Original Article

Development of a Sustainable High-Performance Liquid Chromatography (HPLC) Method for Quantifying Metformin, Sitagliptin, and Empagliflozin in Type 2 Diabetes Treatment

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ABSTRACT

Objective: To develop and validate a green, environmentally sustainable high-performance liquid chromatography (HPLC) method for the simultaneous determination of three commonly used antidiabetic agents: Metformin Hydrochloride (MET), Sitagliptin Phosphate (STG), and Empagliflozin (EMP).

Study Design: Experimental analytical study.

Place and Duration of Study: This study was conducted at the State Company for Drugs Industry and Medical Appliances Samarra (SDI) Iraq from April 2024 to May 2024.

Methods: The chromatographic separation was achieved using an isocratic elution on a C10 column (4.6 × 250 mm, 5 µm) with a mobile phase consisting of phosphate buffer (20 mM), methanol, and acetonitrile in the ratio of 65:30:5 (v/v/v), adjusted to pH 2.9. The flow rate was maintained at 1.0 mL/min, with a column temperature of 30°C and a total run time of 15 minutes. Detection was performed using UV spectroscopy at 208 nm. Linearity was established for MET (0.08–0.13 mg/mL), STG (0.035–0.065 mg/mL), and EMP (0.014–0.026 mg/mL), with correlation coefficients exceeding 0.998 and RSD% not exceeding 1.06%. The method's greenness was evaluated using AGREE and GAPI tools. Additionally, the Blue Applicability Grade Index (BAGI) was employed to assess operational and environmental suitability.

Results: The method exhibited high accuracy, reproducibility, and compliance with green analytical chemistry standards. The AGREE and GAPI assessments confirmed the method's minimal environmental impact. BAGI scoring yielded a value of 82.5, indicating strong sustainability and applicability. The method was successfully applied to the analysis of 13 commercial antidiabetic products, including branded and generic formulations available in the Iraqi market.

Conclusion: The developed HPLC method provides a reliable, accurate, and environmentally sustainable approach for the simultaneous analysis of MET, STG, and EMP. It demonstrates strong analytical performance and practical utility, contributing to green pharmaceutical quality control and sustainable laboratory practices.

Key Words: Green Chemistry; Diabetic mellites; Environmental sustainability; Metformin; Sitagliptin; Empagliflozin; AGREE; GAPI; BAGI

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INTRODUCTION

Diabetes mellitus is a chronic disorder of glucose metabolism caused by insulin deficiency or resistance. Its global prevalence is rising rapidly, with projections estimating 629 million affected adults by 2045 – nearly one in ten people. In response, the WHO designated November 14 as World Diabetes Day.[1-2]

Metformin, or 1,1-dimethylbiguanide hydrochloride, is a well-established antihyperglycemic agent for type 2 diabetes. It works by inhibiting hepatic gluconeogenesis, enhancing peripheral glucose uptake, and reducing intestinal glucose absorption. Recent studies suggest it may also act via the gut microbiome.[3-5] Beyond diabetes, Metformin shows promise in treating certain cancers, infections (including COVID-19 and malaria), aging-related conditions, and polycystic ovary syndrome (PCOS), where it improves insulin sensitivity, menstrual regularity, and fertility.[3,6]

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Sitagliptin, a DPP-4 inhibitor, also acts as an alpha-glucosidase inhibitor. It enhances incretin activity (GLP-1 and GIP) by inhibiting DPP-4, leading to increased insulin secretion and reduced glucagon in a glucose-dependent manner.[7-9] Beyond type 2 diabetes, recent studies highlight its anti-inflammatory and antioxidant effects, including potential benefits in non-diabetic COVID-19 patients through modulation of immune responses and reduction of pro-inflammatory cytokines.[10-12]

Empagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor used in type 2 diabetes management. By blocking SGLT2, it reduces renal glucose reabsorption, lowering the threshold for glucose excretion and increasing urinary glucose output.[13,14] Besides its glucose-lowering effect, Empagliflozin shows diuretic and natriuretic properties by enhancing sodium excretion. Clinical trials report reduced cardiovascular mortality and heart failure hospitalization in diabetic patients with systolic heart failure.[15-17]

Green Analytical Chemistry (GAC) promotes safe, eco-friendly practices based on the 12 principles by Anastas and Warner.[18-20] Tools now assess method greenness, with advances like green HPLC methods for Metformin, Sitagliptin, and Empagliflozin using eco-solvents, miniaturization, and automation to reduce environmental impact.[18,19]

