml	*n*	Average Peak/Height Ratio[b]	Coefficient of Variation, %
0.5	6	0.062	13.9
1.0	6	0.120	5.9
2.5	6	0.246	5.5
5.0	6	0.482	5.2
7.5	6	0.744	7.0
10.0	6	1.021	4.1
15.0	6	1.512	2.7

[a] Mean coefficient of variation = 5.3%, slope = 0.1003x ± 0.0035, and correlation = 0.9992. [b] Trifluoperazine–Prochlorperazine.

[6] Vortex Genie, Fisher Scientific Co., Edmonton, Alberta, Canada.
[7] Evapo-Mix, Fisher Scientific Co., Edmonton, Alberta, Canada.
[8] T-J6 Centrifuge, Beckman Instruments, Toronto, Ontario, Canada.
[9] Thermolyne Dri-Bath, Fisher Scientific Co., Edmonton, Alberta, Canada.
[10] Model 5840, Hewlett-Packard Canada Ltd., Edmonton, Alberta, Canada.
[11] Chromatographic Specialties, Brockville, Ontario, Canada.
[12] OV-17, Chromatographic Specialties, Brockville, Ontario, Canada.
[13] Gas Chrom Q, Chromatographic Specialties, Brockville, Ontario, Canada.
[14] Vacutainer, Becton Dickinson Co., Mississauga, Ontario, Canada.

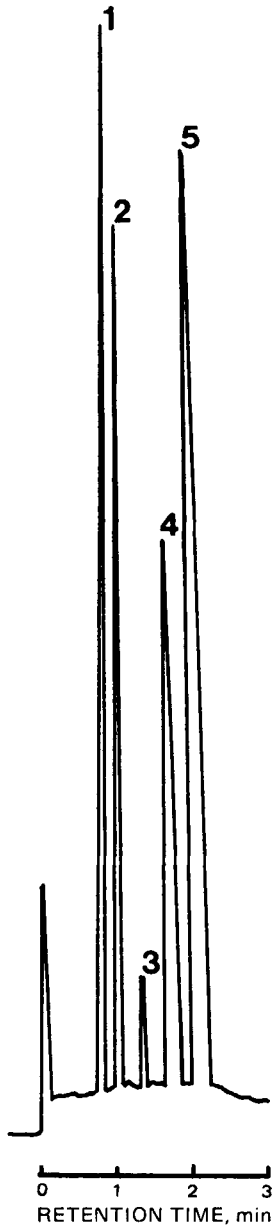

Figure 2—*GLC-NPD separation of trifluoperazine(1), N-desmethyltrifluoperazine (2), 7-hydroxytrifluoperazine (3), prochlorperazine (4), and trifluoperazine sulfoxide (5); OV-17 injector, 300°; oven, 310°; detector, 300°. Approximately 50 ng of each component on-column using nitrogen–phosphorous detection.*

The accuracy and precision of the GLC–NPD assay are demonstrated in Table I. Results are based on six determinations of each concentration of trifluoperazine, ranging from 0.5 to 15 ng/ml of plasma. The overall variation coefficient was 5.3%. The calibration curve obtained by plotting the peak height ratio of the trifluoperazine and prochlorperazine *versus* the concentration of trifluoperazine was linear over the 0.5–15.0-ng range of the drug/ml of plasma. A mean slope value of $0.1003x + 0.0035$ ($r^2 = 0.9996$) was obtained.

The application of the present method to plasma concentration determinations is shown in Table II, where the values obtained by the present method are given along with those obtained by GLC–MS and radioimmunoassay procedures. Because of the sensitivity limitations of the GLC–NPD assay, only concentrations around the peak times are shown from one volunteer. The GLC–NPD values compare well with the GLC–MS method; however, the radioimmunoassay gives values that are slightly higher than those obtained with the chemical methods. This is probably due to the cross-reactivity of the antiserum to the 7-hydroxy and *N*-desmethyl metabolites of trifluoperazine (11).

Table II—Comparison of Plasma Trifluoperazine Concentrations Determined by Three Different Methods

Subject Plasma Sample, hr	Trifluoperazine Plasma Concentration		
	GLC-NPD, ng/ml	Radioimmunoassay, ng/ml	GLC–MS, ng/ml
2.0	0.88	1.00	0.80
3.0	0.91	1.09	0.93
4.5	0.73	1.13	0.71

In conclusion, the described procedure is simple and specific for trifluoperazine. It may not have the sensitivity for single-dose pharmacokinetics and bioavailability studies for the drug, however, steady-state monitoring can be easily carried out. This procedure can also be easily adopted for the quantitative determination of prochlorperazine by using trifluoperazine as the internal standard. The other phenothiazine drugs which have been tested for plasma extraction and sensitivity are promazine, chlorpromazine, butaperazine, and promethazine. Thus, the described procedure has the potential for its applicability in the quantitative analysis of these drugs.

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