LUMBUNG FARMASI ; Jurnal Ilmu Kefarmasian ,Vol 3 No 2, Juli 2022
P-ISSN : 2715-5943
E-ISSN : 2715-5277

UV-Vis Spectrophotometric Method Validation of Cefixime in Phosphate Buffer

Abulkhair Abdullah[a,1*], Mutmainnah[a,2], Ahlan Sangkal[b,1], Rahmat Ismail[b,2]
[a]Universitas Khairun, Ternate Selatan, Ternate, Maluku Utara, Indonesia
[b]Universitas Muhammadiyah Manado, Bunaken, Manado, Sulawesi Utara, Indonesia
[1]abulkhairabd03@gmail.com*; [2] nina.mutmainnah08@gmail.com*; [3] ahlan.sangkalcoc@gmail.com; [4] rahmatismail114@gmail.com
*author correspondence

INFO ARTIKEL

Diterima :
08-02-2022
Direvisi :
22-06-2022
Disetujui :
22-06-2022

Kata kunci:
Cefixime;
Penyangga fosfat;
Validasi metode;
UV-Vis
Spectrophotometric.

ABSTRAK

Penelitian ini bertujuan untuk mengembangkan metode baru untuk mengukur sefiksim dalam buffer fosfat 0,05 M. Pengujian dilakukan dengan menggunakan spektrofotometri UV-Vis Genesys 10S. Akurasi, presisi, LOD & LOQ, linearitas, dan ketahanan metode ini divalidasi. Panjang gelombang sefiksim ditemukan pada 288 nm dengan konsentrasi larutan standar kerja 4-20 g/mL. Hasil validasi menunjukkan koefisien regresi sebesar 0,9981; % RSD akurasi, presisi, dan ketahanan di bawah 2%; LOD 0,843 g/mL; dan LOQ 2.555 g/mL. Kesimpulannya, metode yang dikembangkan dalam penelitian ini akurat, teliti, dan dapat diandalkan. Metode ini divalidasi sesuai pedoman ICH Q2 (R1) untuk akurasi, presisi, batas deteksi, batas kuantisasi, linieritas, dan ketahanan.

Key word:
Cefixime;
Phosphate Buffer;
Method Validation;
UV-Vis
Spectrophotometric.

ABSTRACT

This study aim to develop a new method for measuring cefixime in 0.05 M phosphate buffer. The assay was performed using UV-Vis spectrophotometry Genesys 10S. The accuracy, precision, LOD & LOQ, linearity, and robustness of this method were validated. The wavelength of cefixime was found at 288 nm with a working standard solution concentration of 4-20 µg/mL. The validation results show a regression coefficient of 0.9981; % RSD of accuracy, precision, and robustness are below 2%; LOD 0.843 µg/mL; and LOQ 2,555 µg/mL. In conclusion, the method developed in this study is accurate, thorough, and reliable. The method was validated as per ICH Q2 (R1) guideline for accuracy, precision, detection limit, quantitation limit, linearity, and robustness.

Introduction

Cefixime ($C_{16}H_{15}N_5O_7S_2$) is a third generation cephalosporin, given orally for treatment of susceptible infections such as gonorrhea, otitis media, pharyngitis, lower respiratory tract infections, and urinary tract infections (Sweetman, 2009).

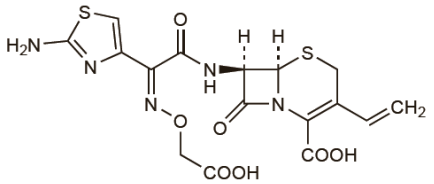

Figure 1. Structure of cefixime (Sweetman, 2009).

Cefixime is available in the form of white or almost white powder which is slightly hygroscopic. Cefixime has poor solubility in water. It is slightly soluble in alcohol, practically insoluble in ethyl acetate, and very soluble in methyl alcohol. Cefixime is classified as class IV in the Biopharmaceutics Classification System (BCS) with low solubility (solubility in water 55.11 mg/L) and permeability (logP value -0.4) (Amidon et al., 2015; Gaur et al., 2008; Wishart et al., 2006).

