Journal of the Association of Arab Universities for Basic and Applied Sciences (2013) **13**, 14–18

University of Bahrain

Journal of the Association of Arab Universities for Basic and Applied Sciences

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فصل وتقدير ثلاثي فلوبيرازين و بروكلوربيرازين في المستحضرات الصيدلانية باستعمال كروماتوغرافيا السائل عالي الأداء (HPLC)

جميل موسى ضباب، سلام عباس حسن، أصف حميد توفيق

قسم الكيمياء، كلية العلوم، الجامعة المستنصرية، بغداد، العراق

الملخص:

في هذه الدراسة لقد تم تطوير طريقة كروماتوغرافيا السائل عالي الأداء (HPLC) ذات الطور العكسي للتحليل المتزامن لثلاثي فلوبيرازين وبروكلوربيرازين للمستحضرات الصيدلانية. هذه الطريقة تم عملها على عمود كربون (C18) باستخدام نتريل المثيل كطور متحرك بمعدل إنسياب 1مل/دقيقة وكاشف الأشعة فوق البنفسجية عند طول موجي 250 نانومتر، وباستعمال هيدروكلوريد كلوربيرازين كمعايرة داخلية. ان ازمنة الاستبقاء للادوية كانت 10.879 دقيقة و 13.708 دقيقة على التوالي. أظهرت الطريقة المستخدمة استجابة خطية عند مدى تركيز بين 5–200 مايكروغرام/مل لثلاثي فلوبيرازين و10–500 مايكروغرام/مل لبروكلوربيرازين. في هذه الدراسة طبقت الطريقة المقترحة بنجاح للحصول على تقدير الكمي لثلاثي فلوبيرازين وبروكلوربيرازين في الأقراص والحقن التجارية وكانت طريقة بسيطة و سريعة ولا تتطلب خطوة فصل لكل نوع من الدواء.

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ORIGINAL ARTICLE

Separation and determination of trifluoperazine and prochlorperazine in pharmaceutical preparations by HPLC

Jameel M. Dhabab, Salam A.H. Al-Ameri *, Assaf H. Taufeeq

Chemistry Department, College of Science, Al-Mustansiriyah University, Baghdad, Iraq

Available online 19 September 2012

KEYWORDS

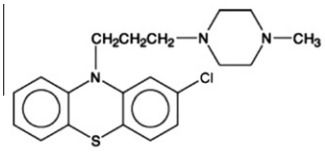
Trifluoperazine;
Prochlorperazine;
HPLC;
Pharmaceutical preparations

Abstract A reverse phase HPLC method is developed for the simultaneous analysis of Trifluoperazine (TFP) and prochlorperazine (PCP) in pharmaceutical preparations. HPLC was carried out on a C18 column using acetonitrile as a mobile phase at 1 mL min^{-1} flow rate and the effluent was monitored at 250 nm. Chlorperazine hydrochloride (CPZ) was used as an internal standard. The retention time of the drugs was 10.879 and 13.708 min, respectively. This method produced a linear response in the concentration range between 5–200 μg ml^{-1} of trifluoperazine and 10–500 μg ml^{-1} of prochlorperazine. In this study, a HPLC method was successfully applied for the quantitative assay of trifluoperazine and prochlorperazine in tablets and ampule of commercial preparations which is simple, rapid and does not require any separation step for each drug.

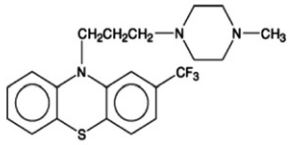
1. Introduction

The safety of a drug is dependent on many factors such as formulation manufacturing process and storage of drug substances (U.S. Food and Drug Admin., 2006; Liu et al., 2007).

