High-Performance Liquid Chromatographic

Determination of Metformin in Human Serum Using Solid-Phase Extraction

Honors Project

In fulfillment of the Requirements for

The University Honors College

University of North Carolina at Pembroke

By

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Chemistry

April 21, 2007

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Date: 5/4/07

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PREFACE/ACKNOWLEDGMENTS

I would like to give special thanks to Esther G. Maynor Honors College and Dr. Jesse Peters for this opportunity to research and explore this topic in my field of study. I am also appreciative to Dr. Flowers for his support and the UNCP Teaching and Learning Center for helping to fund this project. I am especially grateful to Dr. Meredith Storms for her dedication, support, and for allowing me the opportunity to participate in this undergraduate research experience.

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Drug	Retention Time (min)
Acetaminophen	3.650
Alkaseltzer	2.301
Allopurinol	2.909
Amiloride	16.287
Amoxicillin	2.778
Bayer	2.408
Diciofenul	8.763
Maxium Strength Pain Relief	
Maxium Strength Sinus	3.556
Metro	3.842
Mucinex	4.412
PSS	>20

Table 1

LISTOF FIGURES

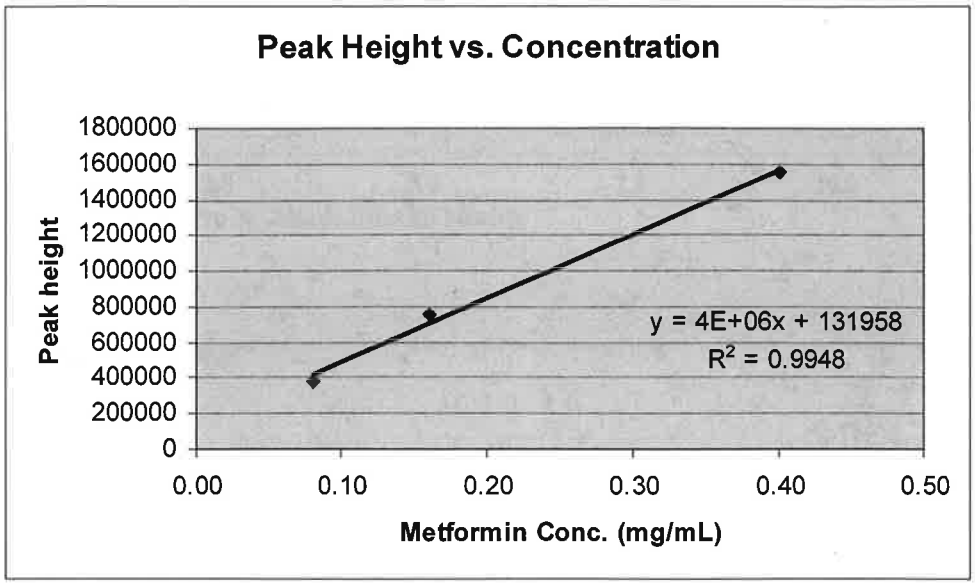


Figure 1 Calibration data human serum spiked with metformin

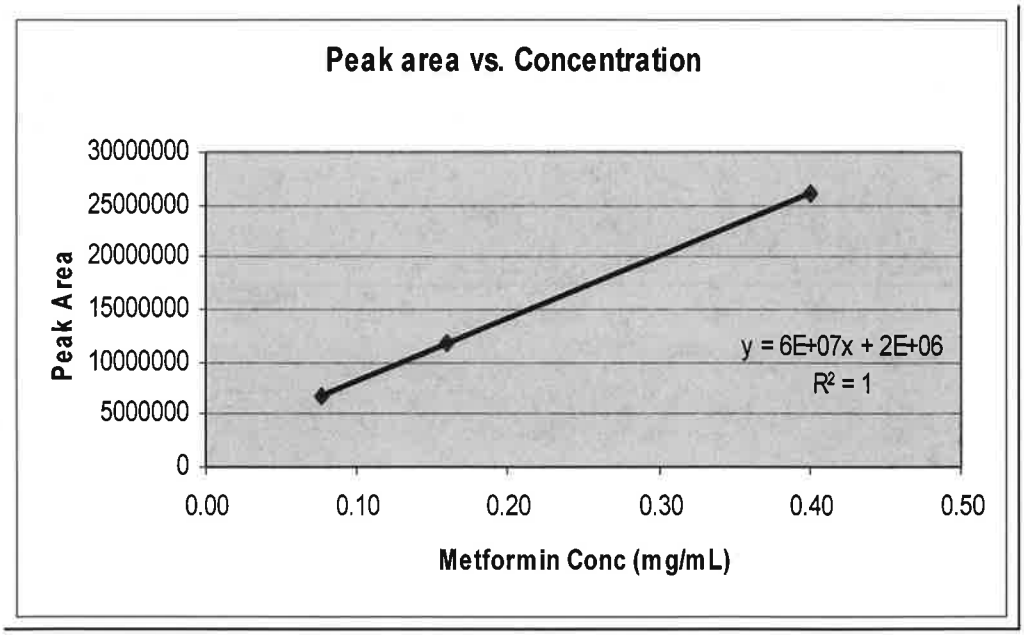


Figure 2 Calibration data human serum spiked with metformin

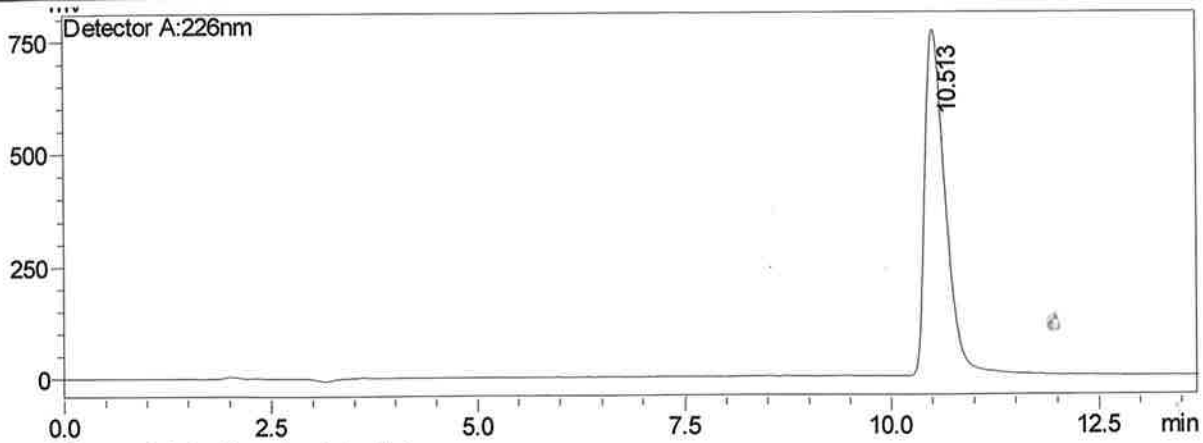


Figure 3 Metformin, blank human serum

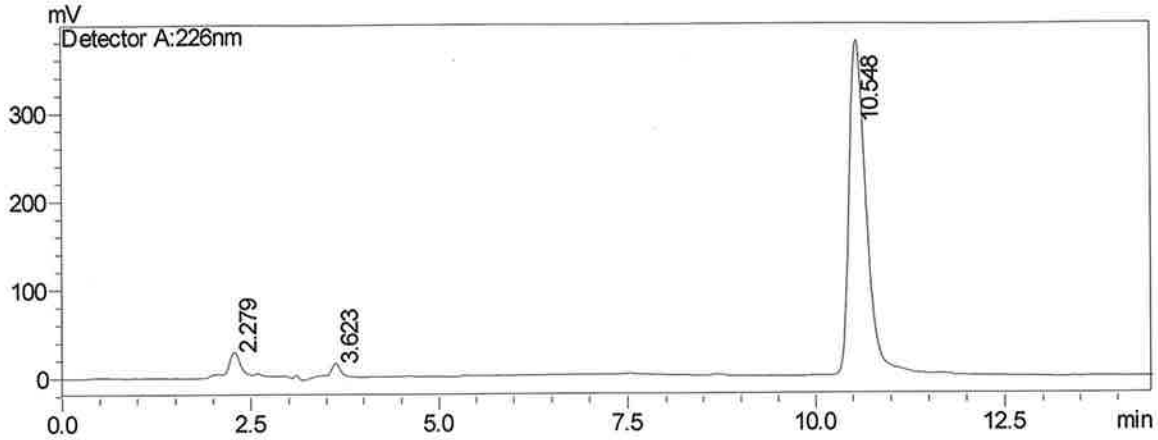


Figure 4 Strata, no wash, ACN elution

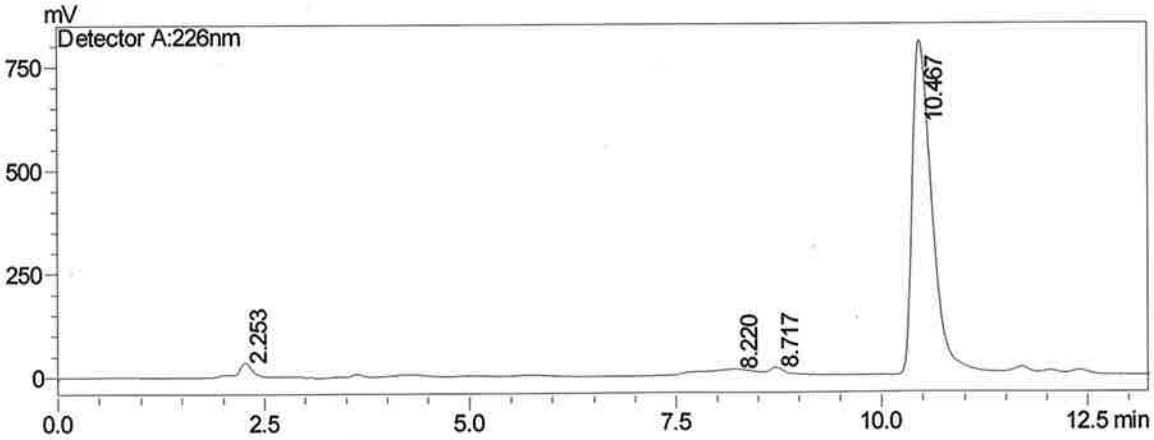
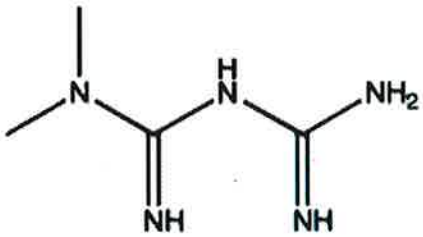


Figure 5 PH, no wash, ACN elution

LIST OF SYMBOLS



Symbol 1 metformin

ABSTRACT

A high-performance liquid chromatography method has been developed for the determination of metformin in human serum. The separation and quantitation are achieved on a 150-cm Luna C18 column using a mobile phase of 60:40% v/v 10 mM sodium phosphate buffer containing 10 mM SDS (pH 5.0) and acetonitrile at a flow rate of 0.5 mL/min with detection of metformin at 226 nm. Recoveries found from a solid-phase extraction (SPE) method for metformin in human serum ranged from 92.4-100.5%. The highest recovery was found using a Pheynl SPE cartridge eluted with 0.75ml ACN while other cartridges yielded lower recoveries.

INTRODUCTION

Metformin (1,1-dimethylbiguanide) is a drug that is administered to help regulate type 2 diabetes mellitus. This type of diabetes is caused by the insulin produced in the pancreas having the inability to get sugar into the cells of the body for it to work appropriately. Metformin is a commonly used drug that has a rare side effect of lactic acidosis [1]. The buildup of lactic acid in the body is lactic acidosis and can lead to the acidification of the blood. Lactic acid is produced by cells in the body when there is not enough oxygen and the cells will use glucose instead [2]. It is important to monitor Metformin in plasma because high concentrations have lead to this rare side effect especially in people who have poor kidney and/or liver function [3]. This condition can be lethal and therefore it is important to monitor it's concentration in plasma.

