

Comparative of Three Titrimetric Determinations of Cefazolin Sodium

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ABSTRACT

Three titration methods for quality evaluation of cefazolin sodium pharmaceutical product were developed and compared among them: acidimetric, iodometric and non-aqueous methods. The titrimetric methods consist of the measured volume of a solution, concentration of knowledge and need to completely react with a species to be quantified. A great advantage is measured without, in most analyzes, the analytical reference standard. Moreover, the smaller volume of solvent and high-end products is performed. The developed methods meets the requirements of Brazilian Pharmacopeia and International Conference on Harmonisation, being fast, suitable, low cost, simple, accurate and can be used as an alternative for analysis of cefazolin sodium pharmaceutical product in laboratories and industry, contributing to improve the quality control of marketed drug.

Keywords: Cefazolin sodium, Analytical methods, Titrimetry.

INTRODUCTION

Cefazolin sodium (CFZ), a β -lactam antimicrobial of the first generation, is commercially available as powder for injection solution. It has high antibacterial activity, demonstrates activity against some species of Enterobacter and can be administered less frequently because of its longer half-life [1-5].

Its efficacy as a therapeutic agent is well recognized, which makes it preferred among these group cephalosporins. Its mechanism of action stems from the inhibition of cell wall synthesis in Gram-positive and Gram-negative bacteria, similar to penicillin, since they have structural similarities [6-10].

Some advantages of CFZ (**Figure 1**) include: (i) efficient penetration of the bacterial cell wall, (ii) a broad spectrum of activity, (iii) extensive tissue penetration, (iv) a high intrinsic activity against the bacterial cell targets, (v) resistance to bacterial enzyme degradation, (vi) very low toxicity, (vii) low degree of serum protein binding and (viii) metabolic stability [11-14].

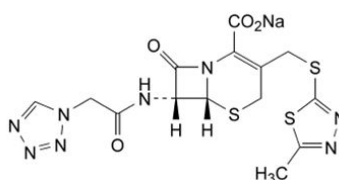


Figure 1. Cefazolin sodium chemical structure.

Cefazolin sodium has some analytical methodologies described in official compendia such as British Pharmacopeia [15], European Pharmacopeia [16], Japanese Pharmacopeia [17], Portuguese Pharmacopeia [18] and US Pharmacopeia [19].

Some analytical methods in the literature for analysis of cephalosporins include spectrophotometry [20-36], high performance liquid chromatography [37-50], capillary electrophoresis [51], fluorimetry [52-56], polarography [57-61] and titrimetry [62].

CFZ is highly researched and researched in the area of antimicrobial activity, pharmacokinetics and pharmacodynamics. However, it has few studies about analytical methods. Thus, researches involving methods of analysis are very important and relevant to ensure the quality of the marketed product [48].

This paper aims to validate and evaluate the equivalence of three methods by titration for quantification of CFZ in pharmaceutical product.

METHODOLOGY

Chemicals

The reagents used were analytical grade and it were prepared according to literature [19].

CFZ reference substance was a secondary standard with a declared purity of 98.2%, obtained from Sigma Aldrich® (St. Louis, USA). CFZ samples were packed in vials, each vial containing 1 g CFZ and they were kindly supplied by ABL Antibioticos do Brasil (Sro Paulo, Brasil).

a) 0.1 M hydrochloric acid: 8.5 mL of hydrochloric acid (Merck™) were diluted with water to 1000 mL. This solution was standardized using 1.5 g of sodium carbonate (Merck™) dried at 270°C for 1 hour dissolved in 100 mL of water. Two Drops of methyl red solution is added as an indicator and titrated with hydrochloric solution to the production of permanent pink color. Each 52.99 mg of sodium carbonate is equivalent to 1 mL of 0.1 M hydrochloric acid.

b) 1 M hydrochloric acid: 85.0 mL of hydrochloric acid (Merck™) were diluted with water to 1000 mL.

c) 0.01 M iodine: 14.0 g of iodine were dissolved in aqueous potassium iodide solution (36%, w/v) and 3 drops of hydrochloric acid was added. This solution was diluted with water to 1000 mL and standardized according to United States Pharmacopeia [19]. An aliquot of 50 mL of this solution was diluted with water to 1000 mL.