Phenothiazine derivatives have been widely used as antipsychotics, antiparkinsonians, and antihistaminic drugs. The drugs are frequently encountered in the field of forensic toxicology because of their relatively narrow safe ranges of therapeutic doses (Ruri et al., 2007). Trifluoperazine(TFP){10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10H- phenothiazine; iralzine} is widely used for the treatment of antipsychotic diseases(British Pharmacopoeia, 2009) while Prochlorperazine(PCP)[2-chloro-10-(3,4′-methylpiperazin-1-yl)pylpropyl) phenothiazine; Stemetil] is widely used for the treatment of nausea, vomiting, migraine, anxiety and sometimes schizophrenia.(Sankey et al., 1982). The drugs are extensively metabolized by the liver and excreted in urine in animals and humans. Fetal intoxication due to these medicines is common because a patient may take several drugs in combination resulting in fetal poisoning; therefore, there is a need for a simple and sensitive method for the analysis of these drugs in pharmaceutical preparations (Einosuke et al., 2007).



Prochlorperazine(PCP)



Trifluoperazine(TFP)

A simple and rapid method for the determination of seven phenothiazine derivatives in human urine was presented, the separation and detection of the extracted compounds were accomplished by liquid chromatography and UV detection, limits of detection were in the range from 21 ng mL^{-1} (thioridazine) to 60 ng mL^{-1} (levomepromazine) and the RSD varied

* Corresponding author. Tel.: +964 07702531868.
E-mail address: alamri_salam@yahoo.com (S.A.H. Al-Ameri).

Peer review under responsibility of University of Bahrain.

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1815-3852
http://dx.doi.org/10.1016/j.jaubas.2012.08.002

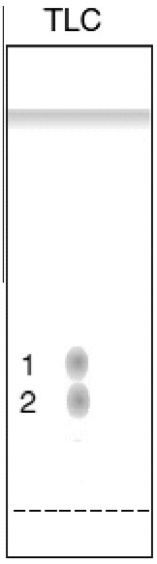

Figure 1 Separation of 1-TFP and 2-PCP by TLC method (methanol as mobile phase).

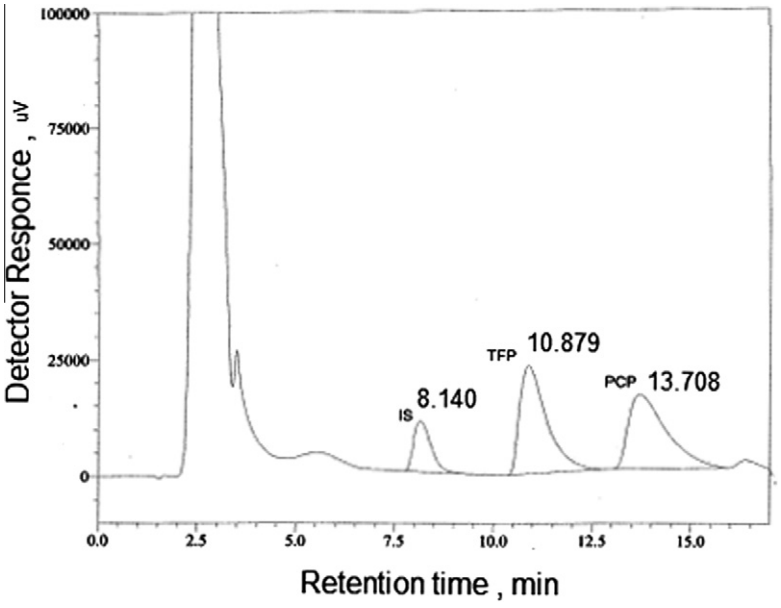

Figure 2 HPLC chromatogram of TFP, PCP and IS (50 μg ml^{-1} as internal standard)at 250 nm.

Table 1 Results of system suitability.

Parameters	Trifluoperazine (TFP)	Prochlorperazine (PCP)
RSD% of retention time	0.229	0.41
Capacity factor, (k)	4.4395	5.854
Selectivity	0.242	0.242
Tailing factor	1.5	0.79
Resolution factor (R)	1.46	1.46
Plate height	0.349	0.420
No. of theoretical plate (N)	717.1	593.9
Peak area	1101858	1051606
Separation factor (α)	1.319	1.319

between 2.2% (levomepromazine) and 3.9% (chlopromazine) (Cruz-Vera et al., 2009). Keisuke et al. was found that the second order derivative spectrophotometry method can be applicable to the determination of partition coefficient of six phenothiazine drugs between human erythrocyte ghosts (HEG) and water without any separation procedures (Keisuke et al., 1998).

