Egypt. J. Chem. **Vol. 67**, No. 10 pp. 307-315 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Eco-Friendly HPLC Method for Quantification of Metformin and Dapagliflozin in Tablets Dosage Form and Spiking Human Plasma Utilizing Solid Phase Extraction

Hassan M. Abdel Fattah[a], Mahmoud A. Mohamed [b], Rehab Mahmoud[a], and Mohamed E.M. Hassouna*[a]

[a] *Department of Chemistry, Faculty of Science, Beni-Suef University, 62511 Beni-Suef, Egypt*
[b] *Hikma Pharmaceutical Company, Beni-Suef 62511, Egypt*

Abstract

Medical quality control produces enormous amounts of dangerous organic waste, which has a critical ecological impact. It was found that mixed-micellar LC was adequate for this purpose. In this study, we developed and validated a simple, accurate, and robust HPLC method for simultaneously determining Metformin hydrochloride (MET) and Dapagliflozin (DAP) in tablet dosage form and human plasma. The components were separated at 30 °C on AGILENT and C18 (150 mm and 4.6 mm). The mobile phase (Mph) was consisting of 2% glacial acetic acid: acetonitrile (85:15, v/v) at 1.0 mL/min flow rate. MET and DAP had retention times of 1.071 and 1.979 minutes, respectively, during the 5-minutes run. DAP and MET showed linear responses over the 1–1000 ng/mL concentration ranges for drug assays and 50–2000 ng/mL for plasma assays. A MET LOD of 0.13 ng/mL and a DAP LOD of 0.18 ng/mL are reported for drug assay, whereas for plasma assay, MET LOD is 0.74 ng/mL and that of DAPA is 0.28 ng/mL. Several validation parameters are calculated by the International Conference on Harmonization Guidelines (ICH), including linearity, precision, accuracy, and specificity. An eco-friendly RP-HPLC method has been constructed to develop a simple, comparatively economical, and fulfilling the aims of green analytical chemistry (GAC). According to the analysis, MET and DAP could be measured simultaneously in tablet dosage form and human plasma using the proposed method.

Keywords: Metformin, Dapagliflozin, Solid Phase Extraction, Spiked plasma, RP-HPLC.

1. Introduction

One of the chronic illnesses that has affected the world the most recently is diabetes. Diabetes has major complications that result in unavoidable human deaths all over the world [1]. Diabetes is a fatal inherited metabolic condition causing hyperglycemia, leading to microvascular complications like retinal impairment, renal retrogradation, neurological illnesses, and frequent macrovascular problems like myocardial infarction and stroke [2]. There are three types of diabetes, with type-1 diabetes affecting primarily youngsters and marked by the full absence of insulin due to the destruction of the pancreatic cells that produce it [3]. Type 2 diabetes, characterized by insulin resistance, is the most common one, with gestational diabetes diagnosed during pregnancy, and the prevalence of diabetes has increased significantly in recent years [3]. DAP is a Sodium Glucose Co-Transporter 2 (SLGT2) inhibitor used to manage Diabetes Mellitus type 2. The drug blocks glucose transport selectively by SGLT2 over SGLT1 in adults, improving glycemic control. In the treatment of non-insulin-dependent diabetes mellitus (NIDDM), MET is a biguanide antihyperglycemic agent (Figure 1). As a result of its reduced hepatic glucose production, absorption, and insulin-mediated glucose uptake, it improves glycemic control in obese NIDDM patients and may induce weight loss; when taken alone, metformin does not cause hypoglycemia, but may enhance insulin and sulfonylurea effects. In addition to treating polycystic ovary syndrome and other insulin-resistant diseases, it is also used to treat obesity. Diabetes mellitus type 2 (T2DM) is treated with DAP and metformin in combination. Combining DAP and MET may be a promising treatment option for patients with T2DM due to its unique mechanism of action and favorable safety profile [4, 5].

