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SENSITIVE GAS CHROMATOGRAPHIC DETERMINATION OF TRIFLUOPERAZINE IN HUMAN PLASMA

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SUMMARY

Plasma trifluoperazine levels of patients taking a single 20-mg dose of trifluoperazine were measured by a sensitive and linear method. The low detection limit of 0.1 ng/ml plasma was obtained through use of a highly sensitive nitrogen—phosphorus detector combined with an efficient extraction method. Recovery of trifluoperazine added to human plasma was 96%. Data are presented on the stability of trifluoperazine in refrigerated human plasma.

INTRODUCTION

Trifluoperazine (TFP) and other phenothiazines are widely used in psychiatric therapy, and are usually given on a long-term basis. Not infrequently, do patients fail to improve when administered "therapeutic" doses of a phenothiazine. A probable cause for inadequate improvement in non-responding patients is the failure to obtain an effective plasma concentration of the drug. Indeed, for patients on chlorpromazine therapy, data suggest that a minimum plasma concentration is necessary for clinical improvement, and that an excessively high value is related to toxicity [1].

Trifluoperazine which is about 80% removed from rat hepatic venous blood by the liver [2] would appear to be a logical candidate for therapeutic monitoring. There appears to be a paucity of methods published for quantification of TFP in human plasma. This is probably due to the small recommended adult

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doses of 2—20 mg daily [3], and the likely small plasma concentrations which would be difficult to measure.

A fluorimetric procedure has been used to quantitate TFP and its sulfoxide (TFP-SO) in human urine [4]. While the analysis was relatively rapid, it was necessary to selectively extract the sulfoxide away from the parent drug, and then to convert the parent to the sulfoxide before determinations could be made. With a detection limit in the vicinity of 0.5 μg/10 ml it would not be sufficiently sensitive for human plasma samples.

A thin-layer chromatography—ultraviolet reflectance photometry method was used to quantitate TFP and metabolites in rat tissues [5], but it also is too insensitive for human samples. West and Vogel [6] measured tritiated TFP and TFP-SO in rat plasma and tissues, but this procedure is not routinely applicable with human subjects.

Other phenothiazines have been analyzed at sensitivities of 1 part per billion or less by gas chromatography (GC) with electron-capture detection [7, 8] or nitrogen—phosphorus detection [9], or by high-performance liquid chromatography with electrochemical detection [10]. These methods, while sensitive, either recovered only 50—70% of added drug or were too involved for routine use. Chlorpromazine has been measured by radioimmunoassay [11], but reagents for TFP analysis by this method are not commercially available.

EXPERIMENTAL

Materials

Trifluoperazine HCl and chlorpromazine HCl were obtained from Smith, Kline & French Labs. (Philadelphia, PA, U.S.A.). Kimax 12-ml conical tubes and 15-ml screw-top culture tubes (Owens-Illinois, Toledo, OH, U.S.A.) were silanized prior to use to avoid drug adsorption to glass by rinsing them with a 5% solution of dimethyldichlorosilane (Sigma, St. Louis, MO, U.S.A.) in toluene. Four liters of hexane (Matheson, Coleman & Bell, Norwood, OH, U.S.A.) were passed through a 85 × 3.0 cm glass column filled with Matheson, Coleman & Bell grade 12 silica gel, 28—200 mesh, prior to use to remove chromatographic interferences. Extraction solvent was prepared by mixing 19 volumes hexane with 1 volume U.S.P. alcohol (U.S. Industrial Chemicals, New York, NY, U.S.A.).

Gas chromatography

A Hewlett-Packard 5710A gas chromatograph equipped with a specific nitrogen—phosphorus detector was used for analyses. Separation was attained with a 2 m × 2 mm I.D. silanized glass column packed with 3% OV-17 on Gas-Chrom Q, 80—100 mesh. The injector port and detector were maintained at 300°C, and the column at 260°C. Flow-rates were 30 ml/min for the helium carrier gas, 50 ml/min for air, and 4 ml/min for hydrogen. Electrometer range and attenuation were set at 1 and 8, respectively, and collector voltage was adjusted between 13 and 15 V to obtain adequate sensitivity.