Several analytical methods have been used for their determination of TFP and PCP such as HPLC (Deepak et al.,2009) potentiometric spectrofluorometric (Aleksandra et al.,2001) conductimetric (Olga and Joanna, 2001) gravimetric (Janina et al., 2001) spectrophotometric (Mohamed et al.,1989), TLC (Luminiţa et al.,2009) voltammetric (Yuxia et al.,2003) and capillary electrophoresis (Fran-

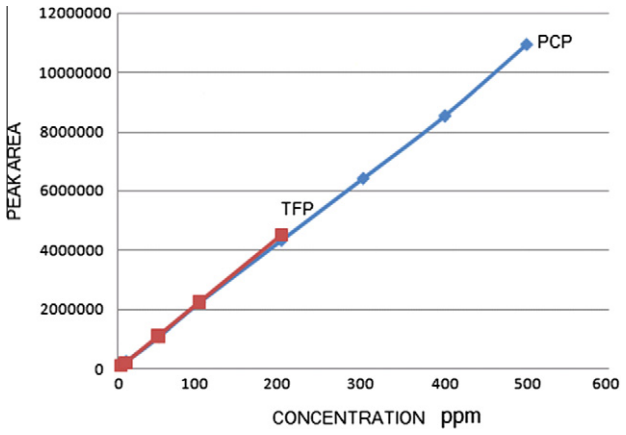

Figure 3 Calibration curve for (TFP) and (PCP) using proposed method. TFP $y = 22417\ x - 4216$, $R^2 = 0.999$, PCP $y = 21631\ x - 13984$, $R^2 = 0.999$.

cisco et al.,2006). HPLC methods are very useful in the determination of drugs in pharmaceutical preparations. It is always required to develop a new method for analysis using HPLC technique (Hérida et al., 1999).

The aim of the present study is to develop and validate a simple, sensitive, selective and non-gradient elution HPLC/UV method for the simultaneous analysis of TFP and PCP in the pharmaceutical preparations without any need for sample pretreatment.

2. Materials and methods

HPLC model Shimadzu LC-2010A with UV detector and auto sampler with two pumps was used. C18 column (250 × 4.6 mm), of 5 µm particle size from phenomenex co. the acetonitrile as mobile phase at 1 ml.min^{-1} flow rate was used with 10 µl injection volume of each solution. All the reagents used in the method of analytical HPLC grade purity were purchased from commercial source. Standard Trifluperazine was supplied from Samara Co. for drug industry-Iraq (SID), standard prochlorperazine and chlorpromaizine were obtained from commercial sources.

2.1. Preparation of stock solution of drugs

A 5000 µg ml^{-1} standard solution of each drug was prepared by transferring 50 gm of accurate weight of TFP and PCP to 10 ml volumetric flask, added with stirring 5 ml of ethanol for 5 min. A series of standard solutions for TFP and PCP at a concentration range between 0.5–500 µg ml^{-1} were prepared by diluting a suitable volume of stock solution with ethanol. These standard solutions were stored in dark and cold places until using.

2.2. In pharmaceutical preparations (ampule dosage form)

Each 1 ml of commercial stemetel ampule contains 12.5 mg ml^{-1} of PCP, 10 ampoules were mixed and then 2 ml volume was taken and diluted with 30 ml of ethanol in 50 ml volumetric flask, stirred for 5 min then the volume completed 50 ml, the concentration must be 500 µg ml^{-1}. Appropriate diluted solutions were prepared by taking suitable volume of solution and adding a constant amount of chlorpromaizine, CPZ as internal standard.

2.3. In pharmaceutical preparations (tablets dosage form)

The trifluoperazin (iralzine tablet) is supplied in two tablet formula, 1 mg and 5 mg. Thirty tablets of each type were powdered and triturated well. A quantity of powdered equivalent to 25 tablets of 1 mg and 5 tablets of 5 mg of TFP was transferred to 50 ml volumetric flask and added with mixing a 30 ml of ethanol for about 10 min, the volume completed to the mark with the same solvent. This solution was filtrated to separate any insoluble matter; the clear filtrate was collected in a clean flask. An appropriate solution was prepared by taking a suitable volume of clear filtrate, adding a 0.1 ml constant amount of chlorpromaizine, CPZ as internal standard.

