Adnan Menderes University, 4th AACD Congress, 29 Sept-3 Oct.2004, Kuşadası-AYDIN/ TURKEY
Proceedings Book 082.DOC

DETERMINATION OF CEFACLOR AND CEPHALEXINE IN BLOOD PLASMA BY HPLC

Trajče Stafilov[1] and Dragica Zendelovska[2]

[1]Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University,
P.O. Box 162, 1001 Skopje, Republic of Macedonia; e-mail: trajcest@iunona.pmf.ukim.edu.mk
[2]Institute of Preclinical and Clinical Pharmacology and Toxicology, St. Cyril and Methodius University,
Medical Faculty, 50 Divizija bb, 1000 Skopje, Republic of Macedonia

Abstract

Cefaclor and cephalexine are cephalosporin antibiotics administered in the treatment of susceptible infections including upper and lower respiratory-tract, skin and urinary-tract. In this paper, a simple high-performance liquid chromatographic (HPLC) method to measure simultaneously the blood plasma concentration of cefaclor and cephalexine has been presented. The mobile phase was 0.025 mol l^{-1} KH_2PO_4 (pH 2.2) and methanol (75:25, *V/V*) using reversed phase C8 column.. Analysis was run at a flow-rate of 1.2 ml min^{-1} and at a detection wavelength of 255 nm. The method was found to be reproducible with a RSD less than 6.0 % over the concentration range 0.2-30.0 μg ml^{-1} for cefaclor and 0.5-50.0 μg ml^{-1} for cephalexine in blood plasma samples. The limits of quantification were 0.2 and 0.5 μg ml^{-1}, respectively.

Introduction

Cefaclor and cephalexine are antibiotic administered in the treatment of susceptible infections including respiratory-tract infections, skin infections, and urinary-tract infections [1]. There are several methods concerning the determination of cefaclor and cephalexine in biological fluids by high-performance liquid chromatography (HPLC) [2-6]. Some methods yielding poor separation of cefaclor and cephalexine from the blood plasma and serum endogenous interference's [2, 6] and require an evaporation step and they are time consuming. In this paper, we report a simple, sensitive, and easy to operate HPLC method using UV detection for the simultaneously determination of cefaclor and cephalexine in human blood plasma.

Experimental

Preparation of standards and samples

Stock solution of cefaclor (Lupin Limited, India) was prepared in water at a concentration of 1000 μg ml^{-1}. Working stock solution of cephalexine (SUN Pharmaceuticals LTD, India) was prepared in methanol at a concentration of 1000 μg ml^{-1} and stored at 4 °C for 1 month. The working solutions were prepared by diluting appropriate portions of these solutions with distilled water.

Human plasma samples were prepared by centrifuging (at 1000 g) of heparinized whole blood samples collected from healthy volunteers who later participated in a bioequivalence study of cefaclor and cephalexine and stored at –20 ^{o}C. Before the analysis blood plasma samples were thawed at 20 °C for about 10 minutes. A 0.25 ml volume of the sample was transferred into a vial and vortexed with 0.25 ml of 6 % trichloroacetic acid for 30 s. After centrifugation, (5 min, 4000 rpm), a 120 μL portion was injected on to HPLC column.

Chromatography

A series of parameters, including composition and pH of mobile phase, column packing, flow rate and detection wavelength, were tested with respect to the location and shape of the peaks of cefaclor and cephalexine in the corresponding chromatograms. The Hibar Lichrospher 100 RP 8 (250 x 4 mm) with 5 μm particle size (thermostated at 25°C) was used as a stationary phase, protected by a guard column Lichrospher 100 RP 8 (5 μm) (Merck). For analysis of samples the composition of the mobile phase was methanol – 0.025 mol/L KH_2PO_4 (25:75, *V/V*) using a pump Perkin Elmer LC series 200. pH of buffer solution (2.2) was adjusted by conc. H_3PO_4. The mobile phase was filtered and degassed with He. The flow-rate of the mobile phase was 1.2 ml min^{-1}. An UV diode array detector (PE LC 235 C) was used for detection at 255 nm for simultaneous determination of cefaclor and cephalexine. The samples (120 μl) were introduced using an autosampler Perkin Elmer LC ISS Series 200.

Results and Discussion

A series of studies were conducted in order to develop a convenient and easy-to-use HPLC method for quantitative analysis of cefaclor and cephalexine in biological fluids. A series of aqueous mobile phases containing buffer solutions with different pH values in combination with different modifiers including acetonitrile, methanol and triethylamine were tested. The results were most satisfactory when mobile phase consisted of 0.025 mol L^{-1} KH_2PO_4 with pH 2.2 and methanol in volume fractions 75:25. C8 packing coloumn showed best separation. Flow-rate of 1.2 ml min^{-1} was the best with respect to location and resolution of the peaks of cefaclor and cephalexine from the interfering peaks. The elution was monitored at wavelength of 255 nm. A typical chromatogram of standard solutions (10 μg ml^{-1}) of cefaclor and cephalexine produced by the developed method is shown in Fig. 1 (b). Retention time of cefaclor and cephalexine are 8.3 min and 9.3 min, respectively. On the other hand, the method has sufficient sensitivity and reproducibility to permit the pharmacokinetic studies. The developed HPLC method can be used for analysis of blood plasma samples from healthy volunteers after oral administration of cefaclor and cephalexine. Typical chromatograms of blood plasma samples of patients before and after administration of 500 mg cefaclor and 1000 mg cephalexine showed no interfering peak at the cefaclor and cephalexine peaks position.