To evaluate the practicality of the proposed analytical method, the Blue Applicability Grade Index (BAGI) was employed. BAGI is a recent color-based metric (dark blue to light blue) that complements green metrics by assessing 10 critical attributes, including analysis type, number of analytes, instrumentation, sample preparation efficiency, throughput, reagents, need for preconcentration, and automation level.[21]

BAGI uses a scoring system from 25 to 100, with higher scores being better. It has user friendly open-source tools to make it easy to use and help researchers identify strengths and weaknesses in methods and get them accepted in the chemical community.[21] Applying these HPLC methods supports the quality of 13 antidiabetic products in Iraq and aligns with global efforts for sustainable pharmaceutical practices.

METHODS

The APIs used in this study were Metformin Hydrochloride (Sohan Healthcare, India, gifted by SDI), Sitagliptin Phosphate (Indexim International, India, gifted by Pioneer), and Empagliflozin (Nanjing Chico Pharmaceutical, China), all with purity ≥99.9%. Placebo excipients included pharmaceutical-grade Maize starch, Sodium starch glycolate, Polyvinylpyrrolidone, Avicel PH 302, Talc, Aerosil, and Magnesium stearate, sourced from Indian suppliers. HPLC-grade Acetonitrile and Methanol (Merck, Germany, 99.95% purity) were used in the mobile phase, and Monopotassium phosphate (A-Z Chem, 95–100.5%) was used for buffer preparation. All pharmaceutical products were obtained from a local pharmacy.

The analytical method was developed and validated using a Shimadzu HPLC system (Japan) comprising an LC-20AD pump, SPD-20A UV-Vis detector, DGU-20A5 degasser, and CTO-20A column oven. Separation was achieved on a reversed-phase L10 (CN) column (4.6 × 250 mm, 5 µm) from MACHEREY-NAGEL (Germany). Supporting equipment included a magnetic stirrer and pH meter (Jenway, Belgium), analytical balance (Sartorius, Germany), ultrasonic bath (ISOLAB, Germany), and UV-Vis spectrophotometer (UV1900, Shimadzu, Japan). Sample preparation involved extraction, dilution, and filtration. The method was validated per regulatory guidelines, with data acquisition and analysis performed using LC Solution software (Shimadzu, Kyoto, Japan) and appropriate statistical methods to support study conclusions. The mobile phase was prepared using 20 mM phosphate buffer (pH 2.35, adjusted with phosphoric acid), mixed with methanol (30% v/v) and acetonitrile (5% v/v), and adjusted to pH 2.9 if needed. The solution was filtered and degassed, contributing to the method's ruggedness.

Standard solutions of Metformin HCl (0.1 mg/mL), Sitagliptin Phosphate (0.05 mg/mL), and Empagliflozin (0.02 mg/mL) were prepared using 70% acetonitrile and ultrasonication, followed by dilution with mobile phase. For test solutions, ten tablets were crushed, average tablet weight calculated, equivalent API amounts transferred to 100 mL flasks, dissolved in 70% acetonitrile (70 mL), sonicated for 5 minutes, diluted to volume with mobile phase, and filtered. Placebo solutions were prepared by dissolving common excipients in the mobile phase, sonicated for 5 minutes, shaken, and filtered.

The simultaneous quantification of Metformin Hydrochloride, Sitagliptin Phosphate, and Empagliflozin was carried out using a Shimadzu HPLC system (Japan) featuring an LC-20AD binary pump, DGU-20A5 degasser, manual injector with 100 µL loop (USA), and a CN reversed-phase column (250 mm). Detection was performed using an SPD-20A UV/VIS detector at 208 nm. The column temperature was maintained at 30°C, with isocratic elution at a flow rate of 1 mL/min. Data acquisition and processing were conducted using LC Solution software and a CBM-20A communication module (Shimadzu, Japan).

To select the optimal wavelength for simultaneous analysis, UV-Vis spectra (190–300 nm) of individual drug solutions revealed a common strong absorbance at 208 nm, suitable for quantifying Metformin HCl, Sitagliptin Phosphate, and Empagliflozin.

