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DEVELOPMENT AND VALIDATION OF A UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF CEPHALEXIN MONOHYDRATE IN HUMAN PLASMA

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Abstract

A simple, rapid and precise spectrophotometric method has been developed for the determination of cephalexin monohydrate in plasma. The method was developed via scan of a standard cephalexin solution at a concentration of 15 µg/ml and obtained a λmax of 261 nm and validated in terms of linearity, accuracy and can be applied for routine estimation of cephalexin monohydrate powder. This method utilized 40 % perchloric acid to precipitate plasma proteins after spiking the plasma with different aliquots of cephalexin standard solution. The supernatant of this solution was measured at 261 nm (λmax) after buffering with phosphate buffer (pH 4) and volume adjustment with distilled water post-centrifugation to construct a 10 point calibration curve. It was linear in the range of 5-150 µg/ml with a correlation coefficient of 0.9994 and the percentage recovery was found to be 84-85.5 %.

Key words: Cephalexin monohydrate, UV spectrophotometer, Method validation

Introduction

Cephalexin is a semi-synthetic first generation oral cephalosporin antibiotic. It is 7-(D-α-amino-α-phenylacetamido)-3-cephem-4-carboxylic acid mxonohydrate (Thomson, 2014). It is widely used in the treatment of certain bacterial infections such as respiratory tract infections, otitis media, urinary tract infections (Thomson, 2014). Cephalexin is also used for the treatment of heart diseases due to its enhanced oral activity (Lai and Wu, 2003).

In literature, various analytical methods, such as Spectrophotometry (Panda *et al*, 2013), High Performance Thin Layer Chromatography (HPTLC) (Jeswani *et al*, 2009), High Performance Liquid Chromatography (HPLC) (Lee *et al*, 1990; Anika *et al*, 1997; Hammami and Rajaa, 2014) and HPLC-MS-MS (Melvin *et al*, 2014) have been developed for determination of cephalexin powder with

some of these methods having cases of low recoveries (82.3 %) (Paul *et al*, 1991). However, to the best of my knowledge there is no method reported for cephalexin monohydrate powder using UV-spectrocopy.

Extraction of an analyte from plasma samples require some degree of pre-treatment due to the matrix formed as a result of binding of this analyte to plasma proteins such as albumin, α-acid glycoproteins, lipoproteins, and γ-globulins (Aerts *et al*,1995). In literature, several precipitating agents have been used to precipitate plasma in order to get a good extract with recoveries greater than 70 % (Fedeniuk and Shand, 1998). For this study, we intend to use 40 % perchloric acid as a precipitating agent based on the fact that the degree of the acidity of this agent does not have much influence on the stability of cephalexin.

There are many substandard and fake drugs in the market and cephalexin monohydrate is not an exception. The quality or potency of which need to be ascertained in order to get a better therapeutic outcome, hence the need for this research work.

The aim of present research work is to develop and validate a simple, precise, and accurate UV-spectrophotometric method for the determination of cephalexin monohydrate in plasma.

Methodology

Apparatus and Materials

The present work was carried out on a Biomate 6 UV/Visible Spectrophotometer Model number 94230 bio 1102E with 1 cm matched quartz cells. Pure cephalexin powder was purchased from Sigma-Aldrich with product number C4895 and batch no. 066M4755V. Distilled water was used as a solvent.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 150 mg of standard cephalexin powder in 100 ml volumetric flask with distilled water to obtain a concentration of 1500 µg/ml solution. From that solution, 1 ml was taken and further diluted up to 10 ml with distilled water (150 µg/ml stock solutions).

Development of the Method

The solution of cephalexin was prepared in distilled water at a concentration of 15 µg/ml. This was scanned in the wavelength range of 200-400 nm and using 60 µg/ml solutions in different pH medium between 3 to 7 to determine the pH of maximum absorption. The wavelength and pH of maximum absorptions were found to be 261 nm and pH 4. The spectral data of the drug is shown in Figure 1.

