Validation of Simple Spectrophotometric Method for Determination of Ceftriaxone in Human Plasma

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ABSTRACT

The use of the ceftriaxone in the treatment of infections of the urinary tract and respiratory tract associated with sepsis both intravenously and intramuscularly is the primary choice in therapy. To support drug therapy monitoring (TDM), a simple, fast, inexpensive and validated analysis method is needed. The availability of UV-Vis spectrophotometer in almost all clinical laboratories is high, so it can be an alternative to apply in TDM of patients. Accuracy, inter-and intra-day precision, linearity, freeze and thaw stability, and short and long-term stability were parameters determined. The maximum wavelength of ceftriaxone was 272 nm, with linearity at the concentration range of 10-30 µg/mL with a correlation coefficient ($r^2$) of 0.99. The accuracy of the method was studied by recovery study. The result showed that %recovery was found in the range of 94-99% with relative standard deviation (RSD) less than 2%. Intra and inter-day precision were accepted with RSD value of < 2%. The spiked human plasma sample containing ceftriaxone remained stable for 4 hours at room temperature and was stable in storage at -20 °C for seven days. The method is simple, accurate and inexpensive. The proposed method can be successfully used for the determination of Ceftriaxone sodium in human plasma.

INTRODUCTION

Ceftriaxone is the third generation of cephalosporin that can be used in a single dose or combination with fluoroquinolone class such as ciprofloxacin in the treatment of infections of the urinary tract and respiratory tract associated with sepsis. The types of bacteria associated with sepsis include Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Enterobacter spp (Dipiro et al., 2014; Minasyan, 2019). Ceftriaxone sodium is a semisynthetic third-generation of cephalosporin that is used
intravenously or intramuscularly. Sodium ceftriaxone (C₁₈H₁₆N₈Na₂O₇S₃·5H₂O) with a molecular weight of 661.59 in the form of yellowish-white crystals, easily soluble in water, slightly soluble in methanol, very slightly soluble in ethanol.

Previously published research related to ceftriaxone determination was by Anion Exchange Chromatography in which ceftriaxone was determined in plasma and tissue samples of Wistar rats (Khasanov et al., 2006). Both single and simultaneous ceftriaxone assay has also been developed using HPLC-UV (Briscoe et al., 2012; Tariq et al., 2010). Other studies to determine the levels of ceftriaxone in human intestine and plasma, as well as dog plasma, has been done by HPLC tandem MS method (Lusk et al., 2016). The UV spectrophotometric method has been successfully developed for the determination of ceftriaxone levels in tablet dosage forms (Reddy and Subbareddy, 2013) and nanoparticle formulations (Ayushi and Mansi, 2018).

This research aims to develop simple an analytical method for determining ceftriaxone levels in human plasma using spectrophotometry UV. The chemical structure of Ceftriaxone sodium is shown in Figure 1.

**MATERIALS AND METHODS**

**Materials**
Spectrophotometer UV-VIS double beam (Shimadzu UV-1700, Shimadzu Corporation, Japan), Centrifuge (Hettich EBA 20), micro pipet, ceftriaxone sodium (pro analysis, Acros Organic), acetonitrile (pro-HPLC/Spectro, Tedia), human plasma, distilled water.

**Preparation of Working Standard of Ceftriaxone**
Weigh accurately 10 mg sodium ceftriaxone, dissolve with distilled water to obtain concentration 100 μg/mL. Working standard prepared were 10, 15, 20, 25 dan 30 μg/mL.

**Sample preparation**
A one mL of plasma transferred into a tube and 50 μL of a standard solution of ceftriaxone, 50 μL of distilled water, and 3 mL of acetonitrile, then vortex for 1 minute. The solution was centrifugation for 10 minutes at 5000 rpm. Then, 2.5 mL of aliquots of supernatants were measured the absorbance.

**Determination of maximum wavelength (λ max)**
Measurement of maximum wavelength was performed by standard scanning solution and standard solution in plasma from sample preparation. Scanning of wavelength was conducted at 200-400 nm. Spectra of standard solution and standard solution in plasma were compared. There were two peaks appear on the spectra, one at 240 nm and 272 nm.

**Validation**
Accuracy was performed as percentage recovery, using three levels concentration of standard solutions, i.e. 10, 20 and 25 μg/mL. Each Concentration was replicated three times. Accuracy accepted if the relative standard deviation (RSD) less than 15% and percentage recovery 95-110%. Precision was evaluated based on the performance of intra and inter-day precision—determination of precision by measured three levels concentration of ceftriaxone triplicates in same days and different days. The precision of the method accepted if the value of relative standard deviation (RSD) less than 15% (European Medicines Agency, 2015).