d) 0.1 M perchloric acid: 8.5 mL of Perchloric acid (Merck™) is mixed with 500 mL of glacial acetic acid (Merck™) and 21 mL of acetic anhydride (Merck™). After this solution cooled, glacial acetic acid was added to 1000 mL. This solution was standardized using 700 mg of potassium biphthalate, previously dried at 120°C for 2 h, dissolved in 50 mL of glacial acetic acid. 2 Drops of crystal violet solution is added as an indicator and titrated with perchloric acid solution until it turns to blue-green color. Each 20.42 mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

e) 0.1 M sodium hydroxide: 4.0 g of sodium hydroxide (Merck™) is dissolved in 150 mL of carbon dioxide-free water. After this solution cooled, it was diluted with carbon dioxide-free water to 1000 mL. This solution was standardized using 5 g of potassium biphthalate, previously dried at 120°C for 2 h, dissolved in 75 mL of carbon dioxide-free water. 2 Drops of phenolphthalein solution is added as an indicator and titrated with the sodium hydroxide solution to the production of permanent pink color. Each 20.42 mg of potassium biphthalate is equivalent to 1 mL of 0.1 M sodium hydroxide.

f) 1 M sodium hydroxide: 40.0 g of sodium hydroxide (Merck™) was dissolved in water to 1000 mL.

g) 0.01 M sodium thiosulphate: 26 g of sodium thiosulphate (Merck™) was dissolved in water to 1000 mL. An aliquot of 50 mL of this solution was diluted in water to 1000 mL.

h) Acetate buffer solution: 5.44 g of sodium acetate (Merck™) and 2.40 g of glacial acetic acid were dissolved in water to 100 mL.

i) Crystal violet 1% (w/w): 100 mg of crystal violet (Merck™) is dissolved in 10 mL of glacial acetic acid.

j) Methyl red 0.1% (w/w): 100 mg of methyl red (Merck™) was dissolved in 100 mL of alcohol. After this process, the solution was filtered.

k) Phenolphthalein 1% (w/w): 100 mg of phenolphthalein (Merck™) was dissolved in 100 mL of alcohol. After this process, the solution was filtered.

l) Starch mucilage: 1 g of starch was mixed with 10 mg of red mercuric iodide and cold water to make a thin paste. 200 mL of boiling water was added with continuous stirring. After cooling, the clear solution was used.

Procedure

Twenty vials of CFZ were individually weighed to determine the average weight. After that, the powder was mixed. The quantity of CFZ was 21.156 g.

a) Acidimetric method: Amount equivalent to 500.0 mg of CFZ was dissolved in 25 ml of purified water free from carbon dioxide was added two drops of phenolphthalein indicator solution and neutralized with 0.1 M HCl. To the above solution, 25 ml of 0.1 M NaOH was added and it was heated on a water bath for 20 minutes at 80°C, taking precaution to

avoid carbon dioxide absorption. It was cooled and the excess of 0.1 M NaOH was titrated with using 0.1 M HCl and phenolphthalein is used as an indicator solution. Repeated operation without the drug, as the difference between two titrations represents the amount of NaOH used, and 1 mol of 0.1 M NaOH corresponds to 47.65 mg of CFZ (measured as C₁₄H₁₃N₈NaO₄S₃).

b) Non-aqueous method: Dissolved exactly an amount equivalent to 250.0 mg of CFZ sample in 30 mL of glacial acetic acid; 2 drops of indicator solution crystal violet is added and stirred till to complete dissolution and titrated with 0.1 M HClO₄ solution and the same titration repeated without drug.

c) Iodometric method: 100.0 mg of the CFZ was weighed and dissolved in water and transferred to 100 mL volumetric flask (1000 µg/mL). (i) An aliquot of 10 mL was transferred to a flask, and 5 mL of 1 M sodium hydroxide is added and after twenty minutes 20 mL of acetate buffer solution, 5 mL of 1 M hydrochloric acid and 25 mL of 0.01 M iodine were added to the flask. The solution is protected from light, after 20 minutes the excess of iodine was titrated with 0.01 M sodium thiosulphate using starch mucilage, added towards the end of the titration, as an indicator. (ii) In the other aliquot of 10 mL of CFZ (1000 µg/mL) was added to the 20 mL of the acetate buffer solution and 25 mL of 0.01 M iodine. After twenty minutes it was titrated with 0.02 M sodium thiosulphate using starch mucilage as indicator, added towards the end of the titration. A blank titration was performed. The difference between titrations represents the volume of 0.01 M iodine equivalent to the CFZ present. Each mL of 0.01 M iodine is equivalent to 4.675 mg of CFZ (measured as C₁₄H₁₃N₈NaO₄S₃).