Standards

A 4 ng/ml assay standard was prepared by adding 0.1 ml of a 1 ng TFP per

μl ethanol solution to a 25-ml volumetric flask, adding 0.5 ml distilled water, filling with human plasma, and mixing thoroughly. Assay standards of 2.0 and 0.5 ng/ml were prepared by diluting portions of the 4 ng/ml standard with human plasma.

Analytical procedure

To 2 ml of standards or samples contained in culture tubes were added 0.5 g sodium chloride, 0.5 ml 2.0 *N* sodium hydroxide, 5 ml extraction solvent and 4 ng chlorpromazine (as the internal standard). The tubes were sealed with PTFE-lined caps and mixed for 5 min on a mechanical shaker. After brief centrifugation, the upper hexane phases were transferred to extraction tubes containing 5 ml 0.1 *N* hydrochloric acid. The tubes were then shaken 5 min, centrifuged, and the hexane separated and discarded. After adjusting the pH to 13 with 2.0 *N* sodium hydroxide, the drugs were extracted into 5 ml extraction solvent. Following centrifugation, the hexane was transferred to 12-ml conical tubes and then evaporated to dryness at 40°C with a stream of nitrogen. To remove drugs from the sides of the tubes, 0.2 ml ethanol was added, vortexed briefly, and then evaporated. The extracts were reconstituted with 30 μl ethanol, and 8 μl were injected on the GC column.

Human samples

Four patients were given a single oral dose of 20 mg TFP (Stelazine^{R}, Smith Kline & French). Blood was drawn into heparinized plastic syringes immediately prior to drug administration, and at several time intervals afterwards. Plasma was separated and stored frozen for seven months until analysis. Assay standards of 0, 0.5, 2.0, and 4.0 ng/ml were routinely processed with patient samples according to the procedure described. Linear regression analysis of peak height ratio versus concentration of standards was performed, and TFP concentrations of patient samples were determined from peak height ratios, and the equation for the standard curve.

TFP recovery

To determine recovery of TFP added to drug-free plasma, TFP standards of 0.2 and 3.0 ng/ml plasma were prepared. These were extracted in the usual manner except that no internal standard was used. Chlorpromazine was added to the final extracts as an external standard prior to evaporation. Non-extracted standards corresponding to 100% recovery were prepared, and the extracted standards were compared to these.

Pharmacokinetic analysis

The concentration of TFP in plasma was fitted according to the equation $C = A(e^{-\beta t} - e^{-\alpha t})$, where C is the plasma concentration at time t, A is a constant, α is the apparent absorption rate constant and β is the terminal elimination rate constant. The terminal half-life of TFP in the plasma was calculated according to the equation $t_{1/2}^{\beta} = 0.693/\beta$.

RESULTS AND DISCUSSION

Linearity and sensitivity

The linearity of the method was demonstrated by correlation coefficients of peak height ratio versus TFP concentration of 0.99 or greater. Sensitivity (defined as a signal-to-noise ratio greater than 2) was 0.1 ng TFP per ml plasma when a 2-ml sample was used.

Recovery

The data in Table I illustrate the high efficiency of the extraction by the nearly complete recovery (96%) in the final extract of TFP added to plasma. Efficiency of the extraction was markedly enhanced by addition of sodium chloride to plasma prior to extraction. When the sodium chloride was omitted, recovery of TFP added to human plasma was only 60%. Addition of salt apparently decreases protein binding of TFP, and thus it is not necessary to add salt when extracting TFP from non-proteinaceous samples. Indeed, spectrophotometric measurements showed that more than 98% of TFP in an aqueous alkaline solution was removed by extraction with an equal volume of hexane.

TABLE I

RECOVERY OF TRIFLUOPERAZINE ADDED TO HUMAN PLASMA

No.	Trifluoperazine concentration (ng/ml)	Recovery (%)	Mean recovery (%)
1	3.0	99.9	
2	3.0	98.4	
3	3.0	91.4	96.6
4	0.2	95.9	
5	0.2	94.6	
6	0.2	97.7	96.1

Precision and accuracy

Precision and accuracy of the method were determined by preparing TFP plasma standards of several concentrations for triplicate analysis. The results presented in Table II show that the overall accuracy of the procedure was quite good. As would be expected, relative variation of the measurement increases as plasma TFP decreases.