*Corresponding author e-mail: Mohamed.hassouna@science.bsu.edu.eg . (Mohamed E.M. Hassouna)
Receive Date: 29 March 2024, Revise Date: 02 May 2024, Accept Date: 28 May 2024
DOI: 10.21608/ejchem.2024.279260.9529

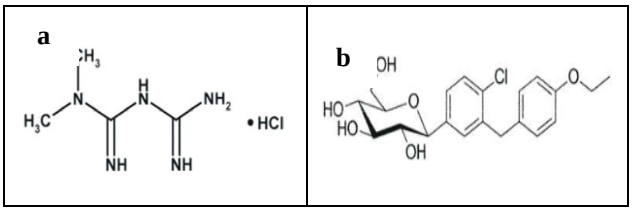


Fig. 1 (a) MET and (b) DAP structures.

A literature survey reveals that different techniques have been utilized for the determination of MET and DAP individually and in combination form. Spectrophotometric [5-8], electrochemical [9], and HPTLC [10]. Several HPLC methods have been applied for the determination of MET or DAP individually and simultaneously [11-23] for both in combination. The collection of the applied techniques for their analysis has been reviewed [24-31]. The efficacy and safety of the treatment with dapagliflozin and metformin compared to metformin monotherapy was tested [32-35]. The pharmacokinetic variables of their combination were assessed [36]. In the present study, an eco-friendly RP-HPLC method has been constructed to develop a simple, rapid, accurate, precise, comparatively economical, and fulfilling the aims of green analytical chemistry (GAC) to develop new analytical methodologies to lower dangerous chemicals as well as chemical waste while enabling faster and more energy-effective chemical analysis. The developed method was validated by using various statistical parameters according to United States Pharmacopeia [37,38], Encyclopedia Britannica [39], and the International Conference on Harmonization Guidelines (ICH) [40].

Existing methods for analyzing MET and DAP, particularly when their degradation products are involved, reveal a significant gap concerning analyst safety and environmental conservation. Despite an extensive literature review, an effective solution still needs to be identified. To align with the principles of green chemistry, a novel HPLC method has been developed that stands out for its innovation, efficiency, and eco-friendliness. This state-of-the-art technique enables the rapid and precise measurement of MET and DAP concentrations. It has been rigorously evaluated across various degradation conditions, including acidic, basic, oxidative, photolytic, and thermal processes, confirming the HPLC method's stability for these drugs. Our detailed examination demonstrates that this new approach is considerably more sustainable than previously established methods as displayed in Table S1. We have delivered quicker and more dependable results by utilizing a shorter column of only 150 mm and reducing the analysis time to merely 5 minutes. It is with assurance that we present this novel HPLC method as a promising resolution to the existing challenges in analyzing MET and DAP drugs.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

(MET and DAP reference standards were kindly supplied by EVA pharm. The tablets (DIAFLOZIMET®,10/500) DAP 10 mg & MET HCl 500 mg of EVA pharm were purchased from a local pharmacy. Glacial acetic acid (GAA), water (DW), and acetonitrile (ACN) of HPLC grade (Honeywell Co, Germany).

2.1.2. *Instruments*

The system consists of an Agilent quaternary pump, a diode array detector, and an autosampler injector, as shown in Table S2.

2.2. Methods and General Procedure

2.2.1. Preparation of Stock and Working Standard Solutions

To prepare stock solutions, 10 mg of each compound was dissolved in 10 mL of DW to obtain a 1 mg/mL concentration. These stock solutions were stable at 5°C for several weeks [17]. The intermediate solution (IM) is prepared by taking 1 mL of the stock solution up to 100 mL of the mobile phase (10 µg/mL). Transfer 1, 2, 10, 20, 100, 200, and 1000 µL from the intermediate solution into 10 mL volumetric flasks to gain the working concentrations (1–1000 ng/mL). The IM solution for plasma assay is prepared by taking 20 µL of the stock solution up to 2 mL of the blank human plasma (10 µg/mL). Transfer 10, 20, 40, 100, 200, and 400 µL from the intermediate solution into 2 mL volumetric flasks to gain the working concentrations (50–2000 ng/mL) in blank plasma, then extracted as mentioned below [37].