UV-Vis spectrophotometry is an analytical method that is widely used today. This method provides a simple way to determine small amounts of a substance. Each method used in the test must be validated. Method validation is an assessment of the

size of certain parameters based on laboratory experiments. Validation is carried out to ensure that the parameters used have met the requirements (Torbeck, 2013).

The aim of this study is to develop a new, simple, accurate, precise, sensitive, and reproducible analytical method for estimating cefixime. A literature survey revealed that a number of spectrophotometric methods had been reported but no method was reported for the estimation of cefixime in 0.05 M phosphate buffer using UV-Vis Spectrophotometer Genesys 10S. Phosphate buffer is buffer solution that is similar to the ion concentration, osmolarity, and pH of human body fluids. This solution is isotonic to human solutions, less likely to cause cellular damage, toxicity, or undesired precipitation in biological, medical, or biochemical research (Attimarad et al., 2012; Dey et al., 2012; Helmenstine, 2019; Kumar et al., 2011; Nayon et al., 2013; Patel et al., 2012; Pekamwar et al., 2015).

Methods

1. Apparatus

UV-Vis spectrophotometer Genesys 10S (Thermo Fisher Scientific, USA) with spectral bandwidth of 1 cm matched quartz shells was used for method validation.

2. Preparation of Phosphate Buffer

0.05 M phosphate buffer was prepared by weighing 6.8 g of KH_2PO_4 then dissolved in 1 L of distilled water and added 1 N NaOH to reach a pH of 7.2.

3. Preparation of Standart Stock Solution

Standard stock solution of cefixime was prepared by dissolving 10 mg of cefixime in 100 ml of 0.05 M phosphate buffer to obtain a concentration of 10 µg/mL.

4. Determination of Maximum Wavelength

An aliquot of the standard stock solution of cefixime was diluted with 0.05 M phosphate buffer to obtain a concentration of 10 µg/mL. The absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength range of 200-400 nm.

5. Preparation of Calibration Curve

An aliquot of the standard stock solution was transferred to 10 mL volumetric and diluted with 0.05 M phosphate buffer to obtain standard solutions with concentrations of 4-20 µg/mL. The absorbance of the solution was measured at the maximum wavelength using a UV-Vis spectrophotometer. A calibration curve was made by regressing the absorbance with the concentration of the solution.

6. UV-Vis Spectrophotometric Method Validation

6.1. Accuracy

Accuracy was obtained by preparing cefixime for three replication. Accurately weighed 10 mg cefixime was transferred to a 100 ml volumetric flask and dissolved by f and diluted with 0.05 M phosphate buffer to obtain concentration of 10 µg/mL. The solution was scanned in the maximum UV wavelength and the recovery was calculated.

6.2. Presicion

Precision was verified by repeatability study using stock solution of three replicate samples of cefixime (10 µg/mL). Each sample was determined by analyzing for three times every two hours in the same day (intraday). Interday precision was determined by analyzing each sample after 24 hours and 48 hours. The precision of data obtained was expressed in terms of % relative standard deviation (% RSD).

6.3. LOD and LOQ

10 mg of cefixime was diluted in 100 ml 0.05 M phosphate buffer. The volume was made up to the mark with 0.05 M phosphate buffer to get concentration of 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL respectively then analyzed by UV spectrophotometer in maximum wavelength. LOD and LOQ were determined by using the formula below:

$$LOD = \frac{3.3 \times SD}{Slope}$$

$$LOQ = \frac{10 \times SD}{Slope}$$

6.4. Linearity

10 mg of cefixime was transferred into a 100 ml volumetric flask and diluted with 0.05 M phosphate buffer. The volume was adjusted with the same up to the mark to give concentration 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL.

