# Abstract

This thesis consists of three parts; each part includes an introduction, literature review and descriptive experimental work for the studied drugs in addition to references and summary in Arabic.

**Part I**: **Determination of Amoxicillin Trihydrate and Dicloxacillin Sodium in Their Binary Mixture and in Presence of Their Common Impurity; 6-Aminopenicillanic acid.**

This part includes:

**Introduction and Literature Review**

This introduction describes the chemical structure, physical properities, pharmacological activity, reported impurities and review of the published methods developed for the analysis of Amoxicillin trihydrate and Dicloxacillin sodium either alone or in their binary mixture.

**Section (A): Spectrophotometric Methods for The Determination of Amoxicillin Trihydrate and Dicloxacillin Sodium in Their Binary Mixture.**

In this section, two simple and sensitive spectrophotometric methods for determination of a binary mixture of Amoxicillin Trihydrate (AMOX) and Dicloxacillin Sodium (DIC) without prior separation and

with minimum sample pretreatment in which Amoxicillin Trihydrate was determined by direct spectrometry at $\lambda_{max}$ 273.6 nm and by first derivative ratio spectrophotometric method. Then, Dicloxacillin Sodium was determined by dual wavelength method and the isoabsorptive spectrophotometry. In dual wavelength method, two wavelengths (223.6 and 237 nm) were selected where the absorbance difference between them is directly proportional to the concentration of Dicloxacillin Sodium and the absorbance of Amoxicillin Trihydrate at these wavelengths is constant. The isoabsorptive spectrophotometry method was based on measuring the absorbance at the isoabsorptive point (230.4 nm) which gives the total concentration. The concentration of AMOX is calculated by direct method at $\lambda_{max}$ 273.6 nm and by first derivative ratio spectrophotometric method, then DIC concentration could be obtained by subtracting.

**<u>Section (B):</u> First Derivative of Ratio Spectra Method for The Determination of Amoxicillin Trihydrate and Dicloxacillin Sodium in The Presence of Their Common Impurity; 6-Aminopenicillanic acid.**

In this section, the first derivative ratio method was successfully applied for determination of Amoxicillin and Dicloxacillin in presence of their common impurity; 6-Aminopenicillanic acid in pure form and pharmaceutical dosage form. For the determination of Amoxicillin, the absorption spectra were divided by the absorption spectrum of 30 µg $mL^{-1}$

of 6-Aminopecillanic acid (as a divisor), and then the obtained ratio spectra were differentiated with respect to wavelength. The peak amplitudes at 227.2 nm were recorded where Dicloxacillin was zero-crossing. . While for the determination of Dicloxacillin, the absorption spectra were divided by the absorption spectrum of 20 µg $mL^{-1}$ of 6-Aminopecillanic acid (as a divisor), and the peak amplitudes at 234.6 nm were recorded where Amoxicillin was zero-crossing.

**<u>Section (C):</u> Partial Least Squares and Linear Support Vector Regression Chemometric Methods for Simultaneous The Determination of Amoxicillin Trihydrate and Dicloxacillin Sodium in The Presence of Their Common Impurity; 6-Aminopenicillanic acid: A Comparative Study.**

The goals of this section are presenting two multivariate chemometric models namely; partial least squares regression (PLSR) and linear support vector regression (SVR), for the analysis of Amoxicillin and Dicloxacillin in presence of their common impurity; 6-Aminopecillanic acid and making a modest comparison between the two models highlighting the advantages and limitations of each. Multilevel multifactor calibration design consisting of 4-levels and 3-factors was performed resulting in training set consisting of 16 mixtures of the three components with orthogonal and rotatable distribution in space. To test the validity and

predictive ability of the developed multivariate chemometric models, an independent test set consisting of 8 mixtures was used. The results obtained give hope for using smart chemometric approaches especially linear SVR for analysis of different pharmaceutical products using cheap and simple instruments like UV spectrophotometer.

**Section (D): HPLC/MS Method for The Simultaneous Determination of Amoxicillin, Dicloxacillin and Their Common Impurity; 6-Aminopenicillanic acid.**

Sensitive and highly selective High Performance Liquid Chromatography- Mass Spectrometry (HPLC/MS) method was developed for simultaneous determination of Amoxicillin, Dicloxacillin and their common impurity 6-Aminopenicillanic acid. The chromatographic separation was achieved by applying the mixture on a $C_{18}$ column (3.5 µm ps, 100 mm x 4.6 id) using acetonitrile: water (65: 35, v/v) as a mobile phase within 4 min. Quantitation was performed on a single quadrupole mass spectrometer employing electrospray ionization technique, operating in selected ion monitoring (SIM) and negative mode. The method was validated in linear range of 2-28 µg $mL^{-1}$, 2-35 µg $mL^{-1}$ and 1-10 µg $mL^{-1}$ for Amoxicillin, Dicloxacillin and 6-Aminopenicillanic acid, respectively. The HPLC/MS method combines the advantage of HPLC separation with specificity of mass detection.

**Part II: Determination of Cefoperazone Sodium in Presence of Its Related Impurities; 7-Aminocephalosporanic acid and 5-Mercapto-1-methyl-tetrazole**

This part includes:

**Introduction and Literature Review**

This introduction comprises a brief idea about the chemical structure, physical properities, pharmacological activity, reported impurities and different methods developed for the analysis of Cefoperazone Sodium.

