ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1639–1644

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

# Photostability-indicating HPLC method for determination of trifluoperazine in bulk form and pharmaceutical formulations

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Received for review 25 September 1995; revised manuscript received 8 January 1996

*Keywords:* Determination; HPLC; Photochemistry; Stability-indication; Tablets; Trifluoperazine

## 1. Introduction

Phenothiazines may cause photosensitization and their photodecay may occur by a free-radical chain process, i.e. auto-oxidation, and/or by involving excited singlet molecular oxygen, i.e. oxygenation [1–4]. Trifluoperazine, [10(3-(4-methyl-1-piperazinyl)-propyl)-2-(trifluoromethyl)-10*H*-phenothiazine], is an antipsychotic tricyclic antidepressant commonly prescribed for treating psychiatric disorders and core symptoms of schizophrenia. Such a drug is capable of photosensitization by both types of mechanism [5]. In-vivo photosensitivity of the phenothiazines indicates that both photoallergy and phototoxicity reactions are mainly due to the decay products formed [3,6]. Early investigations on photo-oxidation of phenothiazines indicated the formation of the corresponding sulfoxides [7]. A number of other photodecomposition products, viz. N-oxides, hydroxy derivatives, dimeric or polymeric products, and excimers, in addition to the sulfoxides and sulfones, of various phenothiazines were isolated and characterized [8–10]. The molecular characteristics/phototoxicity relationship of phenothiazines demonstrated that the tricyclic moiety is essential for phototoxic activity. The importance of phenothiazines has prompted many researchers to establish methods for their identification and quantification. A variety of procedures are based on the oxidation of the drugs, which is usually done chemically [11–19] or photochemically [20–23], followed by manual or automated measurement of the oxidized and unoxidized drug forms.

The versatility of liquid chromatography, especially HPLC, in drug assays is well known. Some HPLC procedures have been described for determination of trifluoperazines singly or with other phenothiazines [24–27]. No studies to determine the stability-indicating characteristics of the described HPLC methods have been reported.

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*PII* S0731-7085(96)01778-5

The main objectives of the present studies were to investigate the photochemical stability of trifluoperazine hydrochloride, in its aqueous solutions and tablets, and to develop a stability-indicating HPLC procedure for selective quantification of the drug in the presence of its all photodegradation products.

## 2. Experimental

### 2.1. Materials

Reference trifluoperazine· 2HCl, assigned purity 100.8% (Smith Kline & French, Pennsylvania, PA,), BN/0492-81110TPP, was utilized without further treatment. Trifluoperazine sulfoxide was prepared by heating 100 mg of the drug (base) with 10 ml of 15% $H_20_2$ and 0.2 ml glacial acetic acid on a water bath at 60°C for 30 min. The solvent was evaporated under vacuum and trifluoperazine sulfoxide was then recrystallized from ethanol. All steps were done with protection from light. $C_{21}H_{24}F_3N_3OS$ (MW = 423.5): calcd. (%): C, 59.50; H, 5.67; N, 9.92; S, 7.56; found (%): C, 59.11; H, 5.72; N, 10.10; S, 7.45. Trifluoperazine-containing preparation: Stelazine™ 5 mg compressed tablets were kindly supplied by the Security Forces Hospital, Riyadh, Saudi Arabia. The tablets, as well as the drug materials, were always protected from light. HiPerSolv™ acetonitrile was obtained from BDH (Poole, UK). Distilled water was bidistilled in an all-glass distillator for all analytical purposes. Orthophosphoric acid 85%, was obtained from E. Merck (Darmstadt, Germany).

### 2.2. Instruments

A chromato UVE light cabinet, Model CC60, with a 60 W lamp model Assy 00-60-SL. (UVP Inc., San Gabrile, CA), was utilized for drug photodegradation. **Warning: avoid long exposure of eyes and skin to direct UV radiation**. A Shimadzu LC-10 AD liquid chromatograph attached to a SPD-10A tunable UV detector, a CTO-10A column oven controller, a DGU-3A mechanical degasser, and a C-R4A Chromatopac data unit (Shimadzu Corp., Anal. Instr. Div., Kyoto, Japan) were used. A fixed loop injector (Rheodyne, 20 $\mu$l) was utilized to carry the samples onto the column (stainless prepacked $\mu$-Bondapak™ C18 (10 $\mu$m, 30 cm × 3.9 mm diameter), Waters Assoc., Bedford, MA).

