Advances in Analytical Chemistry 2012, 2(5): 67-73
DOI: 10.5923/j.aac.20120205.04

Quatitative Methods for the Identification of Cefuroxime Sodium

Daniela Cristina de Macedo Vieira*, Hérida Regina Nunes Salgado

Department of Drugs and Medicines, School of Pharmaceutical Sciences, UNESP – UnivEstadualPaulista, Araraquara, 14801-902, Brazil

Abstract From this study, methods for quantitative analysis were developed to determine cefuroxime sodium in the raw material and in pharmaceutical formulations. These tests included instrumental methods such as spectrophotometry in the ultraviolet, visible and infrared, high performance liquid chromatography and potentiometric determination by microbiological methods (agar diffusion and turbidimetric). The concentration range determined for each method varied greatly, which increases the usability of cefuroxime fori dentifying quality control. In spectrophotometric methods cefuroxime was determined from 5.0µg/ml to 2.0mg/mL.The microbiological methods have determined the drug concentration 30.0-120.0 µg/ml and HPLC ranged from 10.0to15.0µg/ml.The results obtained by analysis of the dosage form were compared to results obtained by analysis of the reference substance and showed significance. These methods proved to be reproducible and rapid determination of cefuroxime can be routinely used in quality control analysis.

Keywords Quantitative Analysis, Quality Control, Cefuroxime

1. Introdution

Cefuroxime is a second generation cephalosporin first commercially available to be widely used in therapy.

In the last decade the study of the cephalosporin molecule has been supported by the acquisition of knowledge of structure-activity relationships of potential sites for replacement of the parent molecule cephalosporin and allows the "construction" of molecules increasingly powerful[1].

Since the first cephalosporin administered orally, cephalexin was licensed in 1968, progress has been made in the development of analogues more stable beta-lactamase with a broad spectrum antibacterial and pharmacokinetic properties and better metabolic: cefuroxime sodium follows this tendency.

Cefuroxime {4 - (carbamoxylomymethyl) -8 -[2-2-furyl-2-methoxyimino-acetyl] amino-7-oxo-2-thia-6-azabicyclo[4 .2.0] oct-4-ene-5-carboxylic acid} (Figura 1) is a second generation cephalosporin injection is structurally similar to other third-generation cephalosporin such as cefotaxime and ceftazidime[2].

It is highly effective and safe for the treatment of respiratory and urinary infections, but not active in vitro against *Pseudomonas*, *Helicobacter* (*Campylobacter*), methicillin resistant *Staphylococcus aureus* and *S. epidermidis*[3].

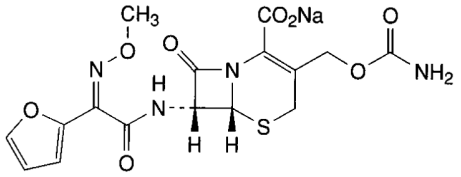

Figure 1. Structure of cefuroxime sodium

The literature highlights several methods for determination of cephalosporin[4-15], but specifically for cefuroxime no review of such methods.

The identification of drugs is a question basic efficacy and safety of the product produced. Failure to comply with the criteria for quality of raw materials used in the production of medicines can interfere with the quality biopharmaceutical, and the realization of fundamental physical and chemical analysisof the raw material to be used in the manufacture of medicines to ensure their suitability for pharmaceutical compendia.

Thus, objective of this study is to select and develop specific procedures for the quantitative determination of cefuroxime sodium in pharmaceutical products andin the presence ofβ-lactam antibiotics other and can be used routinely in analysis ofquality control in pharmaceutical industry.

2. Materials and Methods

2.1. Collection and Sample Preparation

We used reference substance (Batch G060123), kindly

* Corresponding author:
danicmvieira@yahoo.com.br (Daniela Cristina de Macedo Vieira)
Published online at http://journal.sapub.org/aac

provided by *Glaxosmithkline* (Rio de Janeiro, Brazil). The commercial samples (powder for solution for injection) for cefuroxime sodium were purchased from Cellofarm Pharmaceuticals under the trade name Zencef®. Each vial contains cefuroxime sodium equivalent to 750 mg - Batch: 7400444. The reference substance presented declared purity of 97.4%, purified water used in the preparation of the solutions were obtained by the Milli-Q system (Millipore®, California) and all reagents and solvents used were of analytical grade.