RESULTS

According to USP 40, system suitability tests ensure the reliability of the HPLC system. Key parameters include tailing factor (Tf) <2.0 for peak symmetry, and resolution (Rs) ≥1.5 between peaks. The method showed excellent resolution: Rs=10 between Metformin

HCl (Rt=9.01) and Sitagliptin Phosphate, and clear separation from Empagliflozin (Rt=14.75), with all Tf values < 2. Theoretical plates and HETP were calculated per USP guidelines. The average retention times for six injections had %RSD <2%, confirming method consistency (Table 1).

The method showed excellent linearity with correlation coefficients (r) > 0.99. LOD and LOQ were calculated using the standard deviation of response and slope of the calibration curves (Table 2)

The accuracy of the method was validated by spiking known API concentrations at 80%, 100%, and 120% of target levels. Recovery results confirmed high accuracy, with recoveries of 97.7–102.6% for Metformin, 98.8-101.0% for Sitagliptin, and 98.6-101.1% for Empagliflozin. These findings confirm the method's capability for accurate API quantification (Table 3).

The method showed excellent precision, with repeatability assessed over six replicates (n=6) producing RSD% values below 2%. Intermediate precision across different days also yielded RSD% under 2%, confirming consistent performance. The method accurately quantified API content in test samples, with results aligning closely with expected values verifying its accuracy, precision, and reliability for pharmaceutical analysis (Tables 4-6).

Thirteen antidiabetic formulations sourced from Iraqi pharmacies were quantitatively assessed for Metformin HCl (500–1000 mg), Sitagliptin Phosphate (100 mg), and Empagliflozin (25 mg) using a validated RP-HPLC method. Sample solutions (0.1, 0.05, and 0.02 mg/mL, respectively) complied with USP acceptance criteria (90-110%), affirming dosage accuracy and highlighting the necessity of continuous quality assurance (Table 7).

Table No.1: The test of the system suitability method

APIs	Retention time (Rt)(min)	Tailing factors (Tf) NMT 2	Resolutions (Rs) ≥1.5 (USP)	Theoretical plates (N)	HETP (USP) (mm)
Metformin HCl	3.0	1.27	-	1726	87.0
Sitagliptin Phosphate	6.0	1.38	9.01	4334	34.6
Empagliflozin	13.2	1.05	14.75	7300	20.5

Table No.2: Linearity curve of parameter data results

APIs	Regression equation	Linearity range (mg/ml)	Slope	Intercept	Mean (%)	Standard deviation	Relative standard deviation RSD (%)	R-squared value	LOD (mg/ml)	LOQ (mg/ml)
Metformin HCl	y= 269,304,684.7x +12,258,479.156	(0.08-0.13)	269,304, 684.7	12258479.1	99.01	2.7132	$6*10^{-6}$	0.998	0.0266	0.0807
Sitagliptin Phosphate	y= 188,171,535.7x + 207,345.0714	(0.035-0.065)	188,171, 535.7	207345.1	99.9	0.4412	0.441	0.9995	0.0048	0.0147
Empagli-flozin	y=490,796,827.3x 100,663.8810 -	(0.014-0.026)	490,796, 827.3	-100663.8	99.9	0.7006	$7*10^{-6}$	0.9991	0.0023	0.0070

Table No.3: Accuracy data test of Metformin HCl, Sitagliptin Phosphate, and Empagliflozin

APIs	Levels (%)	Theoretical conc. (mg/mL)	Average AUP (mV) N*=3	Recovery (%)	Relative standard deviation RSD (%) +2%
Metformin HCl	80%	0.080	33351885	98.0	0.40
	100%	0.100	39784313	102.2	0.30
	120%	0.120	43952513	98.07	0.20
Sitagliptin Phosphate	80%	0.040	77040450	99.6	0.04
	100%	0.050	96856120	100.7	0.19
	120%	0.060	11366286	98.8	0.02
Empagliflozin	80%	0.016	76520520	98.7	0.08
	100%	0.020	98196600	101.1	0.07
	120%	0.024	11556651	99.0	0.10

Table No.4: Metformin HCl Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Metformin HCl	0.1	39875805	39704636	39704636
		39760814	39559362	39559362
		39866014	38923332	38923332
		39701319	39819463	39819463

		39729076	39997477	39997477
		39874572	39760309	39760309
Mean		39801267	39627429	39894804
Founded (mg/ml)		0.10227	0.10163	0.10262
Recovery (%)		102.3	101.6	102.6
SD		79949.11	373582	79932.1
RSD (%)		0.200871	0.94274	0.20036