### 2.3. Procedures

#### 2.3.1. Drug illumination

Solutions of trifluoperazine hydrochloride in water (10 mg $ml^{-1}$) were irradiated by exposure to UV light (254 nm) in an open container (circular 13.5 cm diameter × 7 cm height, Pyrex™ heat-resistant vessel) at a small-angle ( $\approx 50°$) and a 14 cm exposure distance. Irradiaiton was carried out for different time intervals, while protecting a part of the solution from the light source, which was considered to be the blank. Long periods of irradiation were interrupted by short dark phases to extend the lamp life. An aqueous drug solution (75 $\mu$g $ml^{-1}$) in a closed 1 cm quartz cell was also irradiated at a 20 cm distance and at the same small angle. The spectrum (200–800 nm) of this irradiated solution was recorded against water as a blank at varying time periods. The non-irradiated drug solution was considered to be the control. Photostability studies have also been carried out by subjecting the reference drug substance as well as its powdered tablets, in a watch glass, to normal laboratory (fluorescent and daylight) illumination for 5 days and stressed irradiation with 60 W short UV light at a 10 cm distance for 2 h.

Examination of the components of drug degradation was done on thin layers of silica gel $HF_{254}$ using chloroform–methanol–acetic acid (65:20:1, v/v/v) as the developing solvent. The developed plates were visualized by spraying with 1% aqueous perchloric acid followed by drying with hot air.

The photodegradation products could be separated on laboratory prepared preparative silica plates (2 mm × 20 cm × 20 cm) using the same developing solvent. The developed bands were scraped off the plates, eluted from the silica with acetone, the solvent was evaporated to dryness under vacuum in an atmosphere of pure nitrogen, and the different fractions were analyzed by mass spectrometry (50–300°C, 70 eV).

*2.3.2. Quantification of trifluoperazine in tablets*

All the analytical manipulations were carried out while protecting the reference drug substance, drug solutions and extracts of tablets from direct light the whole time.

*2.3.3. Standard solutions and graphs*

The stock solution of trifluoperazine, 200 $\mu$g ml$^{-1}$, in the mobile phase was diluted to 50 $\mu$g ml$^{-1}$ as working solution. To prepare the standard curve, serial dilutions containing 5–25 $\mu$g ml$^{-1}$ of the drug in the mobile phase were prepared by diluting the working solution. Triplicate injections of each dilution were made and the curve of concentration vs. detector response ($A_{254\ nm}$) was plotted. The consistency of the slope of the prepared standard graphs was checked on different days.

*2.3.4. Drug analysis*

At least 20 tablets were weighed to find the average weight of a tablet. An aliquot of the powdered tablets, claimed to contain 25 mg trifluorperazine, was added to $\approx$ 50 ml of the mobile phase. Extraction was performed by mechanical shaking for 10 min. The filtered drug extract was added to a re-extraction made with another 25 ml of the mobile phase. The combined extract was transferred to a 100 ml calibrated flask before completing the volume with the mobile phase. Separate 1 ml portions of the filtered extract were diluted to give a final concentration of 25 $\mu$g ml$^{-1}$. Replicate injections of each solution were made; the drug content was determined either by referring to the prepared calibration curve or by sample/equivalent standard direct matching.

## 3. Results and discussion

Accelerated drug photolysis has been carried out by irradiating the aqueous solution (10 mg ml$^{-1}$) of the drug, utilizing a 60 W UV lamp (254 nm) at a specified distance in an open glass container. The color of the irradiated solutions gradually became yellowish red to reddish brown. The extent of coloration depends directly on the time of exposure to light. Fig. 1 shows the thin layer chromatographic (TLC) fractionation of a photodegraded sample of trifluoperazine hydrochloride. In addition to the reported photodegradation products of trifluoperazine, a new photodegradate (3-trifluoromethyl(biphenylthiophen)sulfoxide) was characterized only by mass spectrometry, because of its small yield.

