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Research Article

A simple spectrophotometric assay for stability determination of chlorpromazine in veterinary injectable solutions

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Abstract. Objective: To present a simple spectrophotometric method for measuring chlorpromazine (CPZ) concentration in veterinary injectable solutions subjected to accelerated stability testing. Material and methods: Four different injectable solutions of CPZ in their original glass ampoules were subjected to accelerated stability testing by incubating them at 40˚C for 30 days without any exposure to light. The concentration of CPZ was determined from tested ampoules at 0 time (before incubation) and at 30 days after the incubation. The stability of CPZ in the injectable solutions at supposedly storage temperatures of 15˚C and 20˚C was calculated by the Q_{10} method. An aliquot of 0.1 ml of CPZ solution (1 or 2.5%) was diluted with 0.1 N sulfuric acid to 200 ml. Four ml of the diluted CPZ (or CPZ standard 5-80 µg/4m) solutions were mixed well with two ml of 50% sulfuric acid. Then, an aliquot of 0.2 ml of 2% ferric nitrate solution was added to the mixture. The absorbance of the developed color was measured by a spectrophotometer at 530 nm against a water blank. The detection limit (DL) and quantitation limit (QL) were also determined. Results: The calibration curve of CPZ was linear with good correlation coefficient (r=0.9986). The DL and QL of CPZ by the applied method were 4.2 and 12.8 µg/4 ml, respectively. CPZ concentrations following incubation of the four formulations at 40˚C for 30 days decreased to 38, 70, 87 and 86% of their original contents, respectively. The calculated stability periods of CPZ in the injectable solutions at 15˚C were 320, 368, 720 and 608 days, respectively, and at 20˚C they were 160, 184, 360 and 304 days, respectively. The most stable formulations of CPZ were 3 and 4, followed by formulation 2, and the least stable one was no. 1 which showed color change to brownish-red. Conclusion: The method was simple, rapid and accurate for measuring CPZ concentration, as well as for determination of its stability in injectable formulations intended for animal use.

Key Words: phenothiazine tranquilizer, colorimetric method, expiry test, Q_{10} method.

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Introduction

Chlorpromazine HCl (CPZ) is a phenothiazine tranquilizer which antagonizes dopamine-2 receptors in the central nervous system (Posner & Burns 2009). It is used in veterinary practice on the basis of extra-lable drug use (Posner & Burns 2009; Papich 2011). Various pharmaceutical formulations of CPZ such as tablets, capsules, oral liquid forms or injectable solution are available for clinical use (Sweetman 2006).

Several spectrophotometric and high performance chromatographic methods are available for the determination of CPZ concentration in pharmaceutical formulations or biological fluids (Forrest *et al* 1968; Onkubo *et al* 1993; Shetti & Venkatachalam 2010; IPCS 2013). Elaborate methods are needed for extraction, separation and pre-concentration of CPZ from biological or pharmaceutical matrices (Forrest *et al* 1968; Onkubo *et al* 1993; Shetti & Venkatachalam 2010; IPCS 2013). One simple method was reported to be used for determination of CPZ in biological fluids (blood and urine) which also requires prior extraction of CPZ (Leach & Crimmin 1956). In the present report, we describe a simple spectrophotometric assay based on the latter method (Leach & Crimmin 1956) to determine CPZ concentration in injectable solutions subjected to accelerated stability testing without any extraction from the pharmaceutical formulation matrices of the drug.

Material and methods

The chemical compounds used for formulating of four CPZ injectable solutions intended for veterinary use are shown in table 1. The chemicals were kindly donated by the State Company for Drug and Medical Appliances-Ninevah (Mosul, Iraq). The four injectable formulations of CPZ in their original glass ampoules were subjected to accelerated stability testing by incubating them at 40˚C for 30 days without any exposure to light. Then, the concentration of CPZ was determined from the tested ampoules at 0 time (before incubation) and at 30 days after the incubation. The stability of CPZ in the injectable solutions at supposedly storage temperatures of 15˚C and 20˚C was calculated by the Q_{10} method as described before (Allen, Jr *et al* 2005) using the following equation:

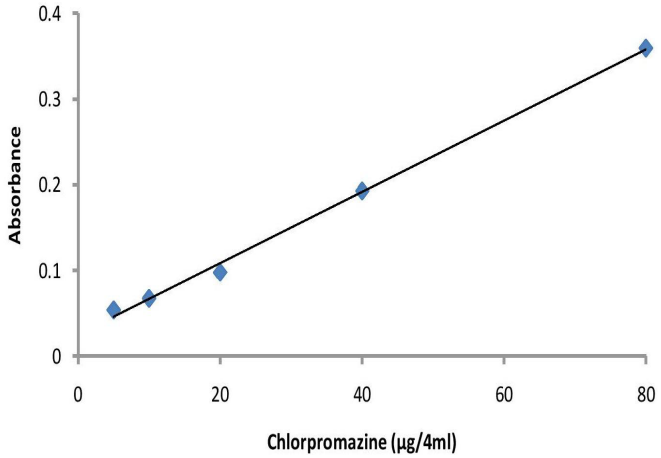

Figure 1. Standard calibration curve of chlorpromazine measured spectrophotometrically at 530 nm. Y = 0.00416X + 0.02545; r = 0.9986.

