A Validated HPLC-PDA Method for Simultaneous Quantitation of Four Oral Antidiabetic Drugs and Application to Pharmaceutical Preparations

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Summary: A simple, rapid, and sensitive HPLC-PDA method has been developed for the simultaneous separation and estimation of metformin (MET), sitagliptin (SIT), glipizide (GPZ), and glibenclamide (GBN). The proposed method utilized Bondapak™ C18 (3.9×150 mm, 10 µm) column and the separation has been achieved within 10 min, with mobile phase consisted of methanol: phosphate buffer 0.05 M (60:40, v/v) adjusted to pH 5, delivered at a flow rate of 0.8 mL/min. Photodiode array detector was used to detect drugs at 260 nm. The developed method was validated according to the ICH guidelines. The method was linear over the range of 0.5-100, 0.5-10, 0.005-10, and 0.5-10 µg/mL for MET, SIT, GPZ, and GBN, respectively, with excellent correlation coefficients ($r \geq 0.9976$). Intra-day and inter-day RSD values were ≤ 2.6. The recoveries of drugs from their tablets ranged from 97-103%. The proposed method was successfully applied to the analysis of MET, SIT, GPZ, and GBN in their tablets, and it is suitable for the rapid quantitation of these drugs in single or combined dosage forms.

Key words: HPLC-PDA; Simultaneous quantitation; Type 2 diabetes; Oral antidiabetics.

Introduction

The number of people diagnosed with type 2 diabetes around the globe continues to grow rapidly. Oral medications are used in the treatment of type 2 diabetes due to their effectiveness and safety.

Metformin (MET) is the only member in the biguanide class. It decreases fasting glucose levels with gastrointestinal side effects. First- and second-generation sulfonylureas act on b-cells in the pancreas to increase insulin release. Hypoglycemia is the major side effect of these agents [1, 2]. Dipeptidyl peptidase-4 (DPP-4) inhibitors have low risk of hypoglycemia, but high cost [3, 4].

The drugs under study have been chosen from the abovementioned classes which are commonly prescribed in combination for treatment of type 2 diabetes. MET is commonly prescribed in combination with sulfonylureas such as Glipizide (GPZ) and Glibenclamide (GBN). Sitagliptin (SIT) (DPP-4 inhibitor) led to reduction in A1C, when taken in addition to MET and sulfonylureas [5, 6].

Literature survey revealed different methods for analysis of MET, SIT, GPZ, and GBN alone or in combined pharmaceutical dosage forms [7-22]. Those methods include spectrophotometry, capillary electrophoresis, and high performance liquid chromatography. HPLC has been widely applied for the determination of these drugs either alone or in combinations. However, no method has been published in the literature so far dealing with the simultaneous analysis of the cited four drugs in a single run.

In this work, RP-HPLC method with photodiode array detection has been chosen to simultaneously separate the four antidiabetic drugs under study. The photodiode array (PDA) detector allows different compounds to be measured at a range of wavelengths concurrently in one run [23].

Therefore, our objective was aimed at developing and validating a simple, sensitive, and rapid RP-HPLC method with PDA detector to separate MET, SIT, GPZ, and GBN in one run.

Experimental

Chemicals and reagents

Reference standard samples were purchased from different pharmaceutical companies (MET from SIGMA-AIDRICH), (GBN from ATLANTIC Research Chemicals), (SIT from TSZCHM), and (GPZ from ALFA AESAR Johnson Mothey Company). The pharmaceutical dosage forms were purchased from a local drug store (Glucovance®, MERCK SANTE, 500 mg MET / 5 mg GBN), (Janumet®, Merck & Co, 850 mg MET / 50 mg SIT), (Minidiab®, Pfizer, 5 mg GPZ). Analytical grade potassium dihydrogen phosphate and

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orthophosphoric acid 99% were purchased from WINLAB (UK). HPLC grade methanol was purchased from Fisher Scientific (UK). Deionized water and a Millipore membrane filter (0.2 mm, Nihon, Millipore) were used throughout the experiments.