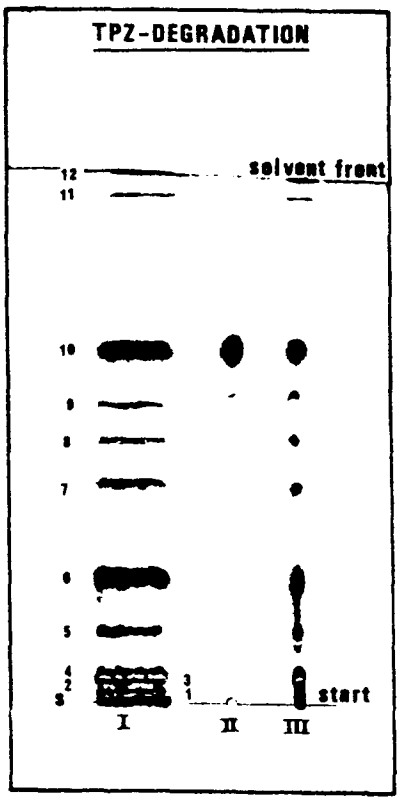
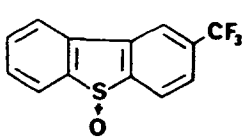



Fig. 1. TLC fractionation of photodegraded trifluoperazine hydrochloride spotted as a band (I) or a spot (III) and compared with fresh drug sample (II).




$C_{13}H_7F_3OS$ (MW = 268): *m/z* [fragment, %]: 269 [(M + 1), 9]; 268 [$M^+$, 31]; 267 [(M − 1), 46]; 248 [(M − HF), 19]; 222 [$C_{13}H_9F_3$, 11]; 203 [($C_9H_5F_3S$ + 1), 8]; 154 [$C_{12}H_{10}$, 9]; 133.5 [$C_8H_6S$, 20]; 108 [$C_4H_5F_3$, 15]; 95 [($C_3H_3F_3$ − 1), 12]; 69

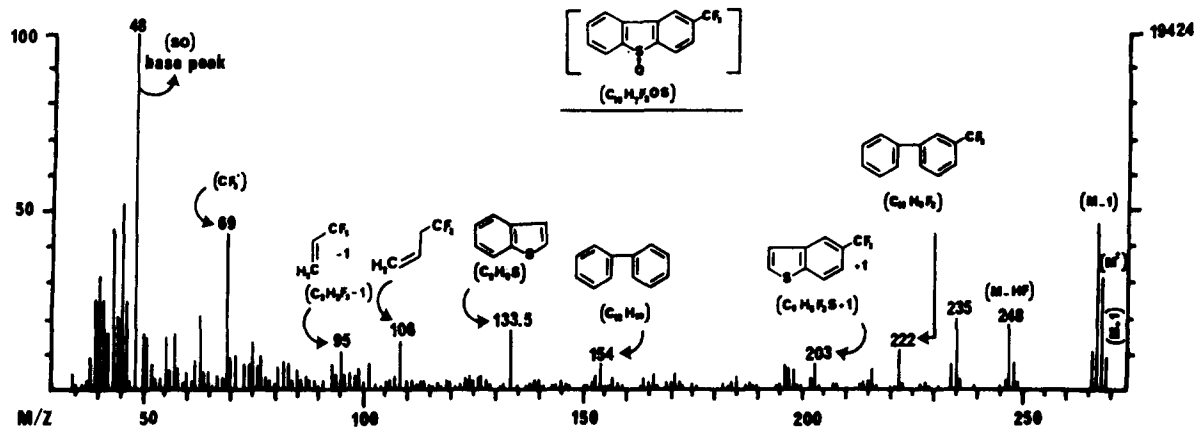



Fig. 2. Mass spectrum of the photodegradate 3-trifluoromethyl(biphenylthiophen)-sulfoxide.

[$CF_3^{\cdot}$, 45]; 48 [SO, 100 (base peak)]. Fig. 2 demonstrates the mass spectrum of the new photodegradate.

