

SIMULTANEOUS SOLID PHASE EXTRACTION OF CHLORPROPAMIDE, GLIPIZIDE AND GLIBENCLAMIDE IN PLASMA, USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: To develop a rapid, clean and simple simultaneous solid phase extraction procedure for three of the most commonly prescribed sulfonylurea drugs (Chlorpropamide, Glipizide and Glibenclamide) using High Performance Liquid Chromatography.

Method: A C-8 solid phase column (100mg) after the column was conditioned, the sample was mixed with three ml buffer (0.1% orthophosphoric acid) and then applied to the column. The drugs were eluted from the column with a half ml (four times) acetonitrile after the washing procedure of the column with one ml buffer, then half ml 20% acetonitrile, then applied to a High Performance Liquid Chromatography, by using the developed method that used a reversed phase C-8 column with a mobile phase consisting of 0.1% orthophosphoric acid pH 2.7: isopropanol: acetonitrile, (45:25:30) operated at ambient temperature, using wave length at 235 nm detector, has been used for the analysis of the extracted drugs (chlorpropamide, glipizide and glibenclamide)

Results: A solid phase extraction method has been developed for the simultaneous extraction of three frequently used sulfonylureas: Chlorpropamide, Glipizide and Glibenclamide. The overall recoveries and relative standard deviations were 98.5%±1.9 for chlorpropamide, 98.9%±2.4 for glipizide, 97.8%±1.7 for glibenclamide, and 97.5%±2.7 for progesterone internal standard. The response on High Performance Liquid Chromatography was linear in the range 1.0- 100µg/ml for Chlorpropamide, Glipizide, and Glibenclamide, with correlation coefficient (r^2) > 0.997 for all drugs. Detection limits were 2ng/ml plasma for Chlorpropamide, 15ng/ml plasma for glipizide and 7ng/ml plasma for glibenclamide, measured at a Signal/Noise (S/N) of three. No interference from administered drugs (Barbiturates, B-blockers, Tranquilizer, Antihypertensive, Histamine antagonist, Antidepressant, Antiemetic, and Anticonvulsant) or endogenous constituents were observed.

Conclusion: The developed method, with its high recoveries for the chlorpropamide, glipizide and glibenclamide, can be applied for clinical, forensic toxicology as well as in the bioavailability or bioequivalence studies or in the study of these drugs in pharmaceutical products.

Key words: HPLC, Sulfonylureas, Solid Phase Extraction, Chlorpropamide, Glipizide, Glibenclamide

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Introduction

A review of drug-induced hypoglycemia in 1,418 cases shows that sulfonylureas account for 70% of

all reported hypoglycemia cases.⁽¹⁻²⁾ Death due to drug-induced hypoglycemia by sulfonylureas was shown in 11.4% of the 220 sulfonylurea drugs

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cases.⁽¹⁻³⁾ In addition to the difficulty and cost in terms of insulinoma diagnosis as instances have been recorded of patients undergoing laparotomy and even subtotal pancreatectomy before the ingestion of sulfonylureas drugs discovered.⁽¹⁻⁵⁾

Sulfonylureas, such as Chlorpropamide, Glipizide and Glibenclamide are encountered in clinical use and drug poisoning. In either of the above situations, a clinical and forensic toxicology analysis might be required. The extraction and analysis should include all the above mentioned drugs, due to the frequent presence of one or more in particular cases.⁽¹⁻⁵⁾

Traditionally, diagnosis of sulfonylureas is achieved by liquid-liquid extraction techniques. This technique could be tedious, time consuming and might result in emulsion formation, hence render separation difficult, in addition to prolonging the procedure and causing substance loss. Also it is not suitable for highly polar molecules.^(1,6) Solid phase extraction on the other hand, is highly efficient, rapid, and reproducible method for drug extraction.^(1,6)

Previously published assays focused only on solid phase extraction of one of the Sulfonylureas.^(1,6) This work was initiated with the objective to develop a rapid, clean and simple simultaneous solid phase extraction procedure for three of the most commonly prescribed sulfonylureas drugs: Chlorpropamide, Glipizide and Glibenclamide in Jordan.

Methods

All solvents used were of High Performance Liquid Chromatography (HPLC) grade and other chemicals used were of analytical grade. The standard drugs were Chlorpropamide (Pfizer, Brussels-Belgium), Glipizide (Farmitalia, Calo Erba), and Glibenclamide (Servier). The internal standard was Progesterone (Solana, Sweden) and C8 (100mg) cartridges were purchased from IST, ISOLUTE™, EEC.