Preparation of calibration curve

From the stock solution, different aliquots in the range 0.17 ml to 5 ml were transferred into series of 10 labeled volumetric flask

containing 1 ml of blank plasma, 20 µL of 40 % perchloric acid was added, vortex mixed for 1 minute and later centrifuged at the rate of 3000 rpm for 10 minutes. The supernatants were transferred to 5ml volumetric flask, buffered with 1 ml phosphate buffer (pH 4) and the final volume made up with distilled water to obtain serial dilutions of the concentrations 5.0-150 µg/ml. Their respective absorbances were determined at 261 nm against the blank. A plot of absorbance against the concentration gave the calibration curve. The plot of Beer's law is shown in Figure 2.

Method Validation

The developed method was validated for its linearity, precision and accuracy. The linearity of measurement was evaluated by

Results

Analyzing different concentrations of standard solution of cephalexin in the range of 5-150 µg/ml. The results are shown in table 1. The precision of the proposed method was determined by analyzing different concentrations (15-40 µg/ml) at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision). The results are shown in table 2 and 3. To ascertain the accuracy of the proposed method, recovery studies were performed by standard addition method. The results are shown in table 4. The LOD and LOQ were calculated from the equations, LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The results are shown in Table 1.

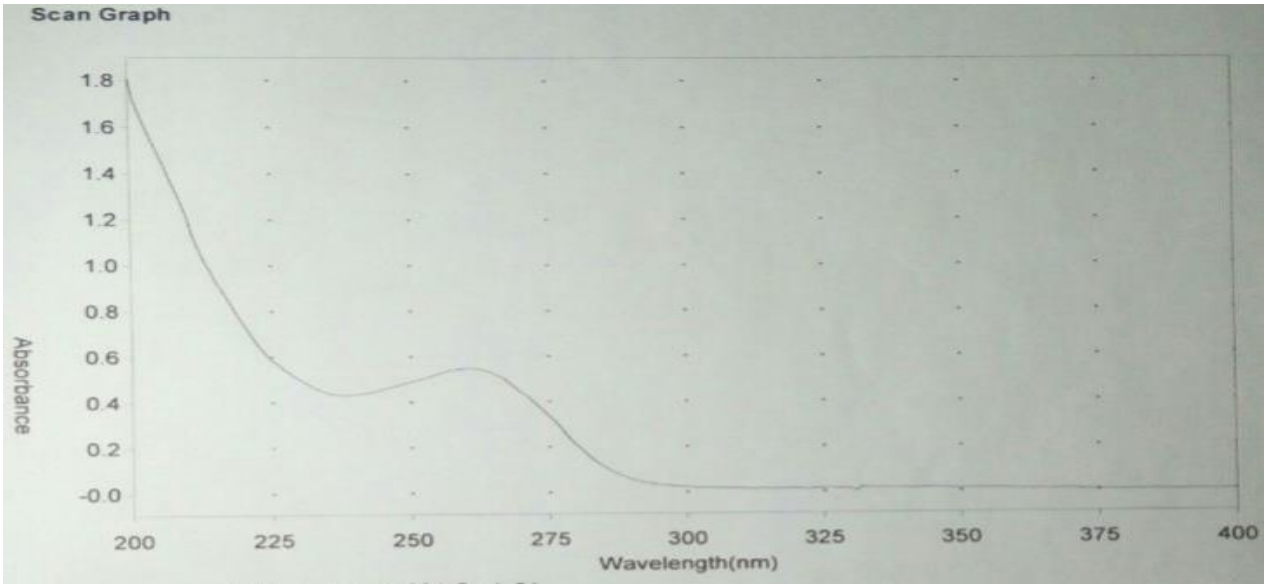

Figure 1: Absorption spectral data (at 261 nm)