6.5. Robustness

Robustness testing was performed by analyzing sample in 25 °C and 18 °C temperature. A 10 mg of cefixime was diluted in 0.05 M phosphate buffer until 100 ml and adjusted to be 10 µg/mL. The % relative standard deviation was calculated after data were obtained.

Results and Discussion

The development of the UV-Vis spectrophotometer method was carried out to determine its validity of the method in measuring samples and to provide accurate, thorough, and reliable results. There are 5 parameters tested

including accuracy, precision, LOD and LOQ, linearity, and durability.

Determination of the maximum wavelength of cefixime was carried out by measuring the absorbance of the standard solution of cefixime (concentration 10 µg/mL) using a UV-Vis spectrophotometer at a wavelength range of 200-400 nm. The maximum wavelength of cefixime was found at 288 nm (see Figure 2).

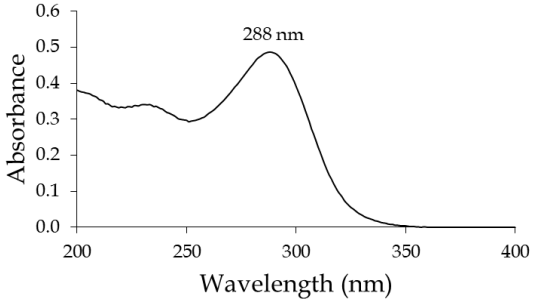

Figure 2. Maximum wavelength of cefixime

Calibration curve of cefixime was carried out by measuring the absorbance of the working standard solutions (concentrations 4-20 µg/mL) using a UV-Vis spectrophotometer at a wavelength of 288 nm. The calibration curve of the difference in absorbance against concentration is plotted (see Figure 3).

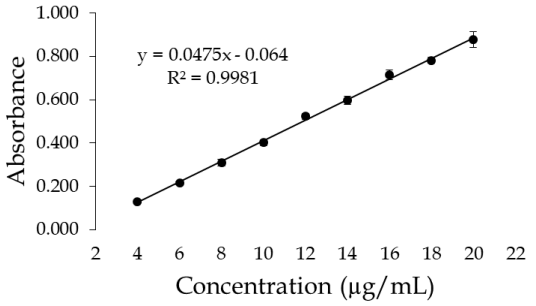

Figure 3. Calibration curve of cefixime

1. Accuracy

The results of the accuracy test are shown in Table I. The % RSD of cefixime was obtained in the range of 0.985-1.267% (less than 2). These results indicate that this method was accurate for determining cefixime.

Table I. Accuracy results of cefixime

Sample	Conc. (µg/mL)	Absorbance	% RSD
A	10	0.317 ± 0.004	1.107
B	10	0.406 ± 0.004	0.985
C	10	0.493 ± 0.006	1.267
Mean			1.119

2. Precision

The results of precision test are shown in Table II. The % RSD of cefixime for intraday and interday were 1.511% and 1.446% respectively (less than 2). These results indicate that this method was precise for determining cefixime.

Table II. Precision results of cefixime

	Sample	Conc. (µg/mL)	Absorbance	Mean	% RSD
Intra-day	A	10	0.395		
	B	10	0.407	0.400	1.511
	C	10	0.399		
	Sample	**Conc. (µg/mL)**	**Absorbance**	**Mean**	**% RSD**
Inter-day	A	10	0.398		
	B	10	0.409	0.403	1.446
	C	10	0.401		

3. LOD and LOQ

The values of detection limit and quantitation limit are shown in Table III. LOD and LOQ obtained for cefixime were 0.843 µg/mL and 2.555 µg/mL respectively.

Table III. LOD and LOQ results of cefixime

Conc. (µg/mL)	Absorbance	Slope	LOD	LOQ
4	0.130 ± 0.007			
6	0.215 ± 0.007			
8	0.309 ± 0.015			
10	0.401 ± 0.015			
12	0.523 ± 0.014	0.048	0.843	2.555
14	0.597 ± 0.018			
16	0.715 ± 0.023			
18	0.781 ± 0.014			
20	0.878 ± 0.036			

4. Linearity

The calibration curve shows good linearity. Cefixime follows Beer-Lambert's law in the range of around 4-20 µg/mL. The linear regression line equation derived was y = 0.0475x - 0.064 with the regression coefficient (R^2) at 0.9981 (see Figure 3).

