Research Article
ISSN: 2456-8473
International Journal of Chemistry, Pharmacy & Technology
Vol. 3, No.1, pp-1-6, 2018

HPLC-UV METHOD FOR THE ESTIMATION OF CEFTRIOXONE AND SULBACTAM- VALIDATED USING ACCURACY PROFILES

J. Dharuman[1], Dr. Rakeshkumar Jat[2] and Dr. S. Mohan[3]

[1]KMCH College of Pharmacy, Coimbatore, India, Research Scholar, JJT University, Rajasthan

[2]Research scholar Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Churela, Rajasthan.

[3]Karpagam College of Pharmacy, Coimbatore, India

Corresponding Author: jdharuman@gmail.com

Received 02-01-18; Revised & Accepted: 25-01-18

ABSTRACT

Generally, accuracy of an analytical procedure is ensured by suitable method validation process. Guidelines provided by the regulatory bodies can be a general framework to assess the validity of a method. Since these guidelines provide marginal accuracy of analytical results, this study was aimed to test a recently evolved strategy that may render analytical method validation more accurate and trustworthy. The concentration range in which reliable analytical results can be obtained is proposed by a Bayesian probability study. Such study renders perfect information about the concentration range of the analyte which may produce accurate analytical results. In order to ensure the applicability of this approach, it was applied for the validation of a HPLC–UV assay method developed for the quantification of ceftrioxone and sulbactam in human plasma. A comparative study between the newer approach and the standard method validation proved that the application of Bayesian analysis at the end of the validation process can produce significant improvement in the analytical results.

Keywords: Reliability, Bayesian, Accuracy, Analysis, validation, statistics

INTRODUCTION

Results accuracy is an important aspect of analytical method validation. The need to check the accuracy of an analytical method is done by analysts in the pharmaceutical industry on an almost daily basis, because adequately validated and reliable methods are a necessity for approvable regulatory filings. Recent developments in analytical instrumentation have resulted in enormous progress in method development. The use of chemometric tools enables the development and optimization of analytical procedures in laboratories and industries worldwide[1]. When advancements in method development are remarkable, the need for innovations in method validation is also important to achieve genuine analytical results. The International Conference on Harmonization (ICH) has issued guidelines for analytical method development and validation. This shows the interest of regulatory agencies for method validation is obvious[2].

Accuracy is a continuous process and the aim is to ensure confidence in the analytical data throughout the analytical method development. It should be designed by the developer or user to ensure repeatability of the method. Methods should be reproducible when used by the other

analysts, on other equivalent equipment, on other days or premises and throughout the life of the drug product. Hence, data that are generated for acceptance, release, stability, or pharmacokinetics will only be trustworthy if the methods used to generate the data are reliable[3].

For over two decades, classical validation parameters were widely used in method validation. In the recent past, implementation of computers and software has created a tremendous change in analytical method validation. However, this is not enough and a further step needs to be performed by means of statistical treatment to enhance the accuracy of the experimental data. Hence, enhancement in method accuracy is highly significant in analytical methods. This study explores the application of a novel strategy that may transform the analytical method more reliable, straightforward and trustworthy.

BASIC PRINCIPLES OF BAYESIAN PROBABILITY ANALYSIS

Generally, Bayesian analysis can be executed by the following four steps.

Step 1: A probability model is formulated initially for the data.

Step 2: The prior distribution that quantifies the uncertainty in the values of the unknown model parameters is decided before the data are observed (the prior distribution).

Step 3: The likelihood function is constructed based on the observed data and the probability model formulated in the first step.

Step 4: The quantities of interest based on the posterior distribution are calculated, after the data are observed (the likelihood function)[3]. These quantities constitute statistical outputs, such as point estimates and intervals.

MATERIALS AND METHODS

Apparatus and chromatography

The HPLC system consisted of a Shimadzu Prominence HPLC (Kyoto, Japan) and chromatography was performed on a Kromasil C8, 5µ, 150mm × 4.6 mm id (Amsterdam, The Netherlands) operated at ambient temperature. Evaporation of the Plasma sample was effected by a Turbo Vap low-volume evaporator (Uppsala, Sweden). The mobile phase used was a combination of phosphate buffer (pH 3.5, solvent A) and acetonitrile (solvent B) (70:30, v/v). The flow rate was 1.2mL/min and the wavelength of detection was set at 220 nm.

Preparation of standard solutions

A stock solution of CF and SM, and internal standard IB was prepared in water (1 mg/mL) and stored at 4±1ºC. Working standards were prepared freshly on each day by appropriate dilution of the stock solution.