Solid-phase extraction (SPE) is one method that will be used; however this is not preferred with metformin in plasma in combination with high-performance liquid chromatographic (HPLC) because of the high polarity of metformin. SPE is an extraction method that uses a liquid and solid phase to isolate one type of analyte from a solution. This type of method is used to clean up the sample before using a method such as chromatographic to quantitate the amount of analyte in the sample [4]. An analyte is the substance being measured in the laboratory. A literature survey revealed that both reversed-phase HPLC with UV detection and cation exchange-based HPLC produce high recoveries of metformin [5], [6]. Literature surveys also revealed that HPLC methods have been reported for the individual determination of metformin in plasma by direct injection [7], protein precipitation [5,6], and liquid-liquid extraction [8,9]; however, solid-phase extraction (SPE) methods are not common. SPE is typically employed in

many therapeutic drug monitoring and pharmacokinetic studies since it often results in a more complete extraction of the analyte (higher recovery), a cleaner sample, and requires less organic solvent (more "green").

A similar project has been researched at the University of North Carolina at Pembroke using SPE and HPLC method to determine metformin in human serum. However, this project will try to improve the SPE method for a smaller sample size and improve the recovery of the analyte. The goal and focus of the project is to develop a solid-phase extraction procedure and HPLC method for the determination of metformin in human serum.

METHODOLOGY

This research was performed and completed in Oxendine Science Building with Dr. Meredith Storms as the mentor. High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE) are the two main components used in this research (see appendix). The HPLC method consists of 0.1mL metformin solution injected into Rheodyne Injector with a flow rate 0.500mL/min, UV detection: 226nm, 50 x 4.8mm i.d. Luna C18 column, an isocratic mobile phase composed of acetonitrile (ACN), and a buffer solution (10mM sodium phosphate dibasic containing 10mM SDS (pH 5) (60:40% v/v)). The SPE method consists of four steps: *Condition:* 1mL distilled H2O and 1mL Methanol, *Load:* 1 mL metformin dissolved in 10 mM phosphate buffer containing SDS (pH 5), *Wash:* None, and *Elute:* 0.75mL acetonitrile (ACN). During the SPE process, more than one method was used to determine the best recovery. The wash step was alternated with no wash or 100μL of H_2O. The elution step consisted of elution solution, methanol, or 0.2mL of ACN.

The reagents and chemicals used during this research were: Metformin, HPLC, drug-free human serum, VAC-ELUT vacuum manifold, Oasis HLB cartridges, Varian Bond Elut cartridges, 150cm Luna C18 column, 10mM sodium phosphate buffer containing 10mM SDS and acetronitrile, Strata SPE cartridge, Phenyl SPE cartridge, 0.45micrometer polymetric filter, phosphoric acid, and methanol.

RESULTS / SUMMARY

The major task for this project is to develop an accurate and precise solid-phase extraction and HPLC method for determining metformin in human serum. Although a similar project has already been explored at the University of North Carolina at Pembroke, the aim was to improve both the SPE method for a smaller sample size and the amount of analyte that will be recovered. The use of solid-phase extraction with HPLC in determination of metformin in plasma is not usually preferred because metformin has a high polarity. The importance of this research is to monitor the concentration of metformin in plasma since high concentrations have led to lactic acidosis in patients who have poor kidney and/or lung function. Pheynl SPE cartridge eluted with 0.75mL of ACN yielded the highest recovery of 100.5% (see appendix). Many of the cartridges also yielded high recovery percentages. The calibration curves were linear with a R^2 value close to one (see figure 1 and 2).

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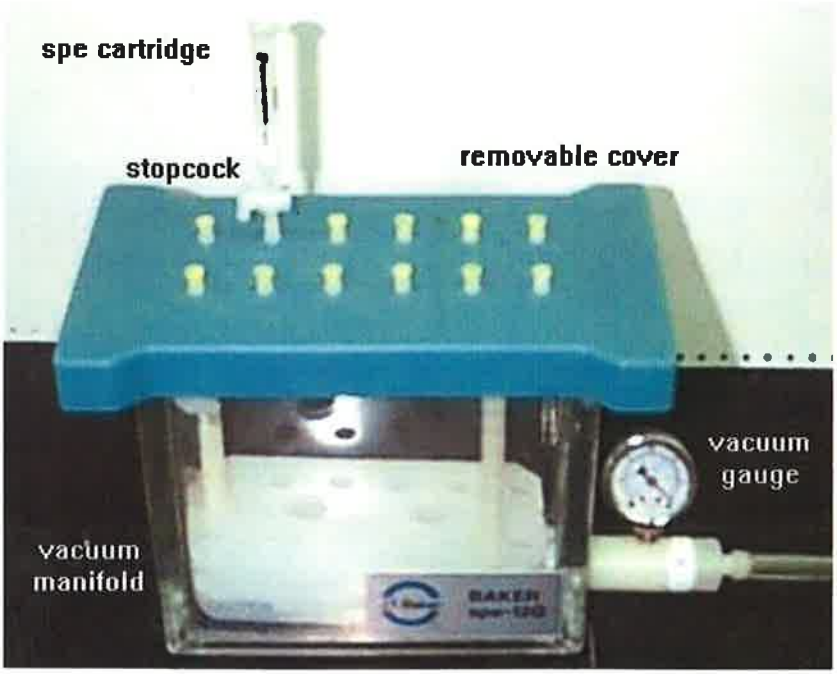
APPENDICES