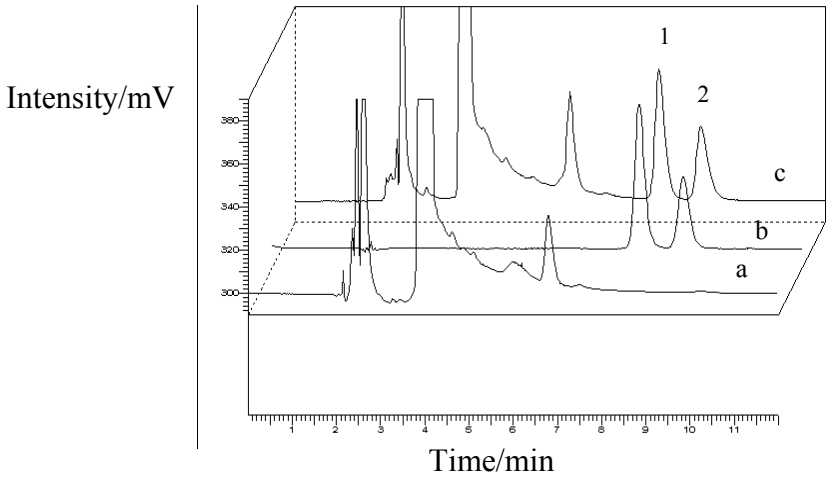

Fig. 2. Chromatograms of blank plasma sample (a), standard solution of cefaclor and cephalexine (b) and spiked plasma sample (c) containing 10 μg ml^{-1} cefaclor (1) and cephalexine (2)

Linearity, precision and accuracy

Linearity was tested in 3 different days at 6 concentration points (with spiked human blood plasma) ranged from 0.2 to 30.0 μg ml^{-1} of cefaclor and 0.5 to 50 μg ml^{-1} of cephalexine. The correlation coefficients were 0.9993 and 0.9996, respectively. The intra- and inter-day variations of the method show that RSDs ranged from 0.91 to 5.71 % for cefaclor and from 1.21 to 6.19 % for cephalexine. For inter-day precision, RSDs ranged from 0.93 to 5.94 % for cefaclor and 1.91 to 6.0 % for cephalexine indicating a considerable degree of precision and reproducibility for the method. Intra- and inter-day accuracy was determined by measuring blood plasma quality control samples at 3 different concentration levels. Relative errors at all 3 concentrations studied for both drugs are <6 % showing that the method is remarkably accurate.

The limit of quantification was defined as signal to noise ratio 1:10 (n=5). The limits of quantification were found to be 0.1 μg ml^{-1} for cefaclor and 0.25 μg ml^{-1} for cephalexine for blood plasma samples.

Stability of cefaclor and cephalexine in blood plasma samples

Stability of cefaclor and cephalexine in blood plasma was investigated using spiked samples at 2 different concentration levels. Spiked samples were analysed after different storage conditions: immediately, after staying in an autosampler for 2, 12 and 24 h, after one and 2 freeze/thaw cycles and after 1 month stored at –20 °C. The results show that both drugs are stable in the different storage conditions except for cefaclor after storage for 1 month at –20 ^{o}C.

Ruggedness

Ruggedness was tested on the second HPLC column of the same type by determining linearity, precision and accuracy. Linearity was performed in the same concentration ranges. The correlation coefficients were 0.9997 and 0.9991 respectively. Intra-day precision and accuracy were determined by measuring 3 series of samples and RSD at 3 concentrations studied were <5.5 %. The relative errors ranged from 0.5 to 3.8 %. Ruggedness expressed as precision of data obtained by 2 columns given as RSD was found to be between 3.5 and 4.8 %. That means that this method is rugged.

Conclusion

A sensitive and rapid HPLC method has been developed for the simultaneous determination of cefaclor and cephalexine in blood plasma samples. The typical assay time is about 12 min. The method is advantageous compared to the other methods because of its simplicity, short time, efficient clean up of the complex biological matrix and high recovery. The validation data demonstrate good precision and accuracy.

References

1. Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 10th ed., McGrow Hill, New York, 2001, p. 1210.
2. Nakagawa, T., Shibukawa, A., Shimono, N., Kawashima, T., Tanaka, H., Haginaka, J. (1987): J. Chromatogr. 420, 297.
3. J. A. McAteer, M. F. Hiltke, B. M. Silber, R. D. Faulkner, Clin. Chem. 33, 1788-1790 (1987).
4. Holt, D. E., De Louvois, J., Hurley, R., Harvey, D. (1990): J. Antimicrob. Chemother. 26, 107-115.
5. Kovach, P. M., Lantz, R. J., Brier, G. (1991): J. Chromatogr. 567, 129.
6. Tutunji, M., Jarrar, O., Musameh, M., Alam, S. M., Quamruzaman, A., Dham, R. (2001): J. Clin. Pharm. Ther. 26, 149-154.