Instrumentation and chromatographic conditions

The development and validation of the method was performed on Waters HPLC instrument equipped with Waters 1525 Binary Pump, Waters 2998 Photodiode array detector (PAD), and Waters 2707 Autosampler (WATERS, USA). The data handling system comprised of a Dell personal computer and Breeze 2 software. The stationary phase used in this study were Bondapak™ C18 (3.9×150 mm, 10 µm) column purchased from WATERS (USA) and Chromolith® reverse phase HPLC column (4.6×100 mm) purchased from Merck (Germany). The mobile phase was methanol: phosphate buffer 0.05 M (60:40, v/v) adjusted to pH 5. The mobile phase was filtered through MS®- nylon membrane filter (pore size 0.45µm, diameter 4.7cm, Membrane solution, USA) and degassed before use. The flow rate was 0.8 mL/min and the detection was performed at 260 nm.

Preparation of stock and standard solutions

Stock solutions containing 1000 µg/mL of individual MET, SIT, GPZ, and GBN were prepared by dissolving 0.01gm in 10 mL HPLC grade methanol. The prepared stock solutions were stored at 4° C and protected from light. Working standard solutions over the range of 0.5-100, 0.5-10, 0.005-10, 0.5-10 µg/mL for MET, SIT, GPZ, and GBN, respectively, were prepared by dilution of individual aliquots of stock solutions with the mobile phase.

Preparation of tablet solutions

Ten tablets from each dosage form (Glucovance®, Janumet®, Minidiab®) were powdered. An accurately weighed portion equivalent to one tablet of Glucovance® was transferred to 100 mL volumetric flask, diluted with methanol, and the solution was sonicated and filtered. Aliquot from this solution was diluted with the mobile phase to give a final concentration of 100 µg/mL MET and 1µg/mL GBN. For Janumet® and Minidiab®, an accurately weighed portion equivalent to one tablet from each dosage form was transferred to 100 mL volumetric flask, diluted with water, and the solution was sonicated and filtered. Aliquots from these solutions were diluted with the mobile phase to give final concentrations of 20 µg/mL MET, 1µg/mL SIT (Janumet®), and 5 µg/mL GPZ (Minidiab®).

Specificity

The specificity of the proposed HPLC method for the determination of MET, SIT, GPZ, and GBN in bulk drugs and in pharmaceutical preparations was investigated under the chromatographic conditions used for this analysis.

Validation

Calibration standard solutions were prepared by making dilutions from the stock solution of each drug over the range of 0.5-100, 0.5-10, 0.005-10, 0.5-10 µg/mL for MET, SIT, GPZ, and GBN, respectively. Calibration plots were constructed and regression equations were derived for each analyte.

The intra-day and inter-day accuracy and precision of the method were determined by analyzing three QC samples of each drug in triplicate over a period of 3 days. Precision and accuracy were reported as % RSD and % recovery, respectively.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of Quantification (LOQ) were determined as 3 and 10 times the baseline noise, respectively.

Recovery

The absolute recoveries of each drug from bulk standard concentrations and pharmaceutical dosage forms, Glucovance®, Janumet®, and Minidiab® were calculated.

Results and Discussions

Method Optimization

Type of stationary phase

A Chromolith® column was used first with different mobile phases, but a complete separation wasn't obtained. Then a Bondapak™ C18 column was used with different mobile phases. A complete separation was achieved with a mobile phase consisting of methanol: phosphate buffer 0.05 M (60:40, v/v) pH 5.

Percentage of organic modifier in mobile phase

The organic modifiers most commonly used for mobile phases in RP-HPLC are acetonitrile and

methanol. A complete separation wasn't obtained with acetonitrile. The effect of methanol added to the phosphate buffer (50-70%) was studied. Rs values more than 4, but with late retention times were observed at concentrations less than 60%. Therefore, 60% of methanol was added to the buffer as optimum percentage.

Concentration of buffer in mobile phase

Different mobile phases have been tested. The best results considering separation and retention time were obtained with phosphate buffer (pH 5). Keeping other parameters constant, buffer concentrations varied from 0.05 to 0.1 M were studied. A high concentration of the buffer did not give good results. A complete resolution of MET, SIT, GPZ, and GBN was observed using 0.05 M phosphate buffer (pH 5).

pH of mobile phase

The effect of pH on the separation was investigated by running the analysis in buffer solutions of varying pH values (4-6). The mobile phase adjusted to pH 5 was the best regarding resolution and retention time.

Finally, a simultaneous separation of MET, SIT, GPZ, and GBN was achieved under the optimized conditions; Bondapak™ C18 (3.9×150 mm, 10 µm), mobile phase consisting of methanol: phosphate buffer 0.05M (60:40, v/v) pH 5, flow rate 0.8 mL/min, and detection wavelength 260 nm. The retention times were 2.06±0.15, 2.78±0.21, 4.26±0.85, and 9.56±1.81 min for MET, SIT, GPZ, and GBN, respectively (Fig. 1).