2.2.2. Analysis of marketed formulations

The pharmaceutical product was prepared by dissolving an accurate amount of drug in DW to obtain a solution at a concentration of (1 mg/mL MET & 50 mg/mL DAP). Variable dilutions in the mobile phase to obtain 20 ng/mL MET& 1000 ng/mL DAP.

2.2.3. Plasma assay

The spiked blank human plasma with standards was set at room temperature after fortification for 10 minutes before solid phase extraction (SPE). At first, SPE cartridges were activated by 1mL ACN, then 1mL of DW slowly (~ 1 drop/ 10 seconds), then 0.5 mL of plasma (pass by gravity without pump pressure), passed air via the cartridges until dryness. Elution takes place with 2 mL ACN slowly under gravity. The obtained eluate was evaporated under a nitrogen

stream at 40°C. Dissolve the dried eluate in 0.5 mL of MPh solution, then filtrate across 0.45 µm before injection into the HPLC system. The plasma concentration of MET & DAP was pointed briefly, as viewed in Figure S1.

2.2.4. Method validation study

The optimized chromatographic conditions were validated according to USP 27- NF 22 (2004) and ICH (2015) by evaluating linearity, precision, accuracy, limits of detection (LOD) and quantification (LOQ), robustness, and system suitability testing.

Linearity: Standard working solutions were diluted in the range of (1–1000 ng/mL) for drug assay and in the range of (50–2000 ng/mL) in plasma assay and injected triplicates into the LC system to plot a calibration curve for the determination of linearity. The slope, intercept, and coefficient of correlation (R) of the plot were determined via linear regression equation (RE).

Specificity: The level of standard and sample were prepared as per the test method at a concentration of 20 ng/mL MET& 1000 ng/mL DAP for drug assay and 100 ng/mL MET & DAP for plasma assay and then injected into the chromatographic system. The chromatograms were recorded and compared.

Forced Degradation Study:

Five types of degradations have been applied to stock solutions in this study:

1) Light degradation: exposure to a UV lamp for 120 min.
2) Heat degradation: exposure to 85ºC for 120 min.
3) Basic hydrolysis: addition of equal volume to the stock solution of 1 N of NaOH. Then placed at room temperature for 120 min., the base hydrolyzed samples were neutralized.
4) Acid hydrolysis: addition of equal volume to the stock solution of 1 N of HCl for acid hydrolysis. Then placed at room temperature for 120 min., the acid hydrolyzed samples were then neutralized.
5) Oxidation: addition of equal volume to the stock solution of 30% of H_2O_2 for oxidation. Then placed at room temperature for 120 min.

After degradation, complete the volume with the mobile phase.

The degradation %= (area of QC sample- area after degradation) × 100.

Precision: The precision was estimated at the method repeatability by calculating the relative standard deviation (RSD) for 6 determinations made under the same experimental conditions and on the same day (intra-day precision) and made at 6 different days (inter-day precision).

Accuracy and recovery: it was tested at three different levels that were 50, 100 and 200%. It was determined by the standard addition method in drug assay and by fortification and spiking method in plasma assay. Accuracy was expressed in terms of percentage recoveries.

Determination of the LOD & LOQ: the determination based on the intercept standard deviation (S) of a linear plot and slope (b). {LOD=3.3*S/ b; LOQ=10*S/b}.

Robustness: this study was performed to evaluate the impact of minor alteration in the chromatographic conditions as the column temperature ± 2 °C (28 and 32°C), UV detector ± 2 nm (228 and 232 nm) and change in mobile phase composition by ± 20%. After each alteration, the sample solution was injected and the % RSD was checked.