2.2. Spectrophotometric Methods

a) Ultraviolet spectrometry: ultraviolet spectrum was obtained in UV-VIS spectrophotometer Shimadzu UV-1240 using mini quartz cells of 1 cm optical path. The reading was performed at 280 nm. The calibration curve was determined in solutions prepared with water in concentrations ranging 5-14 µg/mL of sodium cefuroxime substance. Validation parameters were determined and after they became the determination of cefuroxime sodium in pharmaceutical form in triplicate.

b) Spectrophotometry in the visible region: the spectrum in the visible region was obtained using UV-VIS spectrophotometer equipment Shimadzu UV mini-1240 and quartz cells of 1 cm optical path. Measurements were taken at 510 nm, and the calibration curve determined with solutions prepared in purified water at concentrations ranging from 100.0 to 300.0 µg/mL. Were added to each solution 1 ml of solution of Fe (III) and 0.01 M solution of 500 mL of 0.02 M o-phenanthroline. Validation parameters were determined and after they became the determination of cefuroxime sodium in pharmaceutical form in triplicate.

c) Spectrophotometry in the infrared region: we used conventional spectrophotometer Shimadzu (Columbia, USA) with scan spectra that allows for analysis of electronic files. Equivalent weight were taken at 0.5, 0.7, 1.0, 1.5 and 2.0 mg of cefuroxime sodium reference substance (previously diluted in potassium bromide 1:10) and diluted in potassium bromide (Merck PA) previously dried to constant weight and the pellets were prepared for reading with a total of 250 mg per tablet. The absorbance readings were performed in order to evaluate the intensity of peaks. Validation parameters were determined and after they became the determination of cefuroxime sodium in pharmaceutical form in triplicate.

2.3. Methods Microbiological

a) Agar diffusion assay: the culture media, as well as purified water, were sterilized by autoclaving at 121°C for 15 minutes. Petri dishes were used with 20.0mm high and 100.0 mm diameter stainless steel and templates. These materials were sterilized in an oven at 200°C for 1 hour. Were chosen *Micrococcus luteus* (ATCC 9341) as standard microorganism at a concentration of 2.0%, means No. 1 and No. 2 Grove-Randall for base layer and surface respectively and water as diluent solution concentrations of the drug in 30.0, 60.0 and 120µg/ml. The standardization of the assay was performed using 3 x 3 technique described for other antimicrobial agents[16]. Validation parameters were determined and after they are made to determine the potency of cefuroxime sodium in pharmaceutical form in triplicate.

b) Turbidimetric method: test tubes were used with 20.0 cm high and 4.0 cm in diameter were sterilized by autoclaving at 121°C for 15 minutes, and the purified water. For the incubation of microorganisms used to shaker incubator unit Marconi MA420® model for bacterial culture and greenhouse ECB Digital 1.2 (Odontobrás®). The readings were taken in a spectrophotometer BeckmanDU® 530 model. *Micrococcus luteus* were chosen (ATCC 9341) microorganism as standard at a concentration of 2.0% BHI broth and water as the diluent solution of the drug concentrations 30.0, 60.0 and 120 µg/mL. Validation parameters were determined and after they are made to determine the potency of cefuroxime sodium in pharmaceutical form in triplicate.

2.4. Chromatographic Methods

a) High performance liquid chromatography: analyses were performed on liquid chromatograph Waters 1525 Binary HPLC with UV detection Tump/Vis (Milford, MA), manual injector Rheodyne 7725i Breeze and employed 054,275 Wat C18 column (Milford, MA) dimensions 150 mm x 4.6 mm and particle size of 5 µm and pre-column Waters. The mobile phase was prepared from a mixture of methanol (HPLC grade) and water (purified by reverse osmosis) in the ratio 70:30, v:v, filtered through a membrane of 0.45 µm in pore size and 47 mm diameter, under vacuum, and degassed for 15 minutes. After stabilization of the system were injected preset volumes of 20.0 µL of solution previously filtered through a membrane. The calibration curve was constructed with solutions of concentration between 10.0 to 15.0 µg/ml of cefuroxime sodium. Validation parameters were determined and after they became the determination of cefuroxime sodium in pharmaceutical form in triplicate.