TABLE II

PRECISION AND ACCURACY OF DETERMINATION OF TRIFLUOPERAZINE ADDED TO HUMAN PLASMA

Actual value (ng/ml)	Observed value (ng/ml)	$\overline{X}$	Coefficient of variation (%)
0.2	0.14, 0.25, 0.24	0.21	29.0
0.6	0.49, 0.49, 0.63	0.54	15.1
1.8	1.72, 1.96, 1.66	1.78	8.9

Selectivity

Evidence for the selectivity of the method was furnished by characteristic GC retention times of the reference compounds and the lack of interfering peaks in plasma extracts from subjects not treated with TFP (Fig. 1). Approximate retention times for chlorpromazine and trifluoperazine under the described conditions were 4.4 and 6.0 min, respectively.

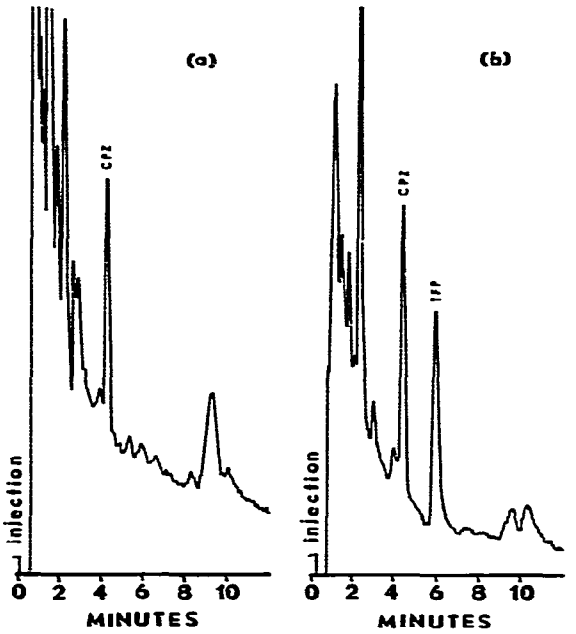

Fig. 1. Chromatograms of trifluoperazine (TFP) and the internal standard chlorpromazine (CPZ) after extraction from plasma. (a) Patient did not receive TFP; (b) patient treated with 20 mg Stelazine^{R} orally.

Patient use of chlorpromazine will interfere with accurate quantitation of TFP. Therefore, it is necessary to ascertain that the patient has not been using chlorpromazine. If this cannot be confirmed, a different internal standard must be selected.

Drugs that might interfere in the assay were tested (Table III). Chlordiazepoxide is not extracted from organic solvents by 0.1 *N* hydrochloric acid, and so would not interfere even though it is not completely resolved from TFP. If diazepam is present, an alternative internal standard should be selected due to its inadequate resolution from chlorpromazine. The other drugs listed do not interfere in the analysis.

Additionally, fluphenazine and perphenazine should not interfere in the assay. Due to their unshielded, highly polar hydroxyl groups, they are strongly adsorbed on the GC column and give very poor response unless derivatized [9, 12].

Even though none of the metabolites of TFP were chromatographed, their interference in this assay does not seem likely. TFP-SO produced by both rats

TABLE III

RETENTION TIMES FOR SOME PSYCHOACTIVE DRUGS RELATIVE TO THAT OF TRIFLUOPERAZINE

Drug	Relative retention time
Trifluoperazine	1.00
Amitriptyline	0.31
Imipramine	0.34
Promazine	0.47
Chlorpromazine	0.74
Diazepam	0.81
Chlordiazepoxide	1.13
Prochlorperazine	2.75
Haloperidol	2.83

[5, 6] and man [4], and 7-hydroxytrifluoperazine produced by rats [2, 5] have higher molecular weights and are more polar than TFP, and so would be expected to elute later than TFP, if present. A mixed function amine oxidase isolated from hog liver microsomes oxidizes the terminal nitrogen atom of the piperazine moiety of TFP, forming the N-oxide [13]. If this metabolite is formed in humans, it is unlikely to interfere in the assay because its higher molecular weight would cause it to elute after the parent drug.