System Suitability testing (SST): carrying out SST on a freshly prepared QC of the MET and DAP to dissect the various optimized parameters. The theoretical plate count, symmetry, and tailing factor were determined for 6 replicates.

3. Results and discussion

3.1. Method development and optimization

To create a new RP-HPLC method, DAP and MET were separated and quantified in medicinal tablets and plasma using various mobile phase compositions. Preliminary experiments were carried out to choose the most suitable and ideal settings to build an efficient procedure for the analysis of the medications. The detection wavelength, column temperature, and mobile phase composition were adjusted to optimize separation and precision, with 2% GAA: ACN in (85: 15 v/v) at 1.0 mL/min. MET and DAP retention times were measured with UV detection at 230 nm and were found to be 1.069 and 1.979 minutes, respectively. Figures 2 and 3 show typical chromatograms of MET and DAP. ICH guidelines were followed to validate the optimized method.

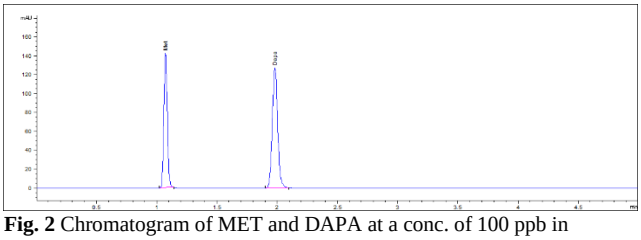


Fig. 2 Chromatogram of MET and DAPA at a conc. of 100 ppb in MPh solution.

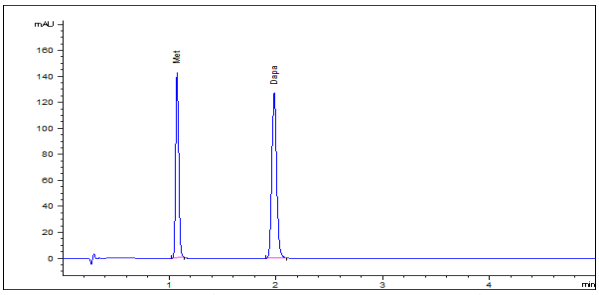


Fig. 3 Chromatogram of MET and DAPA at a conc. of 100 ppb in blank human plasma.

3.2. Method validation

It has been validated that the recommended analytical method for simultaneous estimation of MET and DAP in pharmaceutical formulations is suitable, precise, linear, accurate, and workable for a variety of working ranges.

3.2.1. Linearity and range

The linearity of peak area responses (Y) versus concentrations (X) was demonstrated by linear regression analysis. It was found to be linear in the range of 1–1000 µg/mL, as shown in Table 1 and Figures S2 and S3, and in the range of 50–2000 µg/mL in blank human plasma, as shown in Table 2 and Figures S4 and S5.

Table 1. The concentrations of MET and DAP (µg/mL) in Mph solution and their corresponding area under peak (AUP) using HPLC.

Compound	RT (min.)	Conc. (ppb)	AUP	RE	R
MET	1.071	1	2.816	Y=2.89*X-3.9	0.99996
		2	5.500		
		10	26.467		
		20	54.579		
		100	288.77		
		200	552.1		
		1000	2887.8		
DAP	1.979	1	3.921	Y=4.12*X-6.3	0.99998
		2	7.600		
		10	36.872		
		20	75.9		
		100	401.6		
		200	798.2		
		1000	4120.9		

Table 2. The concentrations of MET and DAP (µg/mL) in blank plasma and their corresponding area under peak (AUP) using HPLC.

Compound	RT (min.)	Conc. (ppb)	AUP	RE	R
MET	1.069	50	140.60	Y=2.89*X-3.9	0.99996
		100	285.77		
		200	572.10		
		500	1389.10		
		1000	2887.80		
		2000	5701.60		
DAP	1.979	50	195.81	Y=4.12*X-6.3	0.99998
		100	399.61		
		200	795.22		
		500	1938.20		
		1000	3968.80		
		2000	7912.00		

3.2.2. Selectivity

The chromatograms were recorded and compared to determine whether there was no interference from excipients or the mobile phase. It is indicated in Figures 4–6 in the drug assay. The chromatograms were recorded and compared to blank reagents and blank plasma for the plasma assay, so there was no plasma or chemical reagent interference, as indicated in Figures 7 and 8.