Extraction of plasma samples

Calibration standards and quality-control (QC) samples were added in a quantity of 25–250µL of plasma in an Eppendorf tube and mixed well. Phosphate buffer (0.01 M) was added in a quantity of 100 µL and centrifuged for 30s; then it was extracted with 300µL of ether. The organic layer was separated from the plasma and evaporated in a low-volume evaporator and reconstituted with 200µL of mobile phase. It was washed with 500µL of n-hexane and a volume of 25µL was injected into the HPLC system.

Validation of the developed HPLC–UV method

Validation process was carried out according to ICH guidelines: validation of analytical procedures: text and methodology Q2 (R1)[5]. The general validation criteria were obtained using e-noval V3.0 software (Arlenda, Liege, Belgium).

Linearity and lower limit of quantization (LLOQ)

The calibration standards were aliquoted and assayed on three successive days to demonstrate the linearity of the method by the OLS method. Least-squares regression analysis of the linear data was performed and the linearity was evaluated from the slope, intercept, and correlation coefficient (r^2) of each curve. The LLOQ was regarded as the lowest analyte concentration that can be determined with a precision of<20% expressed by RSD that produces an S/N of 1:3 between sample and blank.

Precision, accuracy and recovery (extraction efficiency)

The precision and accuracy of the method were assessed at three concentration levels (QC 100, 300, 600 µg/mL of CF and SM). Three replicate samples were prepared and analyzed on the same day for intraday and continuous days (n=4) accuracy and precision according to ICH guidelines. The assay precision was expressed as RSD and accuracy was calculated in terms of bias. The recovery of an analyte is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the pure equivalent of authentic standard. The extraction recovery of CF, SM and IS in plasma was measured at three QC concentration levels (QC 100, 300, 600 ng/mL of CF and OZ).

EVALUATION OF ACCURACY OF ANALYTICAL RESULTS

The concentration range selected from the above procedure was then treated further to obtain the reliable range for future analysis by Bayesian analysis. Bayesian analysis was applied, by fixing the acceptance limits within which the results must fall[6-8]. The acceptance limit was set at a maximum of ±20% around the reference concentration values (T,i) of the validation standards. The minimum accuracy criterion (min) was set at 90. Having set these limits, the profile of accuracy can be framed out for the analytes of interest. The Bayesian accuracy profile was performed with SAS/STAT9.1.3 version (SAS Institute, Cary, NC, USA) pack-age for MCMC sampling.

COMPARISON OF THE NEW VALIDATION PROTOCOL WITH THE USUAL VALIDATION PROTOCOL

Comparison between the new validation protocol and usual validation protocol has been carried out in terms of percentage relative error to study the effect. Information about percentage relative error can be obtained through accuracy profiles for CF and SM conveniently[9].

RESULTS AND DISCUSSION

Results of precision, accuracy and recovery experiments

Results of repeatability, intermediate precision, and accuracy expressed in terms of absolute bias (µg/L) or relative bias (percentage) obtained are summarized inTable1. The within-run and between-run RSDs were <5% for CF and SM. The overall bias was lesser than 5%for both components. The mean (±SD) percentage extraction recoveries of CF, SM and IS at the three concentration levels (8, 16, and 32µg/mL for CF and 1, 2, and 4µg/mL for SM) were studied. The

recovery of CF, SM and IS was consistent and efficient. The mean absolute recovery was 89, 91, and 87 for CF, SM and IS, respectively.

Table.1 General Validation results for determination of CF and SM human plasma

Drug	Conc	Trueness /Relative bias (%) (d = 3, n = 3)* (µg /ml)	Precision /repeatability (d = 3, n = 3)* (%)RSD	Accuracy (d = 3, n = 3)* 90%β-expectation tolerance limits (µg /ml)	LOD (ng/ml)	LOQ (ng/ml)
Ceftrioxone	2.5	2.7	1.3	[2.48, 2.57]	20	56
	5.0	-3.5	0.9	[4.96, 5.21]		
	10.0	-1.4	1.2	[9.96, 10.24]		
	20.0	2.4	1.8	[19.97, 20.15]		
	30.0	2.7	1.4	[29.96, 30.21]		
Sulbactam	1.25	2.0	0.87	[1.21, 1,28]	38	57
	2.5	4.0	1.8	[2.46, 2.58]		
	5.0	-1.4	1. 2	[4.97, 5.16]		
	10.0	−4.0	1.6	[9.87, 10.17]		
	15.0	3.3	1.5	[14.89, 15.21]		

*d = days (runs), n = no. of replicate samples

Results of the Bayesian analysis

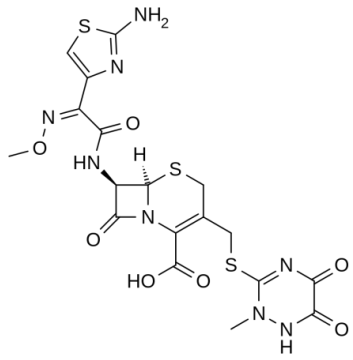
The accuracy of analytical results generated by the quantitative analytical method was obtained by applying the Bayesian procedure by defining the acceptance limits within which the results must fall. Bayesian accuracy profiles obtained for analysis of (A) CF and (B) SM were obtained by Bayesian profiles and were found to be significant for the analysis.

