بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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MONA MAGHRABY

Ain Shams University
Faculty of Science
Chemistry Department

Novel HPLC Methods for the Assessment of Some Cephalosporins

A Thesis
Submitted to Chemistry Department – Faculty of Science – Ain Shams University
In Partial Fulfillment for Requirements of the Master's Degree of Science (M. Sc.) in Chemistry

By

Hassan Ismail Hassan El Shikshaky

B.Sc. in Chemistry, Faculty of Science
Ain Shams University
2008

Under Supervision of

Prof. Dr. Ashraf A. Mohamed
Professor of Analytical Chemistry
Faculty of Science – Ain Shams University

Dr. Mustafa A. Sayed
Lecturer of Chemistry
Faculty of Science – Ain Shams University

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Ain Shams University
Faculty of Science
Chemistry Department

Approval sheet

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This thesis of master's degree approved by

Prof. Dr. Ashraf A. Mohamed ……..
Professor of Analytical Chemistry
Faculty of Science – Ain Shams University

Dr. Mustafa A. Sayed ……..
Lecturer of Chemistry
Faculty of Science – Ain Shams University

Head of Chemistry Department
Prof. Dr. Ayman Ayoub Abdel-Shafi

Student Name: Hassan Ismail Hassan El Shikshaky

Scientific Degree: M.Sc.

Faculty Name: Faculty of Science – Ain Shams University

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Hassan Shikshaky

Aim of Work

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The current work aims at developing and validating new analytical methods for the assessment of some Cephalosporins in human plasma.

Summary

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Herein, a new, simple, sensitive, and reliable three bio-analytical methods were introduced for the determination of two cephalosporins in the human plasma– namely: cefprozil and cephalexin. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) and with ultraviolet (LC-UV) detection methods were developed and validated for the determination of *cis*- and *trans*-cefprozil diastereoisomers. Moreover, LC-UV method was developed and validated for the determination of cephalexin in the human plasma.

For cefprozil determination, the human plasma samples were subjected to protein precipitation after the addition of cephalexin as internal standard. Chromatographic separation of the two diastereoisomers from the endogenous interfering components of the human plasma was achieved using Xbridge C_{18} column (5µm, 4.6×150mm) and maintained at 25° C. The LC-MS/MS method utilized the multiple reaction monitoring (MRM) transitions 390.1 to 208.1 m/z for cefprozil and 348.1 to 158.2 m/z for cephalexin detection, while the wavelength 292 nm was used for the UV detection. Both methods provided good linearity for the determination of *cis*- and *trans*-cefprozil diastereoisomers within the ranges of 0.05-10.00 and 0.02-1.00 µg/ml

respectively. The methods were validated and applied successively to study the bio-equivalence of the two cefprozil diastereoisomers in pharmaceutical products. The maximum plasma levels (C_{max}) detected for cefprozil for the brand and generic products were 10.0 and 9.9 μg/ml, respectively, as determined using the LC-MS/MS method compared to 10.5 and 10.6 μg/ml, respectively, as determined using the LC-UV method. The pharmaceutical products were found to be bio-equivalent after analysis using both methods. The pharmacokinetics data of the reference product were statistically compared over the two methods and resulted in insignificant P-values. This comparison confirm both methods reproducibility, reliability and ability to quantify cefprozil diastereoisomers in the human plasma.

Regarding cephalexin, a LC-UV method was developed and validated for its determination in human plasma. The human plasma samples were subjected to protein precipitation after the addition of sulfamethaxole as internal standard. Chromatographic separation of cephalexin from the endogenous interfering components of the human plasma was achieved using a Nova-pak C_8 column (5μm, 150 x 4.6 mm) and maintained at 30° C. The UV detection of cephalexin and its internal standard was performed at a

wavelength of 254 nm. The developed method provided good linearity and recovery for cephalexin in the range of 0.5 to 50.0 µg/ml.

List of Abbreviations

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$AUC_{0-\infty}$	Area under the concentration-time curve from 0 min to infinity.
AUC_{0-t}	Area under the concentration-time curve from 0 min to the last measured concentration.
B	Brand pharmaceutical product
CEF	Cefprozil
CEF-LC-MS	Liquid chromatography with tandem mass spectrometry detection method for Cefprozil diastereoisomers
CEF-LC-UV	Liquid chromatography with ultraviolet detection method of Cefprozil diastereoisomers
CFE	Cefprozil trans-diastereoisomer
CFZ	Cefprozil cis-diastereoisomer
CL	Clearance
C_{max}	The maximum reachable concentration for the dose in the blood
CPH	Cephalexin
CPH-LC-UV	Liquid chromatography with ultraviolet detection method of Cephalexin
CV	Coefficient of variance
DAD	Diode array detector
EMA	European Medicines Agency
ESI	Electrospray ionization