If desmethyltrifluoperazine, and several other dealkylated primary or secondary amine metabolites produced by rats [5] are found in man, they are likely to elute after TFP due to their more polar nature. Also, hydroxylated metabolites would be expected to give very poor chromatographic response [12].

Stability

The stability of TFP in refrigerated (9°C) plasma was tested. A plasma sample containing 1.80 ng TFP per ml was prepared. Portions of this were tested at various intervals after preparation. Data are presented in Table IV. TFP appears to be sufficiently stable in refrigerated human plasma to allow storage in that condition for several days prior to analysis.

TABLE IV

TRIFLUOPERAZINE STABILITY IN REFRIGERATED PLASMA

A plasma sample containing 1.80 ng trifluoperazine per ml was prepared and stored refrigerated. Trifluoperazine concentration was measured at various intervals after preparation.

Days after preparation	Observed value (ng/ml)
0	1.84
4	1.84
7	1.72
10	1.71
14	1.46
16	1.87

CLINICAL APPLICATIONS

Fig. 2 shows the TFP absorption and disposition profile of a subject given a single 20-mg dose of Stelazine®. Additional data presented in Table V on this and other subjects show large variations in peak TFP plasma concentrations and half-lives. These large variations underscore that the monitoring of individual patient TFP levels may improve the desired pharmacological action of TFP and related compounds.

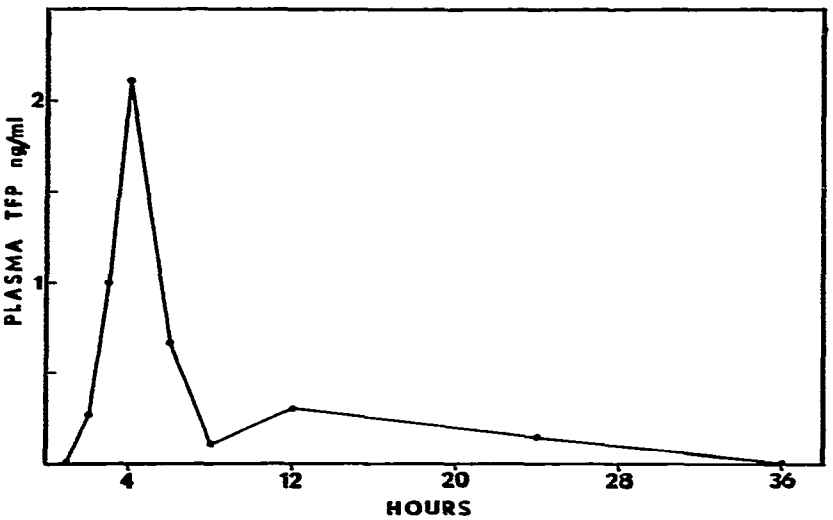

Fig. 2. Representative time course of plasma trifluoperazine (TFP) concentration following oral administration of 20 mg Stelazine® to subject 1.

TABLE V

TFP PEAK PLASMA LEVELS AND HALF-LIVES OF PATIENTS GIVEN 20 mg STELAZINE ORALLY

Numbers in parentheses indicate hour of peak level.

Subject	Peak level		$t^{*}_{½}$
	ng/ml	h	
1	2.12	(4)	8.08
2	0.93	(6)	17.90
3	1.24	(4)	10.88
4	3.97	(3)	7.16

*$t_{½}$ estimated from the β elimination constant which was determined as described in Methods.

The sensitivity of the analysis should permit investigation of the possible relationship between plasma TFP levels and clinical effects. It might also allow one to determine patient compliance. Additionally, the procedure would be useful in comparing bioavailability of different TFP preparations.

It should be noted that when collecting samples for TFP analysis, the blood

should not come into contact with rubber stoppers since plasticizer in some rubber-stoppered blood collection tubes can cause displacement of drug into the red blood cells [11].

CONCLUSIONS

The method described for trifluoperazine quantitation is suitable for measurement of patient plasma levels after a single large dose. It should also be useful for determining levels following administration of multiple lower doses.

Sensitivity in the sub part per billion range was obtained through the use of an extremely sensitive nitrogen—phosphorus detector combined with a highly efficient extraction technique. Specificity was obtained through selective extraction, the selectivity of the nitrogen—phosphorus detector, and chromatographic resolution.

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