LOD and LOQ were determined by statistical method. LOD and LOQ calculated by equations:

$$\text{LOD} = 3, 3 \frac{\sigma}{s}$$

**Figure 1: Structure of Sodium Ceftriaxone**

**Figure 2: UV Sepctra of Ceftriaxone Sodium (15 μg/mL) in distilled water**

**Figure 3: UV spectra of Ceftriaxone 25 μg/mL in distilled water (pink color) vsPlasma (blue color)**
Table 1: Validation parameters of Spectrophotometry UV Method for determination of ceftriaxone in plasma (n=3)

| Parameters       | Ceftriaxone (µg/mL) | Found Concentration (µg/mL) | RSD (%) |
|------------------|---------------------|-----------------------------|---------|
| Accuracy         | 15                  | 14.8 ± 0.2                  | 1.3     |
|                  | 20                  | 18.8 ± 0.7                  | 3.6     |
|                  | 25                  | 24.2 ± 0.4                  | 1.5     |
| Intra-day precision | 15             | 14.8 ± 0.1                  | 1.0     |
|                  | 20                  | 18.4 ± 0.2                  | 1.0     |
|                  | 25                  | 24.4 ± 0.2                  | 0.9     |
| Inter-day precision | 15             | 14.8 ± 0.1                  | 0.7     |
|                  | 20                  | 18.1 ± 0.1                  | 0.7     |
|                  | 25                  | 24.3 ± 0.3                  | 1.1     |
| Linearity        | 10-30               |                             |         |

LOQ = 10 $\frac{\sigma}{s}$

$\sigma$ = standard deviation of responses, $s$ = slope

Stability study was conducted by determining freeze and thaw stability and short term and long-term stability. Evaluation of freeze and thaw stability conducted by measured low and high concentration of ceftriaxone after stored on freeze condition for 24 hours.

Freeze and Thaw Stability
This test was carried out by replicating three times at each low and high concentration level after storage under freeze condition for 24 hours and after being allowed to melt at room temperature (thaw stability). The sample was re-frozen at the same temperature and storage conditions to be re-assigned in the next 24 hours. If the analyte is unstable under the established storage conditions, the sample stability test should be carried out at a storage temperature of -70 °C.

Short term Stability
The study conducted to determine the stability of the ceftriaxone analyte in the time required for sample preparation. In this study, 3 x replication at low and high concentrations of the sample solution was stored at room temperature. Then the analyte concentration was measured after 2 and 4 hours of storage.

Long term Stability
Long-term storage time in the long-term stability evaluation requires that it be done from the first day of sampling to the last days. The test was also carried out three times replication at each low and high concentration level at the same storage temperature as the storage conditions for the test sample. The Concentration obtained from each stage of the stability test, then compared to the Concentration obtained from the sample stability test on the first days of the long-term stability test.

RESULTS AND DISCUSSION

One of the important steps in using spectrophotometer UV is the appropriate use of wavelength. Due to potential interference of plasma at a short wavelength, the wavelength used for determination was 272 nm (Figure 2). Data showed that spectra of ceftriaxone sodium in distilled water and plasma at 272 nm almost coincided (Figure 3).

Linearity

Linearity performed to assess respond of the method in order change of sample concentration increase. Based on the measurement of sample 10-30 μg/mL the method showed a good respond of, it could be due to r value more than 0.98 (Table 1).

Accuracy, precision, LOD and LOQ

Accuracy was determined by recovery study. Based on data, percentage recovery of sample concentration of 15-25 μg/mL obtained were 98.9 ± 0.2; 94.2 ± 0.5; 97.0 ± 0.3%, respectively. Based on the data, the accuracy of the analytical method was acceptable due to percentage recovery and RSD values.

Intra-day and inter-day precision were conducted to evaluate the performance of precision. Data showed that spectrophotometry UV method was precise because RSD value less than 2%.

Based on statistic calculation, the value of Limit of Detection (LOD) and LOQ of the methods consecutively were 1.2 and 7.2 μg/mL.

Freeze and Thaw Stability

This study aims to determine the proper sample storage conditions to guarantee the stability of the ceftriaxone in plasma. Analyte remained stable after repeated freezing and thawing at room temperature for 24 and 48 hours (Table 2).

Short Term and Long-Term Stability

In this study, the ceftriaxone sodium as analyte contained in human plasma was stored at room temperature for 24 hours and after storage at -20°C for 168 hours (7 days) remained stable based on Concentration determined.

Based on validation data and the stability test of analytes ceftriaxone in human plasma which remain stable in storage for seven days, this will support therapeutics drug monitoring of ceftriaxone in the hospital where the methods developed are simple and easy to implement.

CONCLUSION

The maximum wavelength in the determination of ceftriaxone levels in human plasma was carried out at 272 nm and gave a percentage recovery value of 94-99 μg/mL with an RSD of less than 2%. The limit of detection (LOD) and Limit of Quantitation (LOQ) were 1.2 and 7.2 μg/mL, respectively. Human plasma samples were spiked with ceftriaxone are declared stable at room temperature for 4 hours and at storage -20°C for seven days. Rapid and straightforward analytical method of spectrophotometry UV was validated. Accuracy, precision, linearity and stability were meet to the requirement.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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