Method Validation

System Suitability

The chromatographic characteristics of the mixture indicate that the proposed method permitted adequate resolution of drugs within reasonable run-time (Table-1).

Table-1: System suitability parameters for the determination of MET, SIT, GPZ, and GBN by the HPLC-PDA method.

Analyte	Rs[a]	α[b]	K[c]	t_R[d]
MET	**2**	**2.05**	**2.05**	**2.06±0.15**
SIT	**2**	**1.36**	**2.78**	**2.78±0.21**
GPZ	**2.6**	**1.53**	**4.26**	**4.26±0.85**
GBN	**5.9**	**2.25**	**9.56**	**9.56±1.81**

[a] Resolution factor, calculated as $Rs= (t_{R\,2}- t_{R\,1})/0.5(w_1+w_2)$. Where $t_{R\,2}$ and $t_{R\,1}$ are the retention of second and first peaks, w_1 and w_2 are the peak width of first and second peaks.
[b] Separation factor, calculated as K_2/K_1
[C] Retention factor, calculated as T_R-T_o/T_o
[d] Retention time in min (mean ± SD, n=3)

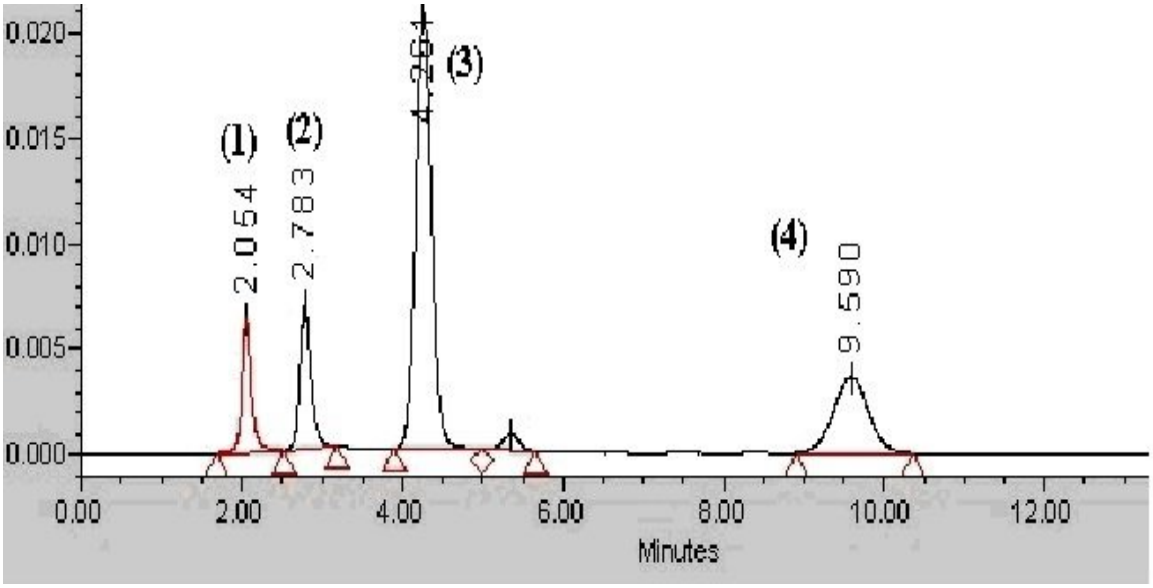

Fig. 1: A representative chromatogram of standard mixture of 10 µg/mL MET (1), 10 µg/mL SIT (2), 10 µg/mL GPZ (3), and 10 µg/mL GBN (4). Mobile phase consisting of methanol: phosphate buffer 0.05 M (pH 5) (60:40, v/v), flow rate 0.8 mL/min, detection wavelength 260 nm.

Linearity

A linear relationship was established by plotting the peak area against the corresponding drug concentration over the range of 0.5–100 µg/mL, 0.5-10 µg/mL, 0.005–10 µg/mL, and 0.5–10 µg/mL for MET, SIT, GPZ, and GBN, respectively. Linear least-square regression analysis was conducted to determine the intercept, slope, and correlation coefficient (r) of calibration curves. The values of the correlation coefficients indicated an excellent linearity (Table-2).