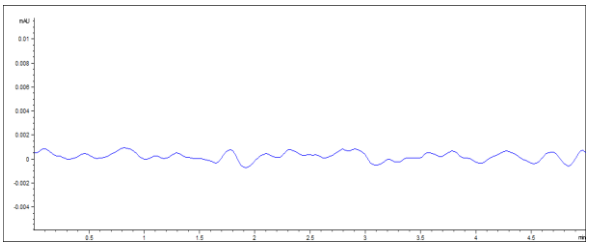


Fig. 4 Chromatogram of blank reagent (MPh).

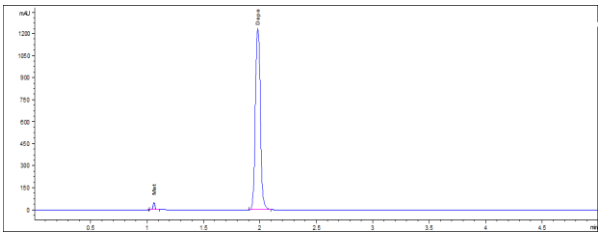


Fig.5 Chromatogram of MET and DAP pure standard at a concentration of 20 &1000 ng/mL, respectively.

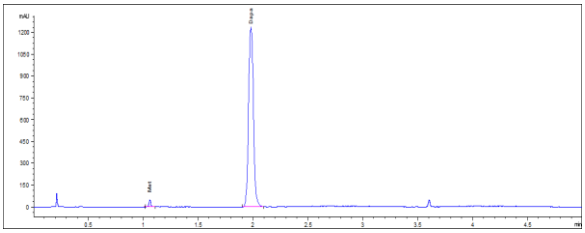


Fig. 6 Chromatogram of MET and DAP in tablet at a concentration of 20 & 1000 ng/mL, respectively.

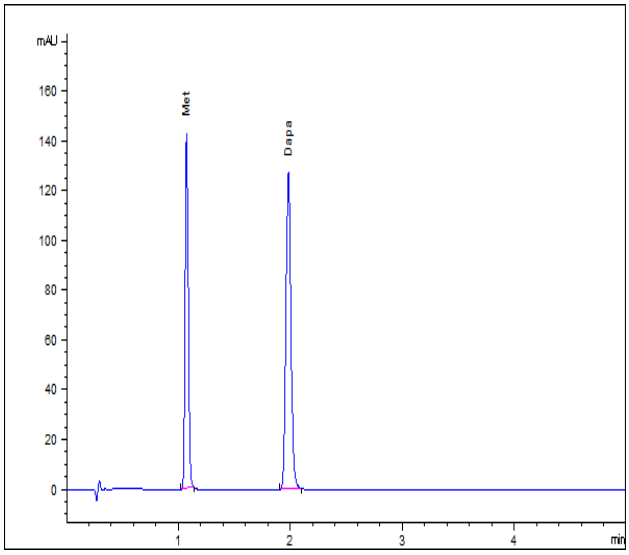


Fig. 7 Chromatogram of MET and DAP standard in blank plasma at a concentration of 100 ng/mL, respectively.