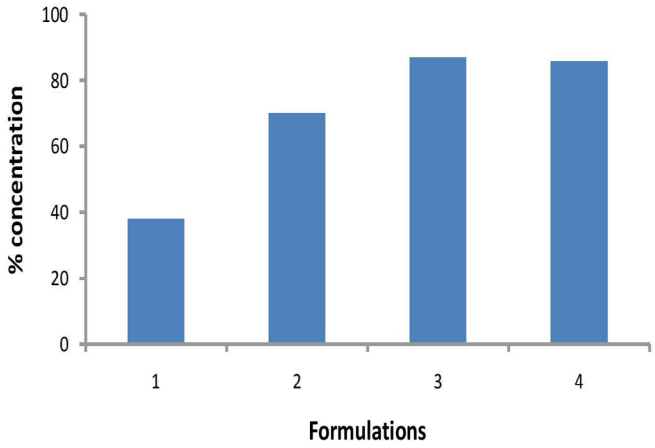

Figure 2. Percentage concentrations of chlorpromazine following incubation of the four formulations at 40˚C for 30 days (accelerated test) compared to their original contents at 0 time.

t_{90} (T_2) = t_{90}(T_1)/$Q_{10}^{(\Delta T/10)}$

Where t_{90} (T_2) is the shelf-life to be estimated at 15˚C or 20˚C, t_{90}(T_1) is the shelf-life at 40˚C, and ΔT is the difference between temperatures T_1 (40˚C) and T_2 (15˚C or 20˚C).

The details of the reagents used and the spectrophotometric procedure applied were as follows:

Reagents: 0.1 N sulfuric acid, 1 N sulfuric acid, 50% sulfuric acid, 2% ferric nitrate in 1 N sulfuric acid.

CPZ standard: The working standard curve of CPZ consisted of the drug concentrations at 5, 10, 20, 40 and 80 µg/4 ml of 0.1 N sulfuric acid.

Steps of CPZ assay: An aliquot of 0.1 ml of CPZ (1 or 2.5%) solutions was diluted with 0.1 N sulfuric acid to 200 ml. Four ml of the diluted CPZ (or CPZ standard) solutions were mixed well with two ml of 50% sulfuric acid. Then, an aliquot of 0.2 ml of 2% ferric nitrate solution was added to the mixture for color development. The absorbance of the developed color was measured by a spectrophotometer (Jenway 6405, Wagteah International, U.K.) at 530 nm against a water blank. All assays were done in duplicate at ambient room temperature. The detection limit (DL) and quantitation limit (QL) of the method were measured according to the ICH guidelines using the following formulae (ICH 2005):

DL = 3.3SD/Slope

QL = 10SD/Slope

Where SD is the standard deviation of the response obtained from analyzing ten blank samples and the slope of CPZ calibration curve was estimated by the regression analysis (Petrie & Watson 2006).

Results

Figure 1 shows a typical standard curve for the determination of CPZ in the range of 5 to 80 µg/4 ml. The calibration curve of CPZ was linear under optimal assay conditions with good correlation coefficient (r=0.9986). The DL and QL of CPZ by the applied method were 4.2 and 12.8 µg/4 ml, respectively. Concentrations of CPZ following incubation of the four formulations at 40˚C for 30 days (accelerated test) decreased by 62, 30, 13 and 14% compared to their original contents, respectively (Figure 2). Using these values, we calculated the stability of CPZ in the injectable solutions at storage temperatures of 15˚C and 20˚C as shown in table 2. The most stable formulation of CPZ were 3 and 4, followed by formulation 2 and the least stable one was no. 1 which showed color change to brownish-red.

Table 1. Chemical compounds in chlorpromazine HCl injectable solutions

Formulation	Chlrorpromazine HCl concentration (%)	Other ingredients
1	1	Ascorbic acid, water for injection
2	2,5	Benzyl alcohol, water for injection
3	2,5	Ascorbic acid, sodium metabisulfite, sodium sulfite, sodium chloride, water for injection
4	2,5	Ascorbic acid, sodium metabisulfite, sodium chloride, benzyl alcohol, water for injection

Table 2. The stability (days) of veterinary chlorpromazine formulations

Formulation	Storage at 15˚C	Storage at 20˚C
1	320	160
2	368	184
3	720	360
4	608	304

The Q_{10} method (Allen, Jr *et al* 2005) was used to determine the stability of the drug after accelerated test (incubation at 40˚C for 30 days).

Discussion

The method of CPZ determination was rapid, accurate and appeared to be robust for measuring CPZ concentration, as well as for the determination of CPZ stability in injectable formulations intended for animals. It was cost effective when compared with other elaborate high cost high performance chromatographic methods (Forrest *et al* 1968; Onkubo *et al* 1993; Shetti & Venkatachalam 2010; IPCS 2013). Both DL and QL were within the acceptable limits according to ICH guidelines (ICH 2005), as the samples of CPZ obtained from the formulations had the drug at concentrations well above the DL and QL and within the linear range of CPZ calibration curve.

The simplicity of the method is that it does not need extraction of CPZ as reported originally when dealing with biological samples (Leach & Crimmin 1956). It appeared that ingredients incorporated into the formulations of CPZ injection (Table 1) did not interfere with the assay. The color change found in formulation 1 after 30-day incubation at 40˚C could be attributed to oxidation of CPZ (IPCS 2013). This was coupled with decreased active ingredient (CPZ) concentration by 62% at the end of the accelerated stability test (Figure 2). In the latter formulation ascorbic acid was the sole antioxidant used, and probably it was not effective in protecting CPZ from the oxidation reaction. Others also have reported the failure of ascorbic acid as an effective antioxidant in zopiclone injection formulation (Swamy *et al* 2008).

Conclusions

In conclusion, the present colorimetric method was simple, rapid and accurate for measuring CPZ concentration, as well as for determination of its stability in injectable formulations intended for animal use. Further experiments are needed to evaluate the suitability of the method for determining CPZ in other pharmaceutical formulations such as tablets and oral dosage liquids.

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