Comparative Determination of Chlorpromazine in Pharmaceutical Injectable Veterinary and Human Formulations by Spectrophotometric and High Performance Liquid Chromatographic Methods

Fouad K. Mohammad[1], Lubna A. Kafi[2], Nabaa K. Al-Hayani[1], Fereal M. Mahdi[3], Ahmed J. Essa[3]

[1]Professor & PhD Student, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, [2]Professor, Department of Pharmacology, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq, [3]Researcher, Veterinary Drugs Research and Production Center, Corporation of Research and Industrial Development, Ministry of Industry and Minerals, Baghdad, Iraq

Abstract

Background: Chlorpromazine (CPZ) is a phenothiazine tranquilizer used in humans and animals. Rapid determination of the drug in pharmaceutical preparations is often needed. The aim of the study was to further examine and ascertain asimple spectrophotometric method in comparison with a high performance liquid chromatographic (HPLC) method for the determination of CPZ concentrations in injectable pharmaceutical formulations used in man and animals. **Methods:** Concentrations of CPZ in injectable pharmaceutical formulations for human and veterinary uses were determined by a modified spectrophotometric method and by an HPLC method with appropriate standard calibration curves. The spectrophotometric method of CPZ determinationwas conducted by diluting 0.1 ml of the veterinary (5%) or 0.3 ml of human (0.5%) formulations to 200 ml of 0.1N sulfuric acid. Four ml of the diluted CPZ samples or the standard solutions were mixed with 2 ml of 50% sulfuric acid. Then an aliquot of 0.2 ml of 2% ferric nitrate was added to the mixture. After 15 minutes, the absorbance was measured at 530 nm against water and vehicle blanks using a spectrophotometer. The HPLC method applied in the present study consisted of reversed phase gradient chromatography with a C_{18} column and the eluent was (A) 50 mmol NaH_2PO_4, pH 2.5 and (B) acetonitrile - 50 mmol NaH_2PO_4, pH 2.5 (60:40 v/v), flow rate at 1 ml/min, with a gradient of 15-55% B in 10 min, to 100% B in 20 min, and 280-nm UV detection. From the calibration curves of CPZ standards, the following were calculated: Limit of detection limit (LOD) = 3.3 x SD/slope; Limit of quantitation (LOQ) = 10 x SD/slope, where SD is the standard deviation of response of CPZ concentrations of the calibration curves.Linear regression analysis and related coefficients of correlations were applied on the calibration curves. **Results:** The calibration curves of CPZ against water or vehicle blanks as determined by the spectrophotometric method or by the HPLC method were linear with strong correlations (r = and / or >0.99). Based on the calibration curves, limit of detection and limit of quantitation the spectrophotometric method were comparable to those of the HPLC one. The contents CPZ in human and veterinary injectable formulations as determined by the spectrophotometricmethod were in accordance (100.6% and 102%, respectively)to the concentrations claimed on the labels of the formulations.

Conclusion: The present results introduce a simple spectrophotometric method that could be used for routine measurement of CPZ concentrations in pharmaceutical formulations, with the added benefits of linearity, precision and cost effectiveness.

Corresponding author:
Prof. Dr. Fouad K. Mohammad, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq. fouadmohammad@yahoo.com

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Introduction

Chlorpromazine (CPZ) is a neuroactive phenothiazine tranquilizer used in humans[1]and in various animal species.[2] The injectable formulation of the drug is applied clinically in veterinary practice on the basis of extra-label use with acceptable therapeutic efficacy and margin of safety.[2,3]Injectable aqueous veterinary formulations of CPZ (1 and 2.5%) have been described, with a modified spectrophotometric method to determine the concentration of the drug in these formulations.[4,5]

Aspectrophotometric method was originally described to determine CPZ in biological fluids after certain elaborate extraction steps.[6]Thereafter, the method wasreportedly furthersimplified and modified to be anadopted procedure under conventional laboratory conditions for rapid determination of CPZ in injectable veterinary formulations.[4,5]Other more elaborate analytical spectrophotometric[6-11]and high performance liquid chromatography[12-14]techniques are available for the determination of CPZas well as other phenothiazine derivatives in pharmaceutical formulations.However, no direct comparison was attempted between the designated spectrophotometric method of CPZ determination[4,5]and a standardized HPLC procedure.