3. Results and Discussion

Ultraviolet spectrophotometry:the ultraviolet absorption spectra are useful tools in the identification of organic compounds and elucidation of its structure, however detailed the relationship between molecular structure with absorption bands is quite extensive, but information relating to a compound of unknown structure can sometimes be obtained by direct comparison of its absorption spectrum with the model compounds of known structure[17]. The calibration curve was made by plotting absorbance values versus the concentration of reference substance and Beer's Law was valid in the range 5.0 to 14.0 µg/mL. The correlation coefficient (0.9996) and the equation of the line slope, $y = 0.0412x - 0.0328$ were calculated using the method of least squares. The molar absorptivity value was $1.15 \times 10^4 L\ mol^{-1} cm^{-1}$. The repeatability of the method was determined

by analysis of six replicates of the sample, each at concentration of 10 µg/mL. At this concentration, the relative standard deviation was 1.40%. The effectiveness of the method was evaluated by calculation of the *F*-test (variance ratio) and compared with the official method[18; 19]. Measurements of *F*-test values were obtained with 95% confidence (Table 1). The results show that the calculated *F* (2.81) does not exceed the theoretical value of 3.11 which is not significantly different from the official method[20].The accuracy was determined by the recovery test. Was obtained in this test recovery of 100.83% which is satisfactory and is a mandatory requirement for the validation of analytical methods. The accuracy of the method was obtained using six determinations of cefuroxime sodium injection, each preparation being carried out in triplicate and the measurements of different preparations. The robustness evaluated the reliability of the method proposed by changing the pH, which rose from 7.2 to 6.8 and by changing the value of that wavelength was changed of 280.0 nm and 283.0 nm. It can be seen that even with the change in these parameters the values found in three determinations of cefuroxime sodium in the product are in the level set that is 99-102% of the content. The average content of cefuroxime sodium in vials was 749.19 mg (99.89%) determined for six consecutive days and in triplicate, and each measurement originates from distinct solutions. The spectrophotometric method has low specificity for compounds containing β-lactam ring which absorb in the range of 250-270 nm[21]. However, the proposed method and the literature[22] show that this method has a good specificity and selectivity for the determination of sodium cefuroxime in the injectable pharmaceutical form.

Table 1. Analysis of variance of the absorbance values determined in obtaining the analytical curve of cefuroxime sodium (reference substance) using the spectrophotometric method in the UV region

Sourcesofvariation	df	SS	MS	F cal	Ftab
between	5	0.3163	0.06326	361.5*	3.11
linear Regression	1	0.3160	0.3160	320.5*	4.75
Deviation from linearity	4	0.002244	0.000561	0.05	3.26
residue	12	0.0021	0.000175		
total	17	0.3184			

Spectrophotometry in the visible region: the region of the visible in pharmaceutical analysis is widely used for identification and assay of drugs and medicines. All spectrophotometric techniques are based on chemical interaction with a radiant energy and, in most cases; the effect of this interaction is the energy absorption by the material being analyzed[23; 24]. Different parameters affect the oxidation reaction and therefore the determination of cefuroxime sodium was optimized. These include the acid, the concentration of solutions of Fe (III) and o-phenanthroline and reaction temperature. The quantitative formation of the complex can be completed in the pH range between 2 and 9[25].Different pH values in the range between 3 and 5 were tested. At pH> 5.0, the solubility of o-phenanthroline was incomplete. The maximum analytical signal was observed at pH 4.2, and then the solution of o-phenanthroline was maintained at this pH value. The effect of acidification of the solution of Fe (III) on the absorbance was observed and the change the concentration of H_2SO_4 (0.3 to 1.4 M) in the absorbance of the formed complex show an increase of absorption to a value of 0.8 M. The influence of the concentration of Fe (III) in the analytical signal was also investigated and it was observed a maximum absorbance obtained with a solution of Fe (III) 0.01 M, which was used for all analysis. Different concentrations of o-phenanthroline in the range of 0.01 to 0.08 M citrate buffer were tested. At low concentrations, the peak separation was observed due to incomplete reaction. With increasing concentration of o-phenanthroline, absorbance also increased showing a maximum concentration of 0.06 M was used throughout the experiment. The absorption spectrum showed absorption peak maximum at 510.0 nm, using an aqueous solution of sodium cefuroxime to 500.0 µg/mL. The linear calibration curve and compliance with Beer's law was proven by values of correlation coefficients, 0.9991 in the concentration range from 100.0 to 300.0 µg/mL. The equation of the line was y = 0.0751 x + 0.179. The intraday precision was evaluated by analysis of five different concentrations and betweendaysprecision was similarly evaluated on three different days. The results in both tests showed high accuracy, and the relative standard deviation did not exceed 2% (Table 2). The method performance was determined by the value will value *F*. The results showed that the calculated values of t and *F* did not exceed the theoretical value with 95% confidence[20].These results indicate that this method does not differ significantly from the official method which is the high performance liquid chromatography. The recovery test was performed with the objective of demonstrating accuracy of the procedure. Thus, known amounts of reference substance were added to solutions of sodium cefuroxime sample for quantification of the substance. The average recovery was 99.98%. With this result proved the correctness of the proposed method, which is one of the mandatory requirements of the analytical methods. The robustness evaluated the reliability of the method proposed by the change of equipment and small changes of the value of that wavelength 510.0 nm and 515.0 nm was changed to. It can be seen that even with changing these parameters, the values found in three determinations of sodium cefuroxime in the product are in the level set that is 99.0-102.0% of the content.