Analysis of the three drugs was performed on a HPLC system consisting of HP1100 isocratic pump and HP1050 ultraviolet-visible spectrophotometer (Hewlett-Packard, CA, USA) in addition to a rheodyne injection valve (Cotati, CA, USA) and a 20 µL fixed filling loop. The analytical column was Luna -C8 (2), 250x4.6 mm I.D (Phenomenex, CA, USA) filled with 5µm C8 sorbent. The effluent of the column was constantly monitored at 235 nm. Data acquisition and integration was performed using HP3395 integrator (Hewlett-Packard, CA, USA).

The mobile phase was helium-conditioned and consisted of (30:25:45v/v/v) mixture of acetonitrile: Isopropanol: 0.1% Orthophosphoric acid (pH = 2.7). It was degassed and filtered through a 0.5µm membrane filter (Millipore, USA). The column temperature was ambient and the flow rate was 1.0ml/min. The eluted drugs were monitored at 235nm. The chart speed was 1.0 cm/min.⁽⁷⁾

A stock solution of the three drugs was prepared by dissolving 100mg of each in 100ml methanol to produce a concentration of 1mg/ml. The working standard solutions of the mixture (concentration range 0.5-1000µg/ml) was made by dilution of the stock solution with methanol. The internal standard (progesterone) of 1mg/ml was prepared in methanol. One hundred micro-liters (µl) of the working standard solution and internal standard were added to 1ml blank plasma concentration of 0.05-100µg/ml of each drug, and 1µg/ml of the internal standard. The samples were placed in conical glass tubes (10ml). Three ml of 0.1% H₃PO₄ was added to the sample then mixed for 30 seconds and centrifuged for five minutes at 3000 rpm. The supernatant of the mixture was applied to the pretreated C8 column and allowed to be adsorbed to the solid phase at a flow rate about 1-2 ml/ minute while applying the vacuum. The column was then washed by 1ml buffer (0.1% H₃PO₄) followed by ½ml 20% acetonitrile at a fast flow rate, and the column was left to dry. Next, the elution step of the drugs was done by applying ½ml X 4 acetonitrile at a flow rate 0.5- 1 ml/minute. The extract evaporated to dryness under a gentle stream of Nitrogen. The residue was reconstituted with 100µl of the mobile phase.

Results

Figures 1-6 shows a HPLC chromatogram for three drug extracts and the standard after extraction by solid phase extraction. The chromatogram showed no interference from the endogenous material present in plasma. Different organic solvents like methanol, acetonitrile, distilled water and buffer (Acetonitrile/ isopropanol/0.1% orthophosphoric acid-30:25:45 respectively) were used to wash the retained drugs on the sorbent bed, and also methanol and acetonitrile with different concentrations were tested to obtain a clean extract and high recovery. These recoveries are summarized in Table I.