5. Robustness

The results of robustness test are shown in Table IV. The % RSD of cefixime at 25 °C and 18 °C were 0.746% and 1.134% respectively (less than 2). These results indicate that this method was robust for determining cefixime.

Table IV. Robustness results of cefixime

Temp. (°C)	Conc. (µg/mL)	Absorbance	% RSD
25	10	0.402 ± 0.003	0.746
18	10	0.417 ± 0.005	1.134

Conclusion

The developed method is suitable for the simultaneous estimation of cefixime in phosphate buffer. The method is a new, simple, accurate, precise, sensitive, and reproducible analytical method for estimating cefixime according to the ICH Q2 (R1) guidelines. Therefore, this method can be used in quality control and routine analysis of cefixime in pharmaceutical dosage forms.

Acknowledgment

The author would like to thank PT. Dexa Medica, Palembang, Indonesia for providing cefixime as a sample for this research

References

Amidon, G., Lipka, E., Reyna, D., Simon, E., Harteg, C., & Hertig, D. (2015). *BCS Database Search*. http://tsrlinc.net/search.cfm

Attimarad, M., Al-Dhubiab, B. E., Alhaider, I. A., Nair, A. B., N, S. H., & K, M. A. (2012). Simultaneous Determination of Moxifloxacin and Cefixime by First and Ratio First Derivative Ultraviolet Spectrophotometry. *Chemistry Central Journal*, *6*(105), 1–7. Chemistry Central Journal

Dey, S., Pradhan, P. K., Upadhayay, U. M., Shah, S., & Goswami, K. (2012). UV Spectrophotometric Determination of Cefixime in Bulk and its Dosage Form. *Journal of Pharmacy Research*, *5*(12), 5419–5422.

Gaur, R., Azizi, M., Gan, J., Hansal, P., Harper, K., Mannan, R., & Rogowska, A. (2008). *British Pharmacopoeia 2009*. HMSO.

Helmenstine, A. M. (2019). *Phosphate-Buffered Saline or PBS Solution*. ThoughtCo. thoughtco.com/phosphate-buffered-saline-pbs-solution-4061933

Kumar, A., Kishore, L., Nair, A., & Kaur, N. (2011). Kinetic spectrophotometric method for the estimation of cefixime in pharmaceutical formulations. *Der Pharma Chemica*, *3*(4), 279–291.

Nayon, M. A. U., Nesa, J.-U., Uddin, M. N., Amran, M. S., & Bushra, U. (2013). Development and Validation of UV Spectrometric Method for the Determination of Cefixime trihydrate in Bulk and Pharmaceutical Formulation. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *3*(22), 1–5.

Patel, D., Goswami, K., & Patel, M. M. (2012). Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefixime and Linezolid in Combined Dosage. *International Journal for Pharmaceutical Research Scholars*, *1*(4), 112–118.

Pekamwar, S. S., Kalyankar, T. M., Tambe, B. V, & Wadher, S. J. (2015). Validated UV-Visible Spectrophotometric Method for Simultaneous Estimation of Cefixime and Moxifloxacin in Pharmaceutical Dosage Form. *Journal of Applied Pharmaceutical Science*, *5*(1), 37–41.

Sweetman, S. C. (2009). *Martindale: The Complete Drug Reference* (36th ed.). Pharmaceutical Press.

Torbeck, L. D. (Ed.). (2013). *Pharmaceutical and Medical Device Validation by Experimental Design*. CRC Press.

Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., Chang, Z., Woolsey, J., & Tg, C. (2006). *DrugBank: A Comprehensive Resource for In Silico Drug Discovery and Exploration*. *34*, 668–672.