Table 2. Quantities determined for the assay sample cefuroxime sodium (750.0 mg) by the spectrophotometric method in the visible region at 510.0 nm

Assays	Abs*	amount found (mg)	percentage	Mean ± m.s.e.	RSD(%)
I	0.295	764.39	101.92		
II	0.299	755.07	100.68		
III	0.299	735.23	98.03	99.82 ± 0.66	1.48
IV	0.299	736.66	98.22		
V	0.297	749.51	99.93		
VI	0.296	751.06	100,14		

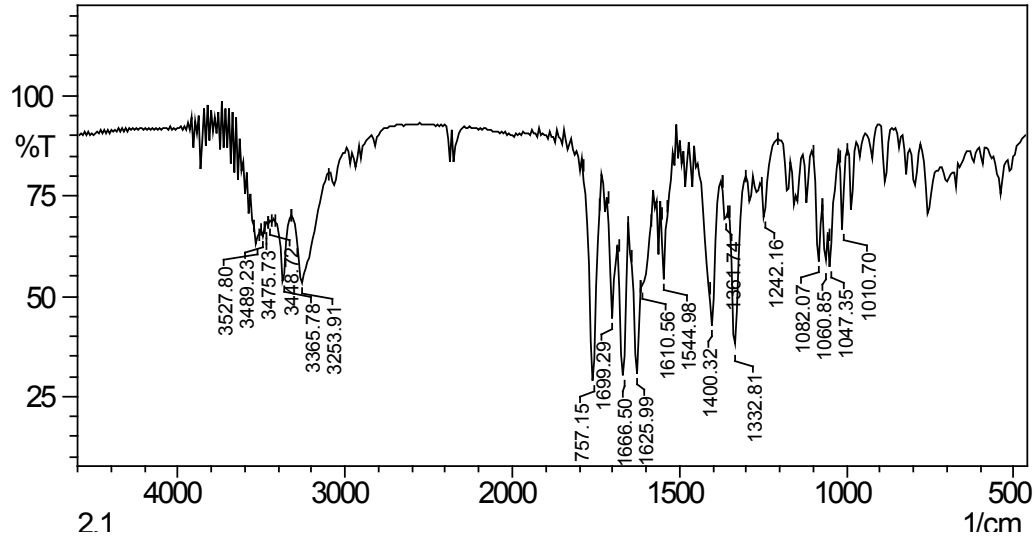

Figure 2. IR spectrum in the region of cefuroxime sodium sample into KBr pellets

The infrared spectrophotometry: in the first step of the development work of the analytical method has been studied the infrared spectrum of cefuroxime sodium (reference substance) to identify the bands of the spectrum, which showed that cefuroxime sodium has an intense band at 3365.78 cm^{-1} (Figure 2) which is assigned to the primary amine grouping of the molecule. The results showed a linear relationship between the concentrations of cefuroxime sodium reference substance used in the preparation of tablets versus absorbance, with the possible construction of the calibration curve, which showed the straight line equation y = 0.5053x - 0.0114 and coefficient of linear regression of 0.9991, indicating the linearity of the method and therefore the possibility for it to be used for quantification of cefuroxime sodium. The results for the drug were 100.25%, showing good accuracy and precision, with relative standard deviation of 1.00%. The recovery test was performed with the objective of verifying the accuracy of the proposed method and was 99.83%. With this result was confirmed the accuracy of the proposed method, which is one of the mandatory requirements of the analytical methods. The relative standard deviation was 0.82%. Currently, the combination of near infrared spectrophotometry and multivariate calibration has been widely used in the determination of various substances including, pharmaceuticals and food products and is pointing to the emerging methods of quantitative analysis[26]. Thus, we conclude that the present investigation demonstrated that infrared spectroscopy, hitherto employed for the identification of substances, can be extended to the quantification of drugs, making possible its application in routine analysis of quality control.