Results for the comparison study of validation protocols

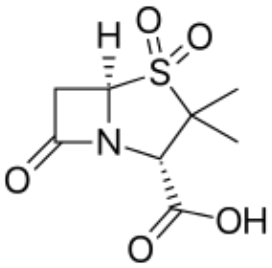
A comparison between the usual validation protocol and the newer protocol in terms of percentage relative error has been carried out to test the applicability of the new strategy. The percentage relative error resulted in usual protocol was more than ±10%, while in the new approach it was within±10%. Accuracy profiles obtained for CF and SM are given in Fig. 2.

Concluding remarks

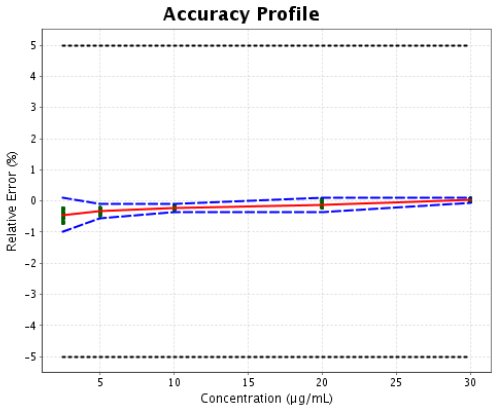
In the proposed research, a HPLC method has been developed and validated using a novel strategy known as Bayesian analysis. In the process, Bayesian analysis determines the concentration range that produces reliable analytical results. Matrices containing the drugs under study were estimated using the optimized chromatographic conditions. A comparison of results in terms of total error between the newer validation strategy and usual validation protocol indicated that the newer approach proposed in this research can produce more accurate results and can convert the process of validation most trustworthy.



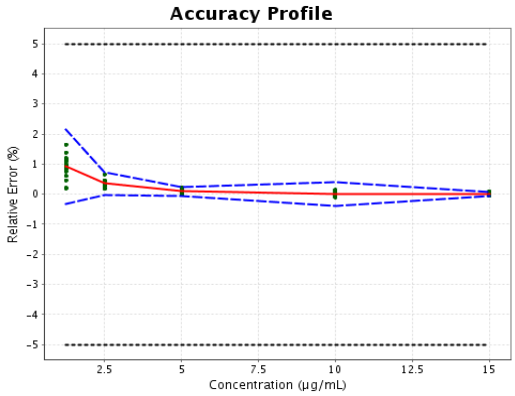
(A)



(B)



(C)



(D)

Figure.1. Chemical structure of a) Ceftrioxone and b) Sulbactam

Figure.2. Accuracy profile of (A) CF and (B) SM analyzed by the usual method of analysis showing larger percentage (negative) bias values.

(C and D) analyzed within Bayesian calculations showing lower percentage bias values for CF and SM respectively.

REFERENCES

[1] Thompson, M et al. Harmonized guidelines for single-laboratory validation of methods of analysis. Pure and Applied Chemistry. 2002; 74: 835–855.

[2] Epshtein, N. A. Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations. Journal of Pharmaceutical Chemistry. 2004; 38: 212–228.

[3] Dharuman JG and Vasudevan M. Reliability-targeted HPLC-UV method validation--a protocol enrichment perspective. Journal of separation science 2014; 37: 228-36.

[4] Rubinstein, Y and Kroese, DP. Simulation and the Monte Carlo Method, Wiley Series in Probability and Statistics, Wiley, New York 2000.

[5] ICH. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartite Guideline, ICH, USA 1996, 1–8.

[6] Rozet, E et al., Evaluating the reliability of analytical results using a probability criterion: a Bayesian perspective. Analytica Chimica Acta. 2011; **705:** 193–206.

[7] Chen, MH et al., Monte Carlo Methods in Bayesian Computation, Springer-Verlag, New York 2000.

[8] Hubert, P et al., Harmonization of strategies for the validation of quantitative analytical procedures. A SFSTP proposal--Part I. Journal of Pharmaceutical and Biomedical Analysis. 2007; 45: 70–81.

[9] M. Feinberg. Validation of analytical methods based on accuracy profiles Journal of Chromatography A. 2007; 1158: 174–183.