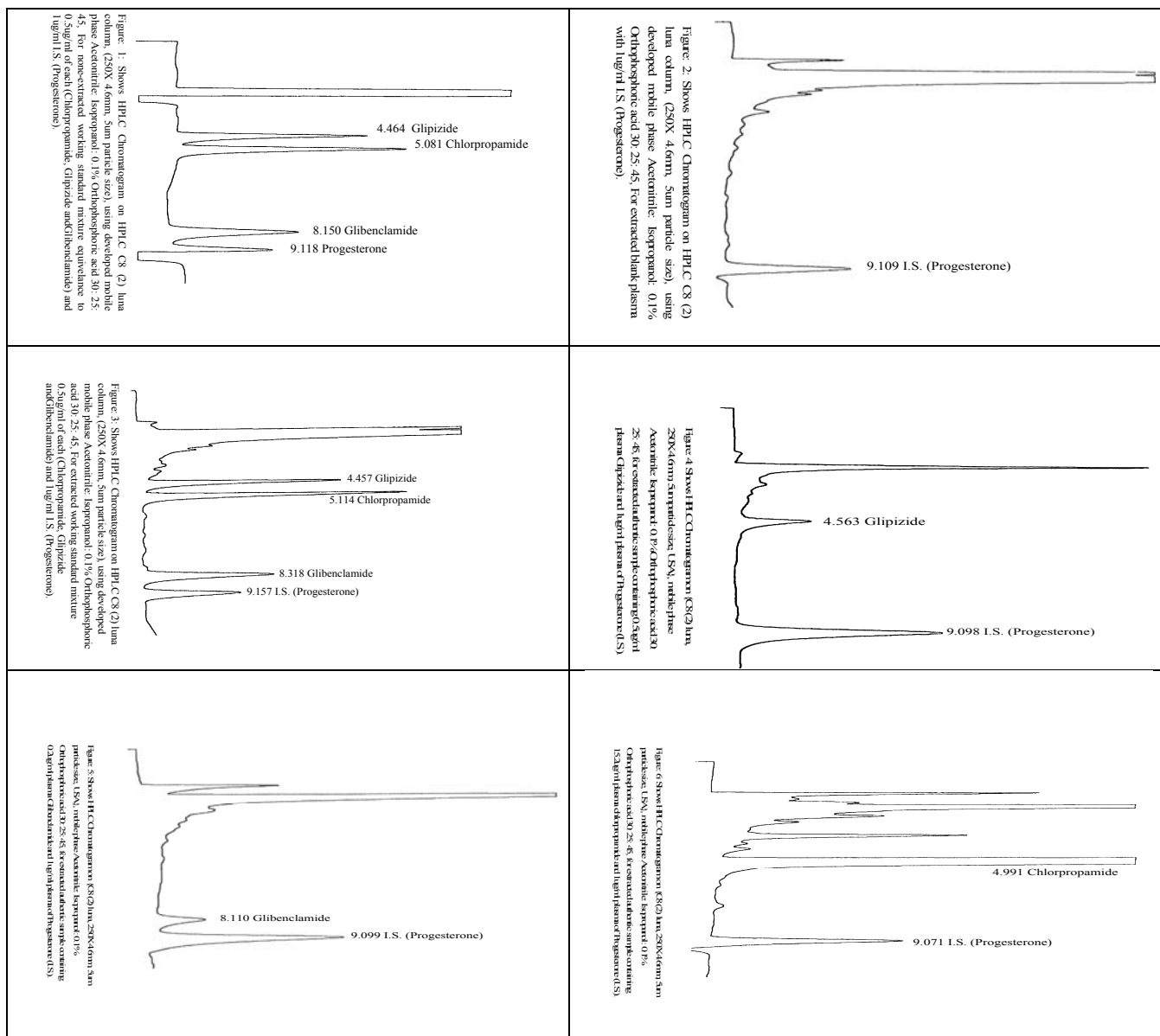


Table I. Extraction on SPE column (C8), mixture 1µg/ml of Chlorpropamide, Glipizide, Glibenclamide and IS progesterone

Washing procedure	Elution system	Average Recovery %			
		Chlorpropamide	Glipizide	Glibenclamide	Progesterone
1 ml buffer, then ½ml 30% methanol	½ ml 60% methanol	54.1	73	38	1.6
1ml D.W, then 1 ml buffer	½ ml x 2 methanol	48.8	136.3	74.4	122.8
1ml D.W., 1ml buffer, then ½ ml 30% acetonitrile	½ ml acetonitrile	78	91.4	77.3	85.1
1ml buffer, then ½ml 20% acetonitrile	1ml acetonitrile	86.1	82.1	84.4	84
1ml buffer, then ½ml 20% acetonitrile	1ml X2 acetonitrile	84.1 ± 6.4 n= 5 C.V*=7.6%	83.8±7.8 n=5 C.V=9.3%	84±10.5 n=5 C.V=5.2%	82.8± 9.2 n= 5 C.V=11.1%
1ml buffer, then ½ml 20% acetonitrile	½ ml X4 acetonitrile	98.5±1.9 n= 5 C.V=1.6%	98.9±6.4 n=5 C.V=1.9%	97.8±1.7 n= 5 C.V=1.6%	97.5 ± 2.7 n= 5 C.V=2.1%

*C.V= coefficient of variation

One ml buffer, then ½ml 20% acetonitrile for washing the eluted drugs, and ½ml four times of acetonitrile to elute the drugs and the internal standard from the sorbent bed showed the highest recovery with accepted coefficient of variation.

Discussion

In this study, the C8 solid phase extraction columns were selected, taking into consideration their similarity to the column that is used in the chromatographic system. Buffering the plasma with 1ml of 0.1% Orthophosphoric acid was enough, but more than 1ml was required to decrease the viscosity of the plasma, and 3ml was found to be adequate for easy passing of the buffered sample through the SPE column. The use of ½ml X 4 acetonitrile obtained high recoveries for all drugs, when other concentrations or lesser volumes of acetonitrile, or different concentration of methanol were used the recovery decreased significantly. In addition, the use of acetonitrile gave a clean chromatogram, while methanol extracted many endogenous materials, that interfered with the drugs (glipizide and IS.), this interference increased the recovery greater than 122% for glipizide and IS. Washing by 1ml buffer and 20% acetonitrile with fast flow rate showed minimal effect on recoveries of drugs of interest (Fig.1-6).

In the literature, solid phase extraction recoveries obtained 101.3% for gliclazide and 99.9% for IS⁽¹⁾ and 100.36%+2.36 for Glibenclamide,⁽⁶⁾ while recoveries obtained by the developed method was 92.2- 97.7% for all drugs of interest. The reason for lower recoveries by the developed method is the simultaneous extraction of the drugs of interest and IS, while in the literature extraction is performed on single drug in addition to IS.