The drug stability study was completed by subjecting the drug in the solid state, i.e. bulk form and powdered tablets, to stress conditions via irradiation with short UV light (60 W), fluorescent light and daylight. The same degradation products could be identified but in relatively appreciable amounts in the case of the short UV radiation. The spectrum (200–800 nm) was monitored during irradiaiton of aqueous solutions of trifluoperazine hydrochloride. Fig. 3 illustrates the spectral changes in the UV region (200–400 nm) which occurred after a short period of illumination. The light absorption by the drug at $\approx 256$ nm ($\lambda_{max}$) decreased progressively with the time of light exposure. This decrease in absorption at the maximum absorption of the drug was accompanied by increases in the absorption in other regions of the drug spectrum. The increase in the light absorption in the visible region at $\approx 523$ nm caused a red coloration, which became more intense on prolonging the period of exposure to the light source. The principal photodegradation products of the phenothiazines are reported to be the corresponding sulfoxides [9,12,28].

Forrest et al. [9] proved the existence of a sunlamp-induced free radical of chlorpromazine (CPZ), distinct from the reported red radical $CPZ^{\cdot +}$ [29–31]. The observed red color in the case of trifluoperazine (TPZ) can be attributed to a similar stable red radical $TPZ^{\cdot +}$ which decomposes on heating. The distribution of $\pi$ electrons in the trifluoperazine ($TPZ^{\cdot\cdot}$) molecule according to its electronic topography may lead to the formation of many radical forms, such as at the S atom, at the $N_{10}$ atom, and between the S and N

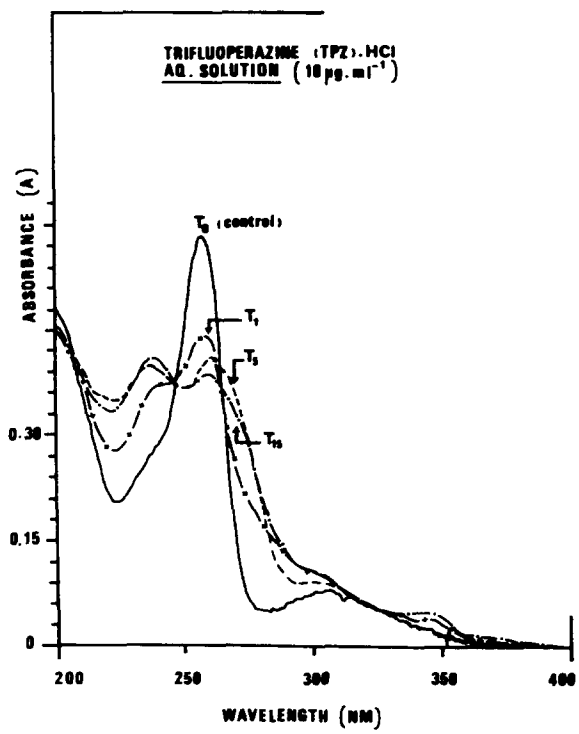



Fig. 3. UV (200–400 nm) scan of aqueous solutions of fresh and photoirradiated trifluorperazine samples.