Microbiological analysis in agar diffusion: the quantification of antimicrobial agents by chemical methods, such as high performance liquid chromatography and spectrophotometry to show accurate, but do not provide true indication of biological activity[27]. An advantage of this is determining not require specialized and expensive equipment, and does not use solvents potentially toxic to the analyst, and for the environment. Although microbiological methods show a relatively simple performance, many aspects must be considered in its development, to ensure they are sufficiently safe and accurate, such as incubation temperature, thickness of the agar, microorganism used, and the diluent solution pH between other characteristics. To ensure the validity of the dissemination of microbiological assay agar, in determining the power of cefuroxime sodium, three different concentrations were used to sample the same as the reference substance, of 3 × 3 design (Figure 3)[16]. These doses are in a geometric progression as the system used has a linear relationship between the logarithm of the concentration of substance tested and the diameters of the inhibition zones. The results obtained analytical curve was constructed with a solution of 30.0, 60.0 and 120.0 µg/mL. The solutions were made in triplicate, with a correlation coefficient equal to 0.9999. The equation of the line was y = 1.8205 Ln (x) + 19.297. The assay method of the cylinder agar diffusion plates developed and validated for cefuroxime sodium proved to be precise, accurate and linear. The recovery test was performed with the objective of verifying the accuracy of the proposed method. Was obtained in this test an average recovery of 100.77%. The accuracy of the method was obtained using six determinations of cefuroxime sodium sample. The relative standard deviation was 0.3376%, and can reach 15% according to official literature [16; 19; 20], and the content of cefuroxime sodium was determined in the sample calculated by the equation Hewitt[28] to obtain an average content of 99.96%, demonstrating the proximity of the results obtained by the proposed method with reference values accepted as that for the finished product should vary no more than 10% the nominal value, according to references pharmacopoeia[16; 19; 20]. Among the variables evaluated in the trial, those who need to be completed to validate the proposed method are linear regression and the range of doses, which should be

significant. The variation between preparations, the deviation from parallelism, linearity deviation and variation between boards should be insignificant. In all tests these conditions are met (Table 3). The proposed method consists of a technique for expressing the potency of the product since it has sensitivity, linearity, accuracy and precision appropriate second validation concepts presented in the Brazilian Pharmacopoeia[16] and Riley and Rosanske[29].

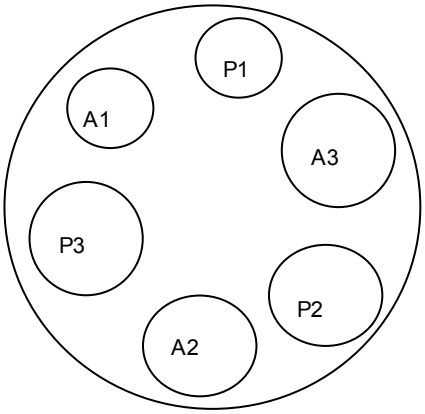

Figure 3. 3 x 3 Design for cefuroxime bioassay, where: S: standard and T: test. S1 (30 µg/mL); S2 (60 µg/mL); S3 (120,0µg/mL); T1 (30 µg/mL); T2 (60 µg/mL) and T3 (120,0 µg/mL)

Analysis by the turbidimetric method: allows the determination of drug potency, by measuring the turbidity (absorbance), caused by inhibition of microorganism by antimicrobial. According to the Brazilian[16] andBritish[20] Pharmacopoeia, the model of parallel lines is used, the two lines dose - response of the sample and the reference substance should be parallel and linear at the concentrations used in the calculation. These conditions are verified by statistical analysis, described in these codes. Statistical analysis showed that there is no deviation from linearity of the calibration curves derived from reference substance and sample. The correlation coefficients are 0.9997 and 0.9991 for the reference substance and the sample, respectively, values close to unity, which are excellent in the case of a bioassay (Figure 4). It was shown statistically that there is no significant difference in the slope of the analytical reference substance and sample.