Table-2: Regression and statistical parameters for the quantitation of MET, SIT, GPZ, and GBN by the HPLC-PDA method.

Parameters	MET	SIT	GPZ	GBN
Linear range (µg/mL)	0.5–100	0.5-10	0.005–10	0.5–10
Intercept (a)	9222.17	810.26	-1397.01	-1589.84
Slope (b)	2435.48	4195.76	24665.09	9242.95
Correlation coefficient (r)	0.9998	0.9976	0.9995	0.9997
LOD (µg /mL)	0.005	0.005	0.001	0.1
LOQ (µg /mL)	0.5	0.5	0.005	0.5

LOQ and LOD

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined according to ICH guidelines for validation of analytical procedure (Table-2).

Accuracy and Precision

The precision of the method was evaluated in terms of repeatability (intra-day) and intermediate precision (inter-day). The relative standard deviation (RSD) values were used as a measure of precision. Accuracy was determined using the % recovery. The acceptance criteria were met in all cases. The good % recoveries and RSD values showed that the proposed method is accurate and precise. A summary of accuracy and precision results are given in Table-3.

Specificity

It is evident that MET, SIT, GPZ, and GBN were well separated under the optimized HPLC conditions. A complete resolution was observed with no interferences from the excipients commonly formulated with studied drugs, and that indicated the high selectivity of the method (Fig. 1).

Method Application

The abovementioned results showed the applicability of the developed HPLC-PDA method for analysis of MET, SIT, GPZ, and GBN in bulk form. As a result, the method was applied to the analysis of the drugs in their tablets. The results were acceptable in terms of accuracy as the recovery values range from 97-103% (Table-4). These data indicated the applicability of the developed method for the accurate and rapid quantitation of studied drugs in pharmaceutical formulations (Fig. 2).

Table-3: Precision and accuracy of the HPLC-PDA method for quantitation of MET, SIT, GPZ, and GBN.

	Analyte	Nominal conc.(µg/mL)	Measured conc. (µg/mL) ± SD	% Recovery	% RSD
Intra-day[a]	MET	2	1.93±0.045	96.5	2.3
		5	5.07±0.112	101.4	2.2
		7	7.05±0.148	100.7	2.1
	SIT	2	1.99±0.044	99.5	2.2
		5	5.15±0.019	103.0	0.4
		7	7.11±0.016	101.6	0.2
	GPZ	2	1.95±0.017	97.5	0.9
		5	4.98±0.010	99.6	0.2
		7	7.17±0.043	102.4	0.6
	GBN	2	1.92±0.032	96.0	1.7
		5	5.06±0.070	101.2	1.4
		7	6.96±0.046	99.4	0.7
Inter-day[b]	MET	2	1.95±0.030	97.5	1.5
		5	5.07±0.112	101.4	2.2
		7	7.05±0.148	100.7	2.1
	SIT	2	1.96±0.050	98.0	2.6
		5	5.04±0.129	100.8	2.6
		7	7.23±0.050	103.3	0.7
	GPZ	2	1.95±0.019	97.5	1.0
		5	4.99±0.013	99.8	0.3
		7	7.14±0.017	102.0	0.2
	GBN	2	1.93±0.026	96.5	1.4
		5	5.03±0.095	100.6	1.9
		7	6.88±0.170	98.3	2.5

[a] Assessed by three replicate injections (n=3).
[b] Assessed by three replicate injections on three consecutive days (n=9).

Table-4: Application of the HPLC-PDA method to the analysis of Janumet® (MET / SIT), Glucovance® (MET / GBN), and Minidiab® (GPZ) formulations.

Drug	Nominal conc. (µg/mL)	Measured conc. (µg/mL) ± SD	% Recovery
MET / SIT	20 / 1	20.57±0.051 / 1.03±0.075	102.9 / 103
MET / GBN	100 / 1	97.36±0.771 / 0.97±0.028	97.4 / 97
GPZ	4	4.05±0.017	101.3