3. Results and discussion

3.1. TLC method

The separation ability for TFP and PCP has been tested by using TLC technique on silica gel thin layer with many mobile phases. Methanol showed best separation Fig. 1. Trifluoperazine scored 3.7 cm while prochlorperazine scored 3.2 cm and the L solvent was 8 cm. The *Rf of TFP was 0.4625 while the Rf of PCP was 0.4 (Luminiţa et al., 2009). The TLC technique shows good separation between trifluoperazine and prochlorperazine:

$$\mathrm{Rf} = \mathrm{L\,compound}/\mathrm{L\,solvent}$$

where *Rf is retardate factor, L is the distance of analyte or solvent, this result encourages us that we can separate these drugs by HPLC method.

Table 2 Least square regression analysis for the determination of TFP and PCP.

Statistical parameters	Trifluoperazine (TFP)	Prochlorperazine (PCP)
Linearity range (ppm)	5–200	5–500
Correlation coefficient (R^2)	0.999	0.999
Slop	24140.5	32059.5
Intercept	20206.5	33685.75
Slop (RSD%)	0.09	0.282
Intercept (RSD%)	4.1	5.4
LOD (ppm)	0.156	0.31
LOQ (ppm)	0.5	1.0
Repeatability (RSD%)	1.99	0.73
Reproducibility (RSD%)	2.03	0.75

Table 3 Analysis of laboratory-prepared TFP and PCP mixture.

Compound	Concentration (ppm)	Amount found (µg mL^{-1})	Recovery (%)	RSD (%)
TFP	50	49.34	98.68%	0.04
PCP	50	49.26	98.52%	0.15

3.2. HPLC method

The present study was aimed to develop a sensitive, precise and accurate HPLC method for the analysis of trifluoperazine and prochlorperazine in pharmaceutical dosage forms at 1 ml min^{-1} flow rate with acetonitrile as the best mobile phase and C18 column (250 × 4.6 mm, 5 μm particle size) at 250 nm optimum wavelength detection. These parameters and analysis condition were used throughout the analysis.

HPLC method precision and accuracy can often be enhanced by using appropriate internal standard, which also serves to correct for fluctuation in the detector response. The structure of CPZ is similar to that of TFP and PCP, therefore it was chosen as internal standard, and also it showed a shorter retention time with better peak shapes and better resolution. The chromatographic peaks which obtained in this system were better defined and resolved with a little tailing. The retention times, t_R were determined as 10.879 min for TFP, 13.708 min for PCP and 8.140 min for CPZ as internal standard, Fig. 2. This method gives results with acceptable accuracy (Rec% > 96%) and precision (RSD% < 2). All tests achieved in this work were carried out according to United State Pharmacopoeia (USP), food and drug administration (FDA) and international conference harmonization (ICH) methods (Suresh et al., 2010).

In this study LOD and LOQ were calculated using the following equations (Monika and Saranjit, 2002): (See Table 1).

Limit of Detection(LOD) : DL = 3(residual SD/slope),

While the Limit of quantification(LOQ) : QL = 10(residual SD/slope).

A good linear relationship (correlation coefficient (R2) > 0.999) was observed between the concentration of each drug and respective peak area. The regression curve was constructed by linear regression fitting and its mathematical expression was $y = 22417\ x - 4216$ for TFP and $y = 21631\ x - 13984$ for PCP (where y is peak area and x is the concentration of drug), Fig. 3. Parameters of the peak area versus concentration of the compounds Table 2 such as statistical data of the regression equation such as LOD and LOQ values, repeatability and reproducibility data, are presented in Table

Table 4 Analysis of TFP and PCP in pharmaceutical preparations.

Compound	Labeled claim (mg per tablet), ampoule	Amount found (%)	RSD % of amount found
TFP (iralzin) S*	1 mg/Tablet	0.7923 mg/Tablet (79.23%)	0.065
TFP (iralzin) S*	5 mg/Tablet	5.598 mg/Tablet (111.96%)	0.765
PCP (stemitil) F*	12.5 (μg mL^{-1}) ampoule	14.3 (μg mL^{-1}) ampoule (114.4%)	0.266
PCP (stemitil) P*	12. 5 a (μg mL^{-1}) ampoule	11.87 (μg mL^{-1}) ampoule (94.96%)	0.061

S* – drug of Iraq, F* – drug of France P* – drug of Pakistan.