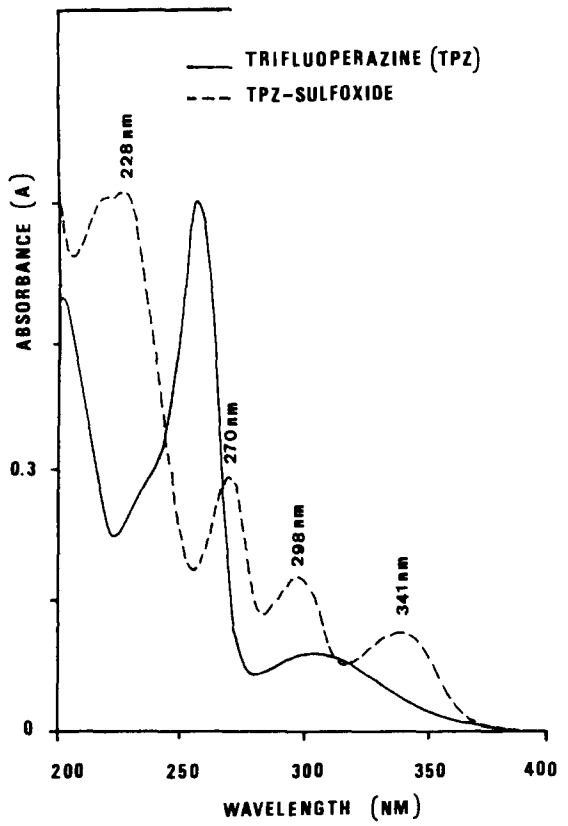



Fig. 4. UV (200–400 nm) scan of aqueous solutions of trifluoperazine and its synthesized sulfoxide.

atoms on the phenothiazine ring, as the result of resonances. The formation of a $TPZS^{\cdot +}$ radical at the S atom is expected under the influence of short-lasting UV irradiation. The trifluoperazine sulfoxide (TPZSO) may be formed by decomposition of the corresponding dimer TPZSO–OSTPZ, generating clear luminescence.

Trifluoperazine sulfoxide was synthesized by acid oxidation and was utilized for matching the spectra of the non-irradiated and irradiated drug solutions. Fig. 4 shows the UV spectra of fresh trifluoperazine and its synthesized sulfoxide. It is clear that the newly developed absorption bands from the irradiated trifluoperazine occur in the same regions as those of the sulfoxide.

Photochemical studies were planned to assess the revalidation of the stationary phase/mobile phase matches of the dscribed HPLC methods [32–35] for the photostability-indicating analysis of the named drug in bulk forms and in dosage formulations. No significant resolution of the unchanged trifluoperazine from its photodegradation products could be achieved. Attempts were therefore made to optimize the chromatographic conditions. Isocratic elution (1 ml $min^{-1}$) with a mobile phase containing acetonitrile and 0.1% aqueous orthophosphoric acid, 57.5:42.5 (v/v), on a reverse-phase $\mu$-Bondapak $C_{18}$ column with UV detection (254 nm) at ambient temperature resolves trifluoperazine completely from its degradation products. Fig. 5 demonstrates a typical HPLC chromatogram of trifluoperazine resolved from its degradation products ($d_1$–$d_{12}$). Complete elution occurs after $\approx$ 15 min with a peak symmetry factor of $\approx$ 1.2 for the drug peak. Diode-array

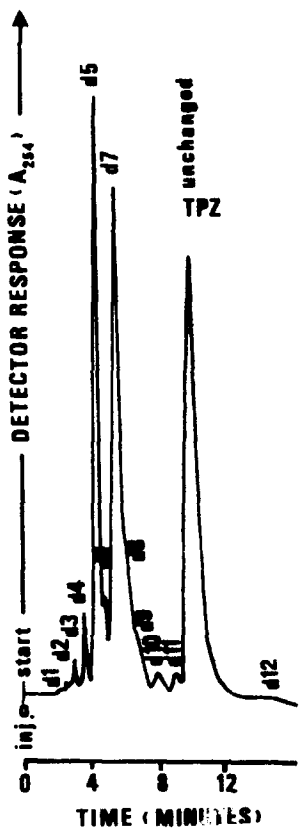



Fig. 5. HPLC resolution of trifluoperazine (TPZ) from its degradation products ($d_1$–$d_{12}$).

detection and/or mass spectrometric coupling seem to be important to clarify any doubt about the presence of degradation product(s) under the parent drug peak.

The method was applied for drug content uniformity testing in Stelazine™ 5 mg tablets. Good results ($X \pm$ %RSD; $n$) were obtained for the drug assay in tablets (98.86 ± 0.74%; 9), with a sensitive limit of detection (15 ng $ml^{-1}$ of unchanged drug). Excellent reproducibility (100.1 ± 0.35%; 14) was also obtained for replicate determinations on the same day. The recovery testing of pure trifluoperazine added to the powdered tablets was also good (100.3 ± 0.25%; 8). The described HPLC method is therefore photostability indicating and can be adopted for drug determination in quality control and routine pharmaceutical analysis.

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