Method

Three titrimetric methods, acidimetric, non-aqueous and iodometric, were developed according to precision and accuracy. The content percentage of CFZ was calculated using the equation proposed by AOAC [63].

a) Precision: Repeatability (intraday) and intermediate precision (interday) were evaluated. Repeatability was studied by the assay of independent samples, during the same day under the same experimental conditions. Intermediate precision was evaluated by comparing the results obtained on 3 different days.

b) Accuracy: This parameter was determined by the recovery study, comparing the theoretical and calculated concentrations of known amounts of CFZ reference added to the sample.

The percentage recovery (R%) was calculated according to AOAC [63], which is demonstrated by Equation 2.

$$R\% = (C_f - C_u / C_A) * 100 \quad (2)$$

Where:

C_f = Concentration of reference + concentration of sample

C_u = Concentration of sample

C_A = Concentration of the reference added to sample

For the acidimetric method, the equivalent to 1000 mg of CFZ reference was dissolved in 100 mL volumetric flask using water as the diluent to produce concentration solution of 10 mg/mL. Amounts of CFZ sample (equivalent to 500 mg of CFZ) were transferred to 250 mL flasks (R1, R2 and R3) and dissolved in 25 mL of carbon dioxide-free water (neutralized with 0.1 M hydrochloric acid). Portions of 2.5, 5.0 and 10.0 mL of CFZ reference (10.0 mg/mL) were added to flasks R1, R2 and R3, respectively. Aliquots of 25.0 mL of 0.1 M sodium hydroxide were added and heated on a water bath at 80°C for 20 minutes. After the solutions were cooled, 2 drops of phenolphthalein solution were added in each flask and the excess of sodium hydroxide was titrated with 0.1 M hydrochloric acid. A blank was performed and the difference between the titrations represented the volume of 0.1 M sodium hydroxide equivalent to CFZ present. This procedure was performed in triplicate. Each mL of 0.1 M sodium hydroxide is equivalent to 47.65 mg of CFZ (as anhydrous base).

For non-aqueous method, the equivalent to 1250.0 mg of CFZ reference was dissolved in glacial acetic acid to produce 100 mL (12.5 mg/mL). Amounts of CFZ sample (equivalent to 250.0 mg of CFZ) were transferred to 250 mL flasks (R1, R2 and R3) and dissolved with 30 mL of glacial acetic acid. Portions of 1, 2 and 4 mL of CFZ reference (12.5 mg/mL) were added to flasks R1, R2 and R3, respectively. After, 2 drops of crystal violet solution were added in each flask and the solutions were titrated with 0.1 N perchloric acid until the blue-green color. A blank was made. This procedure was performed in triplicate. Each mL of 0.1 N perchloric acid is equivalent to 47.65 mg of CFZ (as anhydrous base).

For iodometric method, recoveries were determined by adding known amounts of CFZ reference (0.5, 1.0 and 2.0 mg) to the samples. The equivalent of 500.0 mg CFZ reference was dissolved in water to produce 100 mL (5000 µg/mL). Amounts of 1, 2 and 4 mL of this solution were added to the sample, (i) aliquot of 10 mL, and transferred to a flask and added 5 mL of 1 M sodium hydroxide. After 20 minutes, 20 mL of acetate buffer solution, 5 mL of 1 M hydrochloric acid

and 25 mL of 0.01 M iodine were added and the flask closed. After 20 minutes, protected from light, excess of iodine was titrated with 0.02 M sodium thiosulphate, using starch mucilage, added towards the end of the titration, as indicator. (ii) In the other aliquot of 10 mL of CFZ (1000 µg/mL) was added 20 mL of the acetate buffer solution and 25 mL of 0.01 M iodine. After twenty minutes it was titrated with 0.02 M sodium thiosulphate using starch mucilage as indicator, added towards the end of the titration. The difference between titrations represents the volume of 0.01 M iodine equivalent to the CFZ present.

c) **Method comparison:** Results obtained in this study were compared between of them.