The coefficient of variation of the absorbance obtained by the growth inhibition assay of sample on average 0.95%, less than 5% of the recommended[30]. The selectivity was determined by calculating the homogeneity of variances (*F*) and by comparing the mean (*t*) being the two variables as expected, ie calculated value less than tabulated value. The amount of cefuroxime sodium present in the samples, 99.37%, is in accordance with the official compendium which is from 90.0 to 110.0%[16; 18]. The accuracy was confirmed by the recovery test, estimated the average of 100.81%. The parameters studied to validate the turbidimetric method met the specifications for the proper quantification of cefuroxime sodium in injectable dosage form. The proposed method is a technique for expressing the potency of the product, since it presents sensitivity, linearity, repeatability, accuracy and precision appropriate[16]. It is also advantageous because it does not use toxic reagents and solvents and is faster than the agar diffusion method, as are required only 4 hours of incubation, while the other requires at least 18 hours.

High performance liquid chromatography: a high performance liquid chromatography (HPLC) is a technique frequently used in the quantification of drugs in pharmaceutical formulations, one of the most advanced techniques of separation and quantification of substances on the timescale of a few minutes withhigh resolution, efficiency and sensitivity. According to USP[18], mobile phase used for determining comprises cefuroxime sodium acetate buffer pH 3.4 and acetonitrile, however it is known that the end caps damaging the column over time, which makes the analysis of HPLC more complicated, because the inorganic salts can damage the spine, through the interaction with the silica[25; 31; 32]. Therefore, we chose to use methanol-water (70:30) as mobile phase which was a great improvement in methodology. The linearity of this method was found in the range 10.0 to 15.0 µg/mL. The values for the intercept and slope of the calibration curve were 67.334 and 114.396, respectively, with correlation coefficient of 0.9992 (Figure 5). Statistical analysis showed that no significant deviation from linearity of the concentrations used and that there is linear regression on data obtained at the confidence level of 1%. The average content determined cefuroxime sodium injectable pharmaceutical form was 99.84%, the limits of variation within day of analysis and had a mean 1.04 days between 2.01% for the test accuracy. The accuracy expressed as the percentage recovery is 100.10%.

Table 3. Analysis of variance of data obtained from the assay of cefuroxime sodium by microbiological agar diffusion assay

Sourcesofvariation	df	SS	MS	F cal	Ftab
preparation	1	2,523	2,523	5,019*	4,24
regression	1	217,08	217,08	173,688*	4,24
Deviation of parallelism	1	4,084	4,084	3,267	4,24
quadratic	1	0,005	0,005	0,004	4,24
Quadratic differential	1	0,350	0,350	0,280	4,24
between doses	5	224,043	44,809	35,851*	2,6
between plate	5	1,067	2,613	1,093	2,6
Within (error)	25	31,246	1,250		
total	35	388,36			

* significant p < 0,05

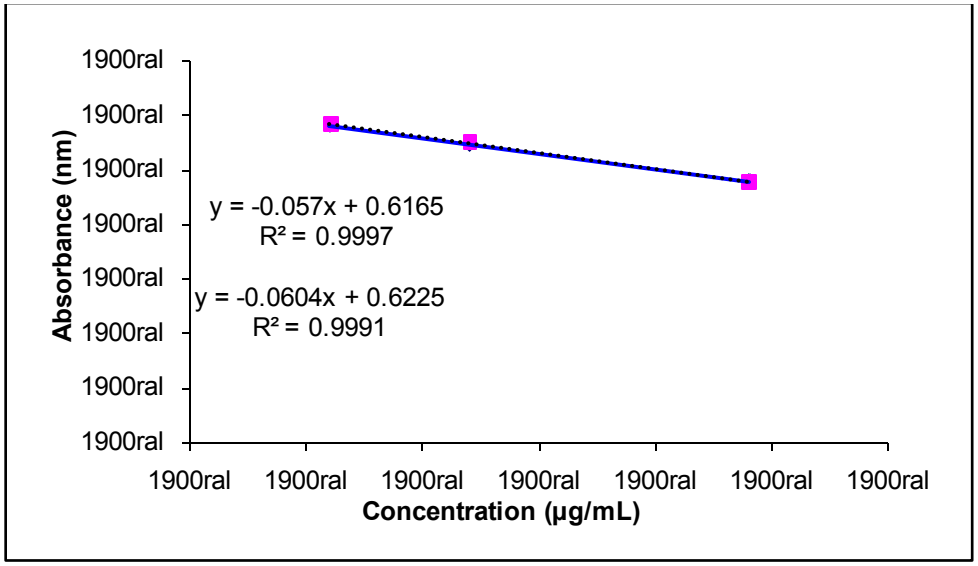

Figure 4. Analytical curves for solutions of sodium cefuroxime reference substance and sample in concentrations of 30.0, 60.0 and 120.0 µg/mL, obtained by turbidimetric method