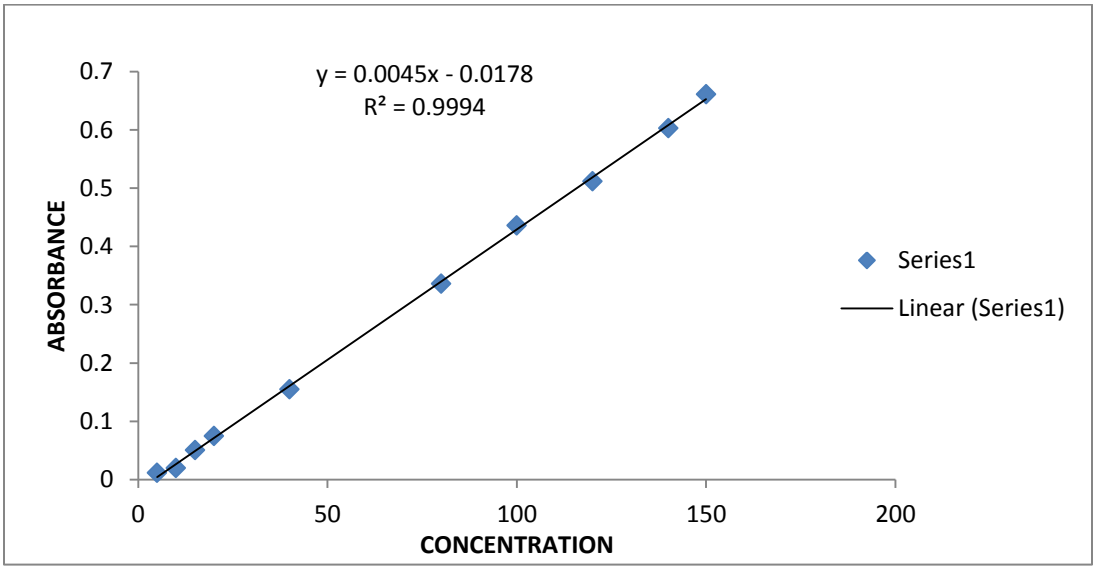

Figure 2: Calibration curve of Cephalexin monohydrate (5-150 µg/mL) at 261 nm

Table 1: Within-day precision using 15, 20 and 40 µg/ml cephalexin solution

Concentration	Absorbance	Mean ± SD, RSD
15	0.051, 0.051, 0.050	0.050 ± 0.0008, 1.6 %
20	0.075, 0.073, 0.070	0.073 ± 0.002, 2.70 %
40	0.150, 0.152, 0.152	0.152 ± 0.001, 0.65 %

Table 2: Between-day precision using 40 µg/ml cephalexin solution

Days	Absorbance	Mean ± SD, RSD
1	0.157, 0.157, 0.159	0.157 ± 0.0012, 0.74 %
2	0.154, 0.155, 0.154	0.154 ± 0.0005, 0.37 %
3	0.153, 0.155, 0.151	0.153 ± 0.0016, 1.06%

Table 3: Accuracy/ recovery studies of standard cephalexin powder spiked in blank plasma

S/No	Amt added (ug/mL) (n=3)	Amt expected (µg/mL)	Amt found (µg/mL) (n=3)	Recovery (%)
1	18	36	34.6	84
2	20	38	36.6	85.5
3	22	40	38.18	84.9

Table 4: Validated parameters of the developed method

S/No	Parameters	Result obtained
1	λ max	261 nm
2	Range	5-150 µg/mL
3	Regression equation	y = 0.0045x - 0.0178
4	Correlation coefficient	0.9994
5	Intercept	0.0178
6	LOD	183.33 ng/mL
7	LOQ	555.56 ng/mL

Discussion

As shown in Figure 1, Cephalexin showed wavelength maxima at 261 nm with the maximum absorption at pH 4. As shown in fig. 2 and table 1, the calibration curve was found to be linear in the range of 5-150 µg/mL with regression equation of y = 0.004x-0.0178; and a correlation coefficient of r^2 = 0.9994 which clearly indicates linearity of the developed method. Results of Intra-day and Inter-day precisions are expressed in % RSD and were found to be 1.65 and 0.72 (averages) respectively which are all within the acceptance limit of <2 as shown in tables 2 and 3. The percentage recovery of this study was found to be 84 to 85.5 % after precipitation with 40 % perchloric acid followed by buffering with phosphate buffer (pH 4) post-centrifugation as shown in table 4. Percentage recovery of 82 % has been reported for cephalexin (Paul *et al*, 1991). The buffering step had the inconvenience of diluting the sample and decreasing the accuracy and sensibility of the method (Ticiano *et al*, 2009).

Conclusion

From the above results it can be concluded that, the UV Spectrophotometric method developed is simple, rapid, precise, specific and economical. Hence it can be use for the quantitative analysis of cephalexin in powdered form. The low recovery of cephalexin observed can be attributed to the instability of β-lactamics such as cephalosporins in different solvents such as water as reported in previous studies

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