RESULTS AND DISCUSSION

The methods used water as solvent. It is environmentally friendly, low cost and easy to handle.

Comparison of methodologies is important to verify equivalence between them, within a given range and whether the variability of procedures differs significantly. USP37 [19] recommends evaluating the accuracy and precision parameters to prove the equivalence of the methodologies.

Table 1 shows the experimental values obtained in the precision parameter for quantification of CFZ using acidimetric, iodometric and non-aqueous methods.

Table 1. Values of precision for the determination of CFZ by the three titration methods.

Sample	CFZ content (%)		
	Acidimetry	Iodometry	Non-aqueous
Day 1	100.04	100.5	100.64
Day 2	100.18	100.21	100.70
Day 3	100.61	100.49	100.32
Mean (intra-day)	100.28	100.40	100.55
R.S.D (%)	0.59	0.31	0.40

R.S.D.=Relative standard deviation

Figure 2 shows the relationship between the levels obtained in the assay CFZ lyophilized powder and their respective standard deviations over the six tests for the methods proposed in this work.

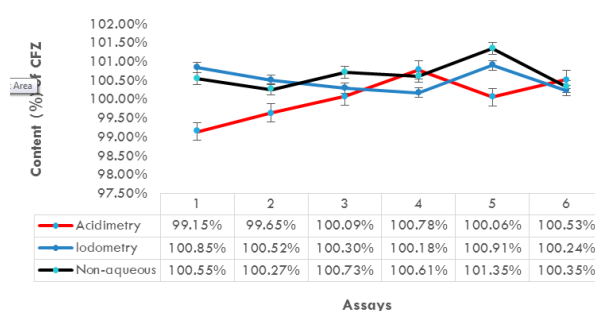


Figure 2. Relationship between the concentrations obtained in the assay CFZ lyophilized powder and their respective standard deviations over the six tests for the methods of acidimetry, iodometry and volume in a non-aqueous medium.

Results obtained in the acidimetric, iodometric and non-aqueous methods showed R.S.D. less than 2%, confirming the precision, according **Table 1**.

The mean recovery of acidimetric, iodometric and non-aqueous methods are shown in the **Table 2**.

Table 2. Values obtained in the recovery test for CFZ by acidimetric, iodometric and non-aqueous methods.

Methods	Added (mg)	Found (mg mL ⁻¹)	Recovery (%)
Acidimetry	25	525.06	100.24

	50	550.07	100.14
	100	600.28	100.68
Iodometry	5	105.03	100.2
	10	110.08	100.5
	20	120.06	100.2
Nonaqueous methods	12.5	262.57	100.56
	25	275.07	100.28
	50	300.29	100.58

Pharmacopeias does not recommend acidimetric, iodometric and non-aqueous methods for cephalosporins analysis, in the case CFZ. But, the proposed methods showed be simple, low cost, fast, easy execution and they did not require of a sophisticated equipment, only glassworks.

The methods can be considered satisfactory because, in comparison, the RSD (%) values for accuracy are less than 5% and close to 100%.

Another way to prove the equivalence of the methods is to compare the dosing data using Fisher's F-Test and Student's t-Test for parametric data [64]. These data are shown in **Table 3**.

The results obtained through the Student's t-test showed no statistical difference between the proposed methods, at a significance level of 5%. So, the methods can be considered equivalent and can be interchangeable for quantification of CFZ sample.

Table 3. Values of Test-F and t-Test for the determination of CFZ sample by acidimetry, iodometry and volumetry in non-aqueous medium.

Methods	F _{tab}	F _{cal}	t _{tab}	t _{cal}
Acidimetry x Iodometry	19	3.26	2.78	-0.63
Iodometry x Volumetryin non-aqueous medium	19	1.54	2.78	1.01
Acidimetry x Volumetryin non-aqueous medium	19	2.11	2.78	-1.33
*Significant at p <0.05				

CONCLUSION

The acidimetric, iodometric and non-aqueous methods are effective and interchangeable for quantification analysis of CFZ powder for solution for injection.

They can also be considered simple to run, low cost, fast and environmentally friendly, making it a great choice for the routine use of laboratories and pharmaceutical industries.

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