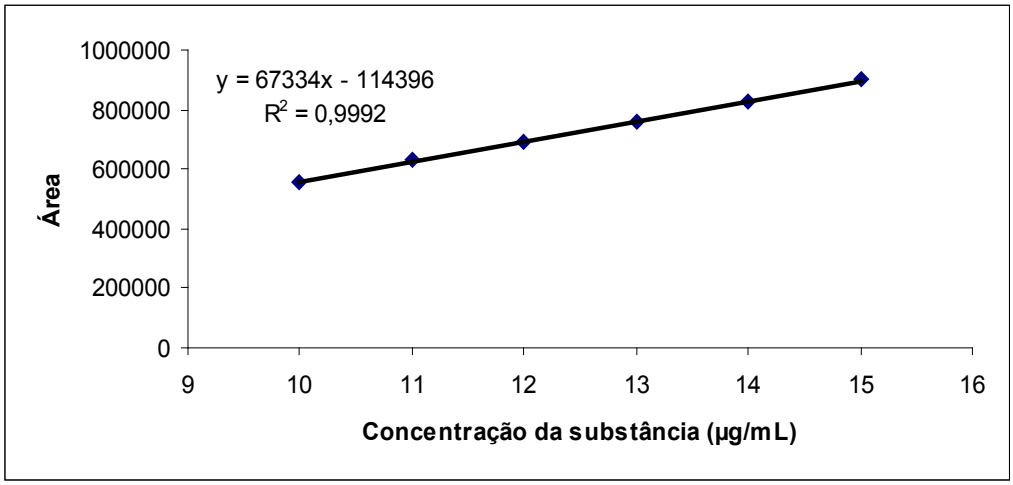

Figure 5. Graphic representation of the analytical curve of the solution in concentrations of cefuroxime sodium 10-15 µg/mL by HPLC

4. Conclusions

The chemical and physicochemical properties of a drug are in many cases determinants in the process of analytical quality control, so that the chemical nature of the molecule can favorably or unfavorably interfere with the mechanisms of interaction with other substrates, making it more selective. Moreover, different methods, simple or complex are emerging every day in an attempt to become more viable and operational routine analysis of a substance. In our work we quantification of cefuroxime (widely sold in the Brazilian market) in raw material and in pharmaceutical preparations, showed to be reproducible to identify it and distinguish it, may be adopted in routine analysis of quality control industrial pharmaceuticals

ACKNOWLEDGEMENTS

Authors thank GlaxoSmithkline Ltda for providing cefuroxime reference substance. This work was supported by PACD-FCFAr-UNESP-Brazil, FUNDUNESP-Brazil, FAP ESP-Brazil and CNPq-Brazil.

REFERENCES

[1] Neu HC. Beta-lactam antibiotics: structural relationships affecting in vitro activity and pharmacologic properties. Rev Infect Dis. 1986; 8: 237-259.

[2] Amin AS, Ragab GH. Spectrophotometric determination of certain cephalosporins in pure form and in pharmaceutical formulations. SpectrochimActa. 2004; 60: 2831-2835.

[3] Brunton LL,Lazo JS, Parker KL. Goodman & Gilman: As Bases Farmacológicas da Terapêutica, New York:McGraw-Hill Book Co.2006.

[4] Adamis G,Papaioannou MG,Giamarellos-Bourboulis EJ, et al.Pharmacokinetic interactions of ceftazidime, imipenem

and aztreonam with amikacin in healthy volunteers. Antimicrob. AgentsChemother2004;23: 144-149.

[5] Altinoz S,Ozer D,Temizer A, et al.Determination of ceftriaxone in aqueous humour and serum samples by differential-pulse adsorptive stripping voltametry. Analyst. 1994; 119: 1575-1577.

[6] AlyFA,Hefnawy MM,Belal, FA. Selective spectro-fluorimetric method for the determination of cephalosporins in biological fluids. Anal. Lett. 1996; 29: 1-10.

[7] Dimitrovska A, Andonovski B, Stojanoski K. Spectrophotometric study of copper(II) ion complexes with cefaclor. Int. J. Pharm. 1996; 134: 213-221.