Table No.5: Sitagliptin Phosphate Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Sitagliptin Phosphate	0.05	9918527	9726208	9929224
		9906403	9747459	9839959
		9831801	9662725	9905762
		9932309	9920181	9934757
		9898239	9911092	9872268
		9953124	9830785	9959943
Mean		9906734	9906734	9906986
Founded (mg/ml)		0.05155	0.05098	0.05098
Recovery (%)		103.1	102.0	103.1
SD		41550.97	104673	44203.3
RSD (%)		0.419422	1.06812	0.44618

Table No.6: Empagliflozin Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Empagliflozin	0.02	9946005	9878348	9910687
		9957256	9766222	9966082
		9972385	9789566	9741066
		9883858	9723323	10021428
		9898239	9913315	9779291
		9963535	9738927	10040576
Mean		9936880	9801617	9909855
Founded (mg/ml)		0.02045	0.02018	0.0204
Recovery (%)		102.3	100.9	102.0
SD		36807.61	77229.7	125088
RSD (%)		0.370414	0.78793	0.78793

Table No.7: Assay of sample tablets (method application)

Samples	Origin	APIs	Samples Conc. (mg/ml)	AUP (mV)	Found Conc. (mg/ml)	Recovery (%)
Glucophage®500mg	Merch,France	Metformin HCl	0.10	38996561	0.099	99.3
Glifor® 1000mg	bilim, Turkey	Metformin HCl	0.10	38772264	0.098	98.5
METFORAL® 500mg	Menarini, Germany	Metformin HCl	0.10	40515318	0.105	104.9
Januvia® 100mg	MSD, USA	Sitagliptin Phosphate	0.05	12643634	0.066	94.5
Sitagla® 100mg	Maddox, Germany	Sitagliptin HCl	0.05	12766454	0.067	95.4
SITAVIA® 100mg	PIONEER, Iraq	Sitagliptin Phosphate	0.05	13324290	0.069	99.7
Jardiance® 25mg	Boehringer Ingelheim, USA	Empagliflozin	0.02	9771733	0.020	100.5
EMPOLI® 25mg	SAMI, Pakistan	Empagliflozin	0.02	10722886	0.022	110.2
EMPO® 25mg	Motakadema, Jordin	Empagliflozin	0.02	9661833	0.019	99.5
Emglif® 25mg	GENIX,Pakistan	Empagliflozin	0.02	10400108	0.021	106.9
Jard® 25mg	Future, Canada	Empagliflozin	0.02	10032391	0.020	103.2

EMPAGIT® 25mg	Getz, Pakistan	Empagliflozin	0.02	10265236	0.021	105.6
Empadil L® 25mg	Ajanta, India	Empagliflozin	0.02	10193317	0.020	104.8

DISCUSSION

The developed HPLC method achieved baseline separation of metformin, sitagliptin, and empagliflozin in 15 minutes using a CN column and isocratic elution (phosphate buffer: methanol: acetonitrile 65:30:5, pH 2.9). Validation studies demonstrated excellent linearity ($r^2 > 0.998$) across concentration ranges of 0.08-0.13 mg/mL (metformin), 0.035-0.065 mg/mL (sitagliptin), and 0.014-0.026 mg/mL (empagliflozin), with LODs of 0.0023-0.0266 mg/mL. Accuracy (98-102% recovery) and precision (<2% RSD) met pharmacopeial requirements. It is suitable for routine quality control and meets USP and ICH standards.[22]

The method showed good environmental performance (AGREE score 0.65, BAGI 82.5) with low solvent consumption (15 mL/analysis).[23] Successful application to 13 commercial formulations confirmed robustness against minor operational variations (±0.2 pH units, ±5% flow rate, ±2°C temperature). This validated approach combines rapid analysis with green chemistry principles for reliable quality control of antidiabetic medications. Future work could explore biological sample applications or alternative detection methods.[24]

CONCLUSION

The developed RP-HPLC method effectively quantified metformin HCl, sitagliptin phosphate and empagliflozin in pharmaceutical forms, showing excellent linearity ($r^2 \geq 0.998$), precision (RSD% <2%), and ruggedness (RSD% ≤1%). Greenness assessment gave an AGREE score of 0.65 and a GAPI profile with 13 green, 9 yellow, and 3 red zones. The method is suitable for routine quality control, with potential for further environmental optimization

Author's Contribution:

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Drafting or Revising Critically:	Mahmood Shakir Al Samarrai, Eman Thiab Al Samarrai
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Agreement to accountable for all aspects of work:	All the above authors

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