These are based on the 1:50 dilution and peak area.

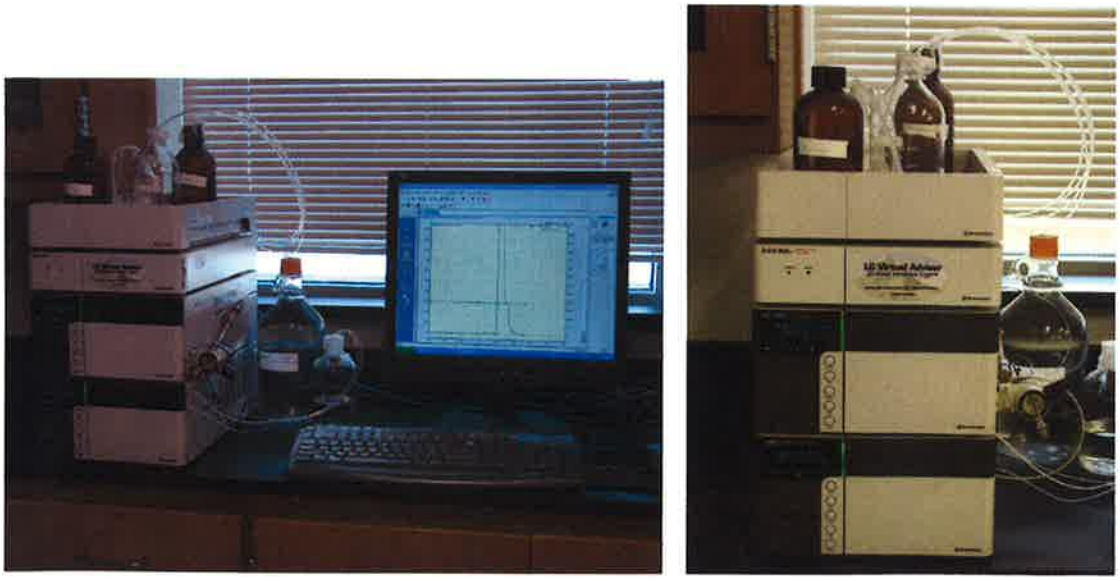
Extractions	Pk Area	Concentration Extracted	% Recovery
PH no wash step (elution solution)	4634838	54.19803	67.74754
Strata no wash step (elution solution)	4902235	57.32487	71.65608
PH no wash step (eluted with methanol)	7946479	92.9231	116.1539
Strata no wash step (eluted with ACN)	6820500	79.75633	99.69541
Strata no wash step (eluted with ACN)	6055851	70.81481	88.51852
PH no wash step (eluted with ACN)	6325516	73.96817	92.46022
1:50 Dilution (80ug/mL)	68413381		

These are based on the stock solution and peak height. Peak height should be used because the peaks are large and the area is not as accurate.

	Pk Height	Concentration Extracted	% Recovery
Strata with wash step of 100uL of water	3859321	4.248374	106.2094
PH with wash step of 100 uL of water	3924437	4.320055	108.0014
PH elution step (ACN of 0.75mL)	3659154	4.028029	100.7007
PH elution step (ACN of 0.2mL)	3867773	4.257678	106.442
Strata elution step (ACN of 0.2mL)	3846147	4.233872	105.8468
Strata elution step (ACN of 0.75mL)	3888915	4.280952	107.0238
4mg/mL solution of metformin	3633692	4	



SPE apparatus



HPLC apparatus