The simultaneous extraction of Sulfonylureas (chlorpropamide, glipizide and glibenclamide) from plasma has the advantage of providing a screening of the above drugs in one step for forensic or clinical cases.

A practical point observed in the procedure, was that heparinized plasma was the best sample for Sulfonylureas analysis. On the other hand, serum from SST® GEL and clot activator tube showed many interference peaks on the chromatogram. C8 solid phase extraction columns were selected taking into consideration their similarity to the column used in the chromatographic system. Buffering the plasma with 1ml 0.1% orthophosphoric acid was enough, but the need for more than 1ml required to

decrease the viscosity of the plasma, 3ml was found to be adequate for easy passing of the buffered sample through the SPE column.

The simultaneous solid phase extraction of Sulfonylureas (chlorpropamide, glipizide and glibenclamide) from plasma has the advantage of providing rapid, clean, safe and reproducible results of the above drugs in one step for forensic or clinical cases. The efficiency of the procedure was measured by comparison of area under the peaks ratio obtained for the three drugs after extraction using the assay procedure with those obtained by direct injection of pure standard solution of the drugs. The Table I shows that the errors were random over the range of the assay. Five replicates, were each at concentration of 10ug/ml. The mean Recovery and standard deviations for the concentrations were 98.5±1.9% for chlorpropamide, 98.9±2.4% for glipizide, 97.8±1.7% for glibenclamide, and 97.5±2.7% for progesterone (internal standard). In the literature review using solid phase extraction, recoveries obtained were 101.3% for gliclazide and 99.9% for IS⁽¹⁾ and 100.36 + 2.36 for glibenclamide,⁽⁵⁾ while recoveries obtained by the developed method were 97.5- 98.9% for all drugs of interest. The reason for the decrease in the recoveries by the developed method was that we concentrated to extract all of the drugs of interest and the IS simultaneously, while in literature review only a single drug including IS was extracted.

Conclusion

The aim of this research was to develop a method for simultaneous solid phase extraction of chlorpropamide, glipizide and glibenclamide in plasma, using HPLC determination for its suitable separation, while solid phase sorbent was used for its acceptable extraction capacity of drugs from biological fluids. A simple, rapid, relatively low cost, specific and sensitive procedure for the analysis of Sulfonylureas from plasma samples was presented. The mobile phase was 30:25:45 mixture of acetonitrile: isopropanol: 0.1% orthophosphoric acid. The drugs were separated on C8 (250X 4.6mm I.D. 5µm particle size, Luna) and the effluent of the column was monitored at 235nm, at flow rate of 1ml/min. The technique gave high reproducibility of retention times for the separated drugs. Detection limits for plasma samples were 2ng/ml, 15ng/ml, and 7ng/ml for chlorpropamide, glipizide, and glibenclamide respectively; they were measured at a signal /noise of 3. The intra-inter days variation for

all drugs were <4.1%. No interference from barbiturates, B-blockers, tranquilizers, antihypertensives, histamine antagonists, antidepressants, antiemetics, and anticonvulsants or endogenous constituents were observed (Table II). The overall recoveries and relative standard deviations were 98.5±1.9% for chlorpropamide, 98.9±2.4% for glipizide, 97.8±1.7% for glibenclamide, and 97.5±2.7% for progesterone (internal standard).

The response on HPLC was linear in the range of 1.0-100µg/ml for chlorpropamide, glipizide, and glibenclamide, with $r^2 > 0.997$ for all drugs.

Table II. The retention times of the drug interference study, on HPLC, C8, using the developed mobile phase Acetonitrile: Isopropanol: 0.1% orthophosphoric acid (30: 25: 45) and the retention times of glipizide, chlorpropamide, glibenclamide, and progesterone: 4.456, 5.109, 8.315, and 9.155 min. respectively

Drug	Retention Time (min.)	Drug	Retention Time (min.)
Diclofenol	13.669	Bromazepam	3.739
Phenbarbitone	3.900	Ibuprofen	14.210
Propranolol	3.078	Chlorpromazine	3.525
Diazepam	6.172	Midazolam	2.727
Rantidine	2.142	Thiopentone	6.683
Haloperidol	3.027	Carbamezipine	4.017
Cloimpramine	3.633	Digoxin	3.489
Captopril	3.291	Frusemide	3.482
Domperidone	2.954	Warfarin	6.320
Gliclazide	6.320		

This method can be applied for clinical, forensic toxicology, bioavailability or bioequivalence studies as well as for the study of these drugs in pharmaceutical products.

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