**Section (A): Double Divisor Ratio Derivative Spectrophotometric Method for The Determination of Cefoperazone Sodium in The Presence of Its Related Impurities; 7-Amino cephalosporanic acid and 5-Mercapto-1-methyl-tetrazole.**

This section describes simple, sensitive and cost-effective double divisor ratio derivative spectrophotometric method (DDRD) for determination of Cefoperazone Sodium in presence of its process-related impurities; 7-Aminocephalosporanic acid and 5-Mercapto-1-methyl-tetrazole without prior separation. The absorption spectra of the solutions prepared of different concentrations of pure Cefoperazone and the ternary mixture were recorded and divided by the absorption spectra of the mixed

solution of 7-ACA and5-MERwith the same concentrations (10μgmL$^{-1}$ each as a double divisor). First derivative of these ratio spectra were obtained at Δλ=4 and then the peak amplitudes values at 246.6nm were plotted against the corresponding concentrations of Cefoperazone to obtain the calibration curve with the corresponding regression equation.

**Section (B): Linear Support Vector Regression and Partial Least Squares Chemometric Methods for The Determination of Cefoperazone Sodium in The Presence of Related Impurities.**

The two models (PLSR and Linear SVR) are used to analyze Cefoperazone Sodium (CEF) in presence of its reported impurities; 7-aminocephalosporanic acid (7-ACA) and 5-mercapto-1-methyl-tetrazole (5-MER) in raw materials and in pharmaceutical dosage form via handling UV spectral data. For optimum analysis, a 3 factor 4 level experimental design was established resulting in a training set of 16 mixtures containing different ratios of interfering species. To validate the prediction ability of the suggested models; an independent test set consisting of 9 mixtures was used. SVR model gives more accurate results with lower prediction error compared to PLSR model and high generalization ability, however, PLSR model is easy to handle and fast to optimize.

**Section (C): HPLC/DAD Method for The Determination of Cefoperazone Sodium in The Presence of Related Impurities.**

In this section, a validated HPLC-DAD technique was applied for the quantitative determination of Cefoperazone sodium (CEF) in presence of its reported impurities; 7-aminocephalosporanic acid (7-ACA) and 5-mercapto-1-methyl-tetrazole (5-MER) in pure form and pharmaceutical formulations in addition to separation of the ternary mixture. The mixture of CEF and the reported impurities; 7-ACA and 5-MER were separated on a C8 column (5 µm ps, 250 mm x 4.6 id) using methanol : 0.05 M $KH_2PO_4$ buffer (22.5: 77.5 v/v , pH=7.5) as a mobile phase. The three components were detected at 254 nm with a concentration range of 10-90 µg $mL^{-1}$. The proposed HPLC method is the first developed one to analyze this ternary mixture using single wavelength detection in short analysis time and has the advantage of being more reproducible.

**Section (D): HPTLC Method for The Determination of Cefoperazone Sodium in The Presence of Related Impurities.**

Highly selective and sensitive HPTLC technique was developed for quantitative determination of CEF in presence of its reported impurities; 7-ACA and 5-MER. Mixture of CEF and its reported impurities were separated on silica gel HPTLC $F_{254}$ plates using (acetone: methanol: ethyl

acetate: 2% sodium lauryl sulphate: glacial acetic acid) (3: 2: 3: 0.8: 0.2, by volume) as a developing system and scanning at 254 nm over a concentration range of 1-10 µg/band. The HPTLC method has the advantage of high sensitivity and higher resolution ability beside using a small quantity of developing system, upon using HPTLC plates with smaller particle size. The suggested method was successfully applied for the analysis of CEF in pure form and the pharmaceutical formulations.

**Part III: Stability Indicating Assay Methods for The Determination of Acemetacin in The Presence of Its Degradation Product; Indomethacin.**

This part includes:

**Introduction and Literature Review**

This introduction presents the chemical structure, physical properities, pharmacological activity, stress stability study and different methods developed for the analysis of Acemetacin.

**Section (A): Stability Indicating First Derivative of Ratio Spectra Method for The Determination of Acemetacin in The Presence of Its Degradation Product.**

In this section, the first derivative ratio spectrophotometric method was successfully applied for determination of Acemetacin (ACM) in presence of its degradation product (DEG) in pure form and pharmaceutical dosage form. The $^{1}$DD method was based on measuring the peak amplitude of the first derivative of the ratio spectra at 244 nm using 20 µg $mL^{-1}$ of DEG as divisor. Being a spectrophotometric method; it is simple, less time consuming and economic stability indicating method compared to published LC methods.

**<u>Section (B):</u> Stability Indicating Mean Centering of Ratio Spectra Method for The Determination of Acemetacin in The Presence of Its Degradation Product.**

In this section, a new and very simple method was developed for the determination of ACM in presence of its degradation product (DEG). The recorded absorption spectra of ACM were divided by the absorption spectrum of DEG (20 µg $mL^{-1}$) to obtain the ratio spectra which were then mean centered. These mean centered values of the ratio spectra at 234 nm were recorded and plotted versus the corresponding concentrations. Compared to the derivative method, MCR eliminates the derivative step and so the signal-to-noise ratio is enhanced. Moreover, it was more selective than the $^{1}$DD spectrophotometric method.

**<u>Section (C):</u> Stability Indicating HPTLC Method for The Determination of Acemetacin in The Presence of Its Degradation Product.**

A sensitive and highly selective HPTLC method was successfully applied as stability indicating assay method for separation of ACM and its degradation product (DEG) as well as in its pharmaceutical preparation. Separation of ACM from DEG was achieved on preactivated silica gel 60 $F_{254}$ HPTLC plates using hexane: ethyl acetate: glacial acetic acid (6:4:0.3, by volume) as developing system followed by scanning at 254nm over a concentration range of 0.4-1.4 µg/band. It can be considered as highly sensitive and selective stability indicating assay method, in addition to separation of ACM from DEG.

**This thesis refers to 170 references; contains 48 figures and 69 tables and ends with a summary in Arabic.**