[8] Yang JH, et al. Simultaneous determination of cephalexin and cephadroxil by using the coupling technique of synchronous fluorimetry and H-point standard additions method. Anal. Chim. Acta. 1996; 325: 195-200.

[9] Reddy GVS, Reddy SJ. Estimation of cephalosporin antibiotics by differential pulse polarography. Talanta. 1997; 44: 627-631.

[10] El-Mammly MY. Spectrophotometric determination of flucoxacillin in pharmaceutical preparations some nitrophenols as a complexing agent. Spectrochim. Acta. 2003; 59: 771-776.

[11] Adamis G,et al. Pharmacokinetic interactions of ceftazidime, imipenem and aztreonam with amikacin in healthy volunteers. Antimicrob. Agents Chemother. 2004;23: 144-149.

[12] Zivanovic L. et al. Investigation of chromatographic conditions for the separation of cefuroxime axetil and its geometric isomer.J. Chromatogr. B. 2004; 800: 175-179.

[13] Tozo GCG, Salgado HRN. Determination of lomefloxacin in raw material and tablet preparations by liquid chromatography.J. AOAC Int. 2006; 89: 1305-1308.

[14] Aly, HM, Amin AS. Utilization of ion exchanger and spectrophotometry for assaying amoxycillin and flucloxacillin in dosage form. Int. J. Pharm.2007; 338: 225–230.

[15] Tozo GCG, Salgado HRN. Microbiological assay for cefoxitin sodium in dosage form. J. AOAC Int.2007; 90: 452-455.

[16] FARMACOPEIA brasileira. 5.ed. São Paulo: Atheneu, 2010. 5.5.3.3.1, p. 264.

[17] Ewing GW. Métodos instrumentais de análise química. São Paulo: Edgar Blücher, 2001.

[18] THE UNITED STATES PHARMACOPEIA (USP 32). The National Formulary (NF 34). By authority of United States PharmacopeialConvention. Rockville, MD: United States Pharmacopoeial Convention, INC, 2011b. p. 683.

[19] BRITISH Pharmacopoeia 2010. London: The Stationary Office, 2010. v. 2, p. 2475.

[20] Rodrigues PC. Bioestatística. Rio de Janeiro: Universitária, 1986. p. 98-100.

[21] Martinèz LG, Falcó PC, Cabeza AS. Comparison of several methods used for the determination of cephalosporins. Analysis of cephalexin in pharmaceutical samples. J. Pharm. Biomed. Anal. 2002; 29: 405-423.

[22] Chaudhari, et al. Simultaneous UV spectrophotometric method for the estimation of cefuroxime axetil and probenecid from solid dosage forms. Indian J. Pharm. Sci. 2006; 68: 59-63.

[23] Watson, DG. Pharmaceutical analysis: a textbook for pharmacy students and pharmaceuticals chemists. London: Churchill Livingstone, 1999. 337p.

[24] Ohannesian; S. Handbook of Pharmaceutical Analysis. New York: Informa HealthCare, 2002. 585p.

[25] Al-Momani, I.F. Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. J. Pharm. Biomed. Anal. 2001; 25: 751-757.

[26] Morgano MA, et al. Determinação de proteína em café cru por espectroscopia NIR e regressão PLS. Cienc. Tecnol. Alim, 2005; 25: 25-31.

[27] Adams E, et al. Neomycin: microbiological assay or liquid chromatography? J. Pharm. Biomed. Anal. 1998; 17: 757-766.

[28] Hewitt, W. Microbiological assay for pharmaceuticals analysis: a rational approach. New York: Academic Press, 2004.

[29] Riley CM,Rosanske TW. MicroAnalytica. Anal. Chim. Acta. 1997; 338: 362.

[30] BRASIL. Leis, decretos, etc. Resolução nº 899 de 29 de maio de 2003. Guia para validação de métodos analíticos e bioanalíticos. Diário Oficial da República Federativa do Brasil, DF, 02 jun. 2003.

[31] Snyder LR, Kirkland JJ,Glajch JL. Practical HPLC method development. 2nd. ed. New York: John Wiley & Sons, 1997. v. 1, p. 292-316.

[32] Watson, D.G. Pharmaceutical analysis: a textbook for pharmacy students and pharmaceuticals chemists. London: Churchill Livingstone, 1999. 337p.