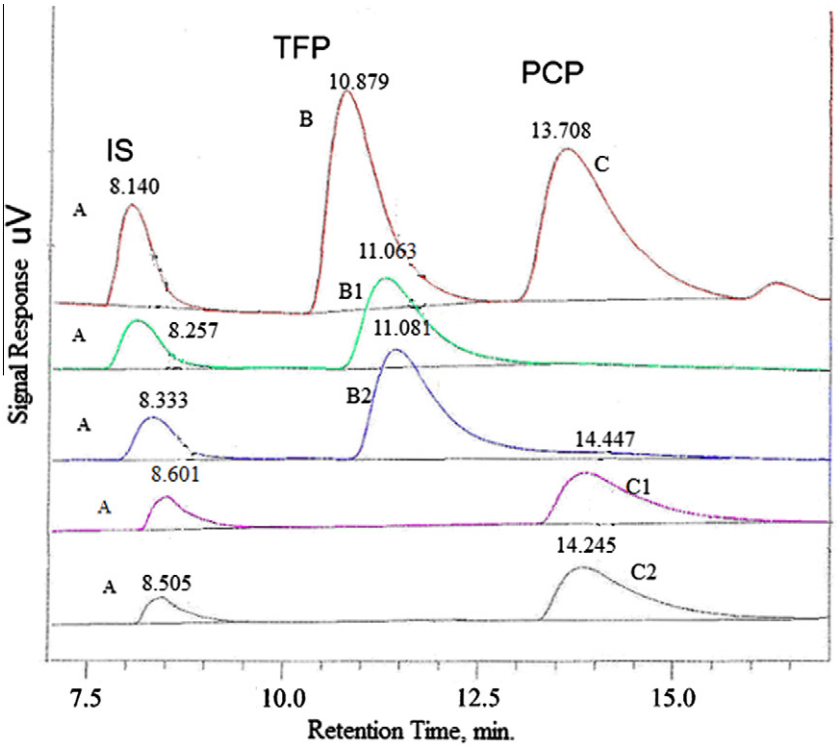

Figure 4 Chromatograms of application drugs in pharmaceutical preparations. A-(IS), B-TFP standard, B1 = iralzine tablet 1 mg, B2 = iralzine tablet 5 mg (SID) C-PCP standard, C1-stemetel ampoule, France, C2 = stemetel vial, (Pakistan).

2. According to these results, no significant difference for the assay was tested by RSD% of repeatability and reproducibility (> 1.9), (James et el., 2000; Riley and Rosanski, 1996).

In order to demonstrate the validity and application of the proposed HPLC method, recovery tests carried out by analyzing a synthetic mixture of these compounds in different composition ratios of drugs are listed in Table 3. A recovery experiment using the developed procedure indicates the absence of interferences from common pharmaceutical preparation used in the selective formulation. The proposed method was verified by means of replicate estimations of pharmaceutical forms and the results obtained were evaluated statistically, Table 4. The typical chromatogram was obtained from tablets and ampoules of TFP and PCP and constant level of internal standard (IS), Fig. 4. The results obtained for the analysis ampoules and tablets for each drug by HPLC method can be used in routine analysis with high efficiency.

The HPLC method was simple, rapid (all compounds were elutes only in 17 min), and accurate, so it can be used for the determination of TFP and PCP in its pharmaceutical formulation.

The calculated *F*-values for the drugs (iralzine 1 mg/tablet 1.33) and (12.5 mg/ampoule 4.77) were less, according to the variance ratio *F*-test than the tabulated values at 95% confidence level, while the theoretical value is 19.0.

4. Conclusions

In this study, a RP-HPLC method has been described to measure TFP and PCP in pharmaceutical dosage form, which is simple, rapid and does not require any separation step. This procedure can be easily adopted for routine quality control analysis of tablet and ampoule dosage forms without any interference from the matrix or each other.

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