The purpose of the present study was to further examine and ascertainthe previously described spectrophotometric method forCPZ measurement.[4,5] Then, to apply the present spectrophotometric method in comparison with a reference HPLC method[13]for the determination ofCPZ concentration in injectable pharmaceutical formulations intended for human and veterinary uses. Such a comparison has not been done previously.

Materials and Methods

All chemicals and reagents used were of analytical grades obtained from well-known suppliers. A locally available injectable brand of aqueous CPZsolution (25 mg/5ml, 0.5%) was purchased from a local pharmacy. A veterinary injectable aqueous CPZ solution (5%) was prepared at the Veterinary Drugs Research and Production Center, Corporation of Research and Industrial Development, Ministry of Industry and Minerals, Baghdad, Iraq.

The concentrations of CPZ in the injectable pharmaceutical formulations were determined by a modified spectrophotometric method[4,5]and by an HPLC method[13]as described earlier with appropriate standard calibration curves. For the spectrophotometric determination of CPZ, the working reagents were0.1 N, 1 N and 50% solutions of sulfuric acid as well as ferric nitrate (2%) in 1 N sulfuric acid. The calibration curve of CPZ was prepared by dissolving CPZ powder in 0.1 N sulfuric acid at concentrations of5, 10, 20, 40 and 80 µg/4ml. The modified spectrophotometric method of CPZ determinationwas conducted by diluting0.1 ml of the veterinary (5%) or 0.3 ml of human (0.5%) formulations to 200 ml of 0.1N sulfuric acid. Four ml of the diluted CPZ samples or the standard solutions were mixed with 2 ml of 50% sulfuric acid. Then an aliquot of 0.2 ml of 2% ferric nitrate was added to the mixture. After 15 minutes, the absorbance was measured at 530 nm against water and vehicle blanks using a spectrophotometer (T 80, Biotech Engineering Management Co., U.K.). All determinations were done in duplicate at ambient room temperature.

The HPLC method applied in the present studyconsisted of reversed phase gradient chromatography with 250 x 4 mm Nucleosil C_{18} column; the eluent was (A) 50 mmol NaH_2PO_4, pH 2.5 and (B) acetonitrile - 50 mmol NaH_2PO_4, pH 2.5 (60:40 v/v), flow rate at 1 ml/min, with a gradient of 15-55% B in 10 min, to 100% B in 20 min, and 280-nm UV detection at room temperature.[13] The external CPZ standard was calibrated between0.625 to 20 µg/ml.

From the calibration curves of CPZ standards, the following were calculated[15,16]asfollows:

Limit of detection limit (LOD) = 3.3 x SD/slope; Limit of quantitation (LOQ) = 10 x SD/slope, where SD is the standard deviation of response of CPZ concentrations of the calibration curves.

Linear regression analysis and related coefficients of correlations were applied on the calibration curves using the statistical package Past 4.03 (https://folk.universitetetioslo.no/ohammer/past)

Results

The linear calibration curves of CPZ standards determined by the spectrophotometric methodare shown in figure 1 (a, water blank; b, vehicle blank) and in figure 2 by the HPLC method. The range of CPZ concentrations by the spectrophotometric method was between 5 to 80 µg/4ml (Figure 1), whereas that of the HPLC method was between 0.625 to 20 µg/ml (Figure 2). All the standard curves showed strong correlations with an r value = and / or> 0.99 (Figures 1a,b and 2).

The calculated LOD and LOQ of both methods are shown in table 1. In referenceto calculations of CPZ standard concentrations on the basis of µg/ml, as well as the estimated values of LOD and LOQ, the ranges of the calibration curves of CPZ determination by both methods were comparable to each other (Figures 1a,b and 2; Table 1).

The concentrations of CPZ in human and veterinary injectable formulations as determined by both methods are presented in table 2. The concentrations of CPZ determined by the spectrophotometric method were in agreement with of those of the HPLC method, andCPZ contents were in accordance (100.6% and 102%, respectively) with the concentrations claimed on the labelsof both formulations (Table 2).