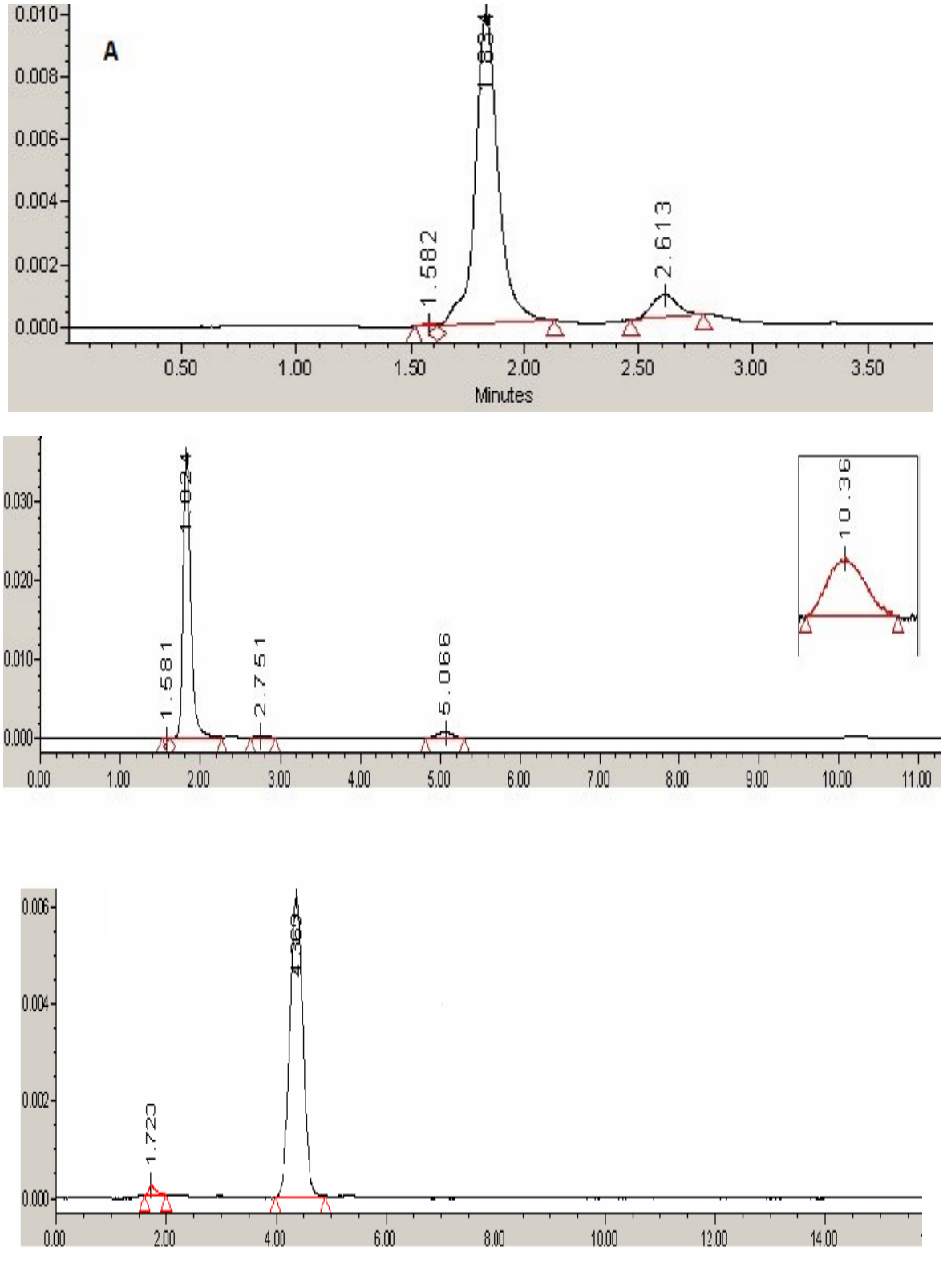

Fig. 2: Chromatograms of Janumet® (MET / SIT) (A), Glucovance® (MET / GBN) (B), and Minidiab® (GPZ) (C). Mobile phase consisting of methanol: phosphate buffer 0.05 M (pH 5) (60:40, v/v), flow rate 0.8 mL/min, detection wavelength 260 nm.

Conclusions

A simple, sensitive, and rapid RP-HPLC method with photodiode array detector was developed to separate MET, SIT, GPZ, and GBN in one single run. Complete separation of the four drugs was achieved under the optimized conditions in less than 10 min. The developed method was validated according to the ICH guidelines and showed good results with respect to linearity, selectivity, precision, and accuracy. The use of the PDA detector offered the advantage of peak purity verification. The method was successfully applied for the analysis of MET, SIT, GPZ, and GBN in single and combined pharmaceutical formulations.

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