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Development and Validation of HPLC Methods for Determination of Some Cephalosporins in Pure and Dosage Forms

A Thesis Presented

By

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Summary

The present work aims to develop and validate simple, rapid, accurate, sensitive method for simultaneous separation and determination of some cephalosporins from different generations (Cefepime Hydrochloride, Ceftazidime, Ceftiofur Sodium, Cefotaxime Sodium, Ceftriaxone Sodium, Cefoperazone Sodium, Cephradine and Cefazolin Sodium) as important class of antibiotics used today by both humans and animals which commonly used to treat pathogens and prevent disease outbreaks due to their large range of antibacterial action, this by trying different methods to reach the suitable method of separation for simultaneous determination of selected cephalosporins with lower limits of detection and quantification.

This thesis consists mainly of three chapters as follows:

Chapter (1): Introduction and literature review.

Contain two parts:

The first part represented general introduction about the process of development and validation of analytical methods and its importance, also the international agencies which concerned with the requirements and the guide lines of the validation of analytical methods as International Conference for Harmonization of technical requirements for pharmaceuticals for human use (ICH). This part provides short notes about physical and chemical properties of β- Lactams and Cephalosporins besides their mode of action and uses. Also give attention to the different analytical methods used for separation which include chromatographic methods in addition to the meaning of chromatography, HPLC instrument and its types.

The second part includes a literature survey of the previous works carried out by different analytical techniques which include chromatographic methods for determination of the drugs in different matrices.

Chapter (2): Experimental

It includes the experimental part, where the materials, reagent, preparation of various solutions and the equipment used are presented. This chapter also includes the optimum chromatographic conditions for separation of selected drugs and also preparation of standard solutions, samples solutions and mobile phase used in the validation criteria of ICH.

Chapter (3): Result and discussion

Reviewing and analyzing the results and divided into three parts:

(a) The optimization of chromatographic condition for determination of cephalosporins in pure and dosage forms:

1. Selection of optimum detection wavelength: In this study wide range of wavelengths was tested to monitor the mixed cephalosporins. The wave length 250 nm was selected as optimum wavelength for determination of these group of cephalosporins.
2. Selection of suitable mobile phase: As we tried a number of mobile phases with different flow rates to achieve best separation. The isocratic mobile phase consists of 0.1M ammonium acetate, acetonitrile with pH 5.6 was chosen to use throughout this study.
3. Selection of stationary phase: C8 column was used for separation of these group of cephalosporins.

(b) Validation of the suggested method:

This process established by some laboratory studies which include:

1. Specificity: There is no interference between the pure standard and peaks of any impurities.

2. Linearity and range: linearity was evaluated by analysis of different concentrations of mixed standard solutions by HPLC to determine (R2) from the linear calibration curve of each drug which found to be 0.9999.
3. Limit of detection and quantification:The results showed that the suggested method achieved lower limits of detection and quantification of selected cephalosporins.
4. Method precision: Six replicate injections of 100% of test concentration (10 µg/mL) for each drug prepared and injected triply for each sample on the same day and also on different days. Precision was expressed as relative standard deviation which found to meet the acceptance crieteria of method precision of ICH.
5. Accuracy and recovery: The accuracy is calculated from the obtained results as percentage recovery of mixed sample solutions versus mixed standard solutions.
6. Robustness: By analyzing the samples of mixed cephalosporins with varying procedure parameters and observing to which extent it will affect on the analyte analysis, the number of replicates (3) for the concentration level 100% (10µg/mL) and evaluated based on system suitability parameters on recovered amounts, compared to data obtained using the original chromatographic parameters of the method and the results showed that the method is robust according to ICH acceptance criteria.
7. System Suitability Tests

Finally, the proposed RP-HPLC method is simple, specific, precise, accurate, and reproducible for simultaneous analysis of some cephalosporins antibiotics from different generations. The simultaneous quantification of this important group of cephalosporins with isocratic solvent system in the same run not only saves the

solvent but also with short run time makes it better choice for the analysis of these drugs as in quality control and research lab.