Table 1: Limits of detection and quantitation of the assays of chlorpromazine by spectrophotometric and HPLC methods

Variable	Spectrophotometry µg/4ml	HPLC µg/ml
Limit of detection	4.0	2.8
Limit of quantitation	12.1	8.3

Table 2: Percentages of chlorpromazine contents recovered from commercial human and veterinary formulations as determine by spectrophotometric and HPLC methods

Formulation	Concentration claimed on label (% w/v)	Spectrophotometry (% w/v)	HPLC (% w/v)
Human	0.5	0.503	0.535
Veterinary	5	5.10	5.05

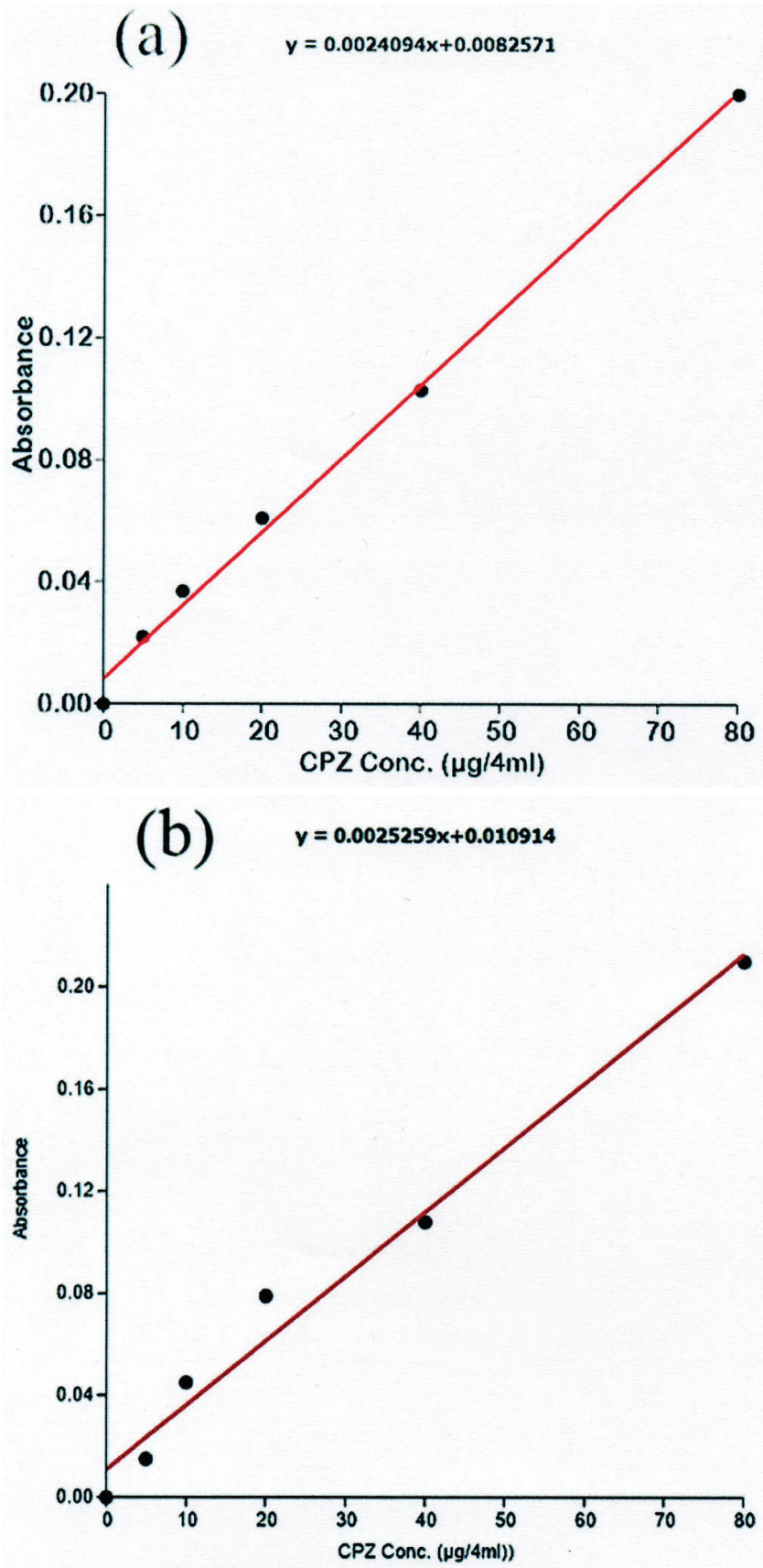

Figure 1: Standard calibration curves of chlorpromazine (CPZ) with water (a) or vehicle (b) blanks determined spectrophotometrically at 530 nm, r = 0.998 and 0.99, respectively.

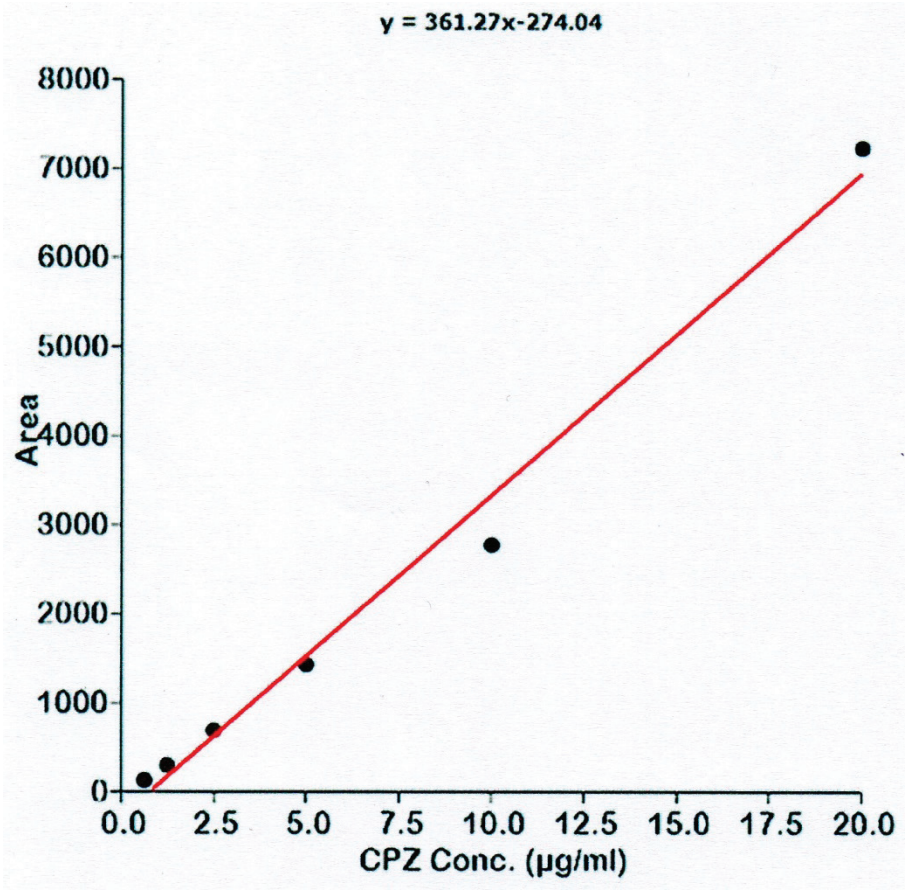

Figure 2: Standard calibration curve of chlorpromazine (CPZ) determined by HPLC, r = 0.994.

Discussion

The present study introduces a useful, simple and accurate spectrophotometric method for determination of CPZ concentrations in pharmaceutical formulations in a manner comparable to a highly sensitive HPLC method.[13] The results further ascertain previous findings reported on the application of the present spectrophotometric method for the measurement of CPZ in aqueous pharmaceutical formulations.[4,5]The LOD and LOQ as determined by both spectrophotometric and HPLC methods are within the acceptable ranges for the drug assay.[4,5,15,16]

The contents of CPZ in both human and veterinary formulations of the present study as estimated by the spectrophotometric methodwere in accordance with those of the HPLC method. They were also in good agreement with the concentrations (100.6% and 102%, respectively) claimed by the manufacturers on the labelsof both formulations. These results of the modified spectrophotometric method for measurement of CPZ concentrations in aqueous formulations, support the findings reported earlier.[4,5] Furthermore, the added benefit of the present study was that the spectrophotometric method was very much comparable to a highly sensitive and standardized HPLC method[13], as there wasgood agreement between both methods. Further studies, however, are needed using the present simple spectrophotometric method on routine batch analysis of CPZ in pharmaceutical industries.

Conclusions

A simple spectrophotometric method is presented herewith,possibly,for routine measurement of CPZ concentrations in pharmaceutical formulations, with the added benefits of linearity, precision and cost

effectiveness.

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Ethical Clearance-The authors followed the institutional scientific research ethics as a well the research protocols and guideline of the Ministry of Industry and Minerals**.**

Competing Interests: The authors declare that they have no competing interests.

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