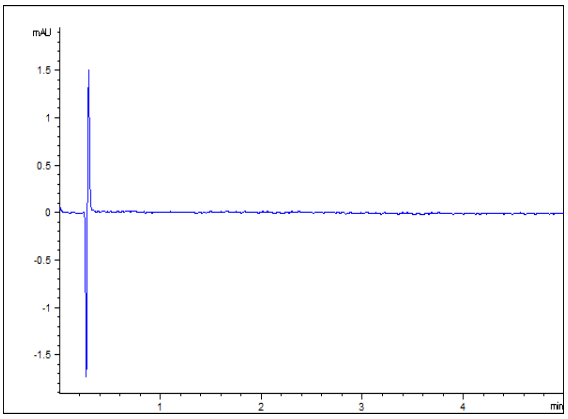
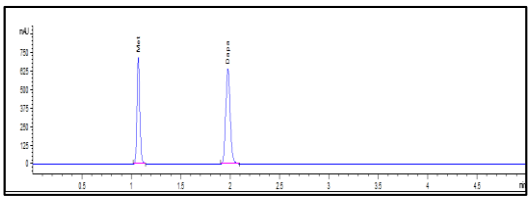


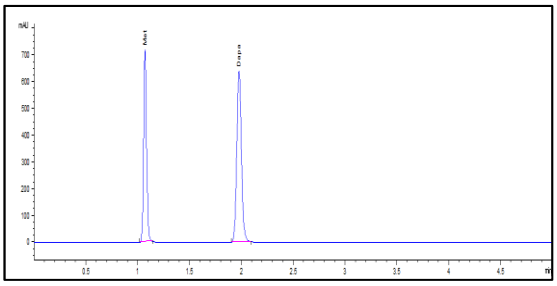
Fig. 8 Chromatogram of blank plasma

3.2.3. *Forced Degradation*

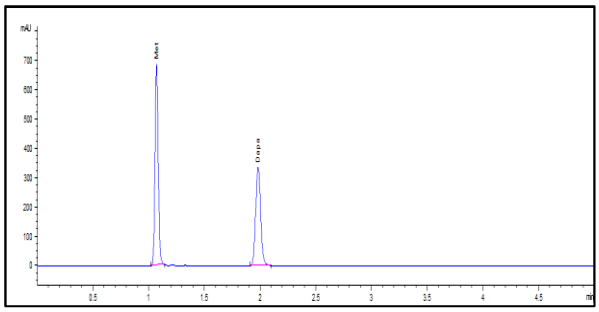
The International Conference on Harmonization (ICH) guideline entitled stability testing of drug substances that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The findings of forced degradation are displayed in Table 3 and the graphs are depicted in Figure 9.



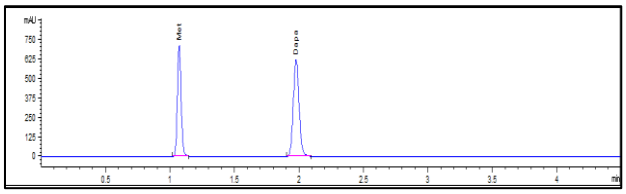
(a)



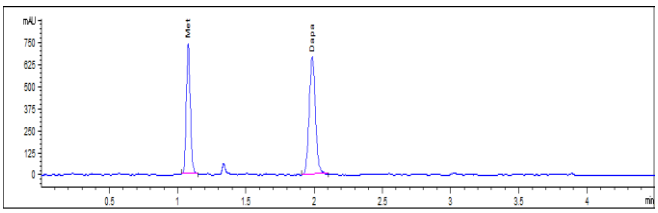
(b)



(c)



(d)



(e)

Fig. 9 Chromatograms of MET and DPA after exposure to (a) light, (b) heat, (c) acid, (d) base, and (e) oxidation.

Table 3. Degradation studies results for 120 min.

Items	Condition	Degradation%	
		MET	DAP
Standard		-----	-----
Photo	UV	0.4	2.8
Thermal	85ºC	1.4	3.6
Acid (HCl)	1N	11	23
Alkaline (NaOH)	1N	6	14
Peroxide (H_2O_2)	30%	8	15

3.2.4. Precision

The intra- and inter-day precisions of drug assay were summarized in Tables 3-6 showing that RSD% is 0.59 & 1.3 %; respectively for drug assay and 0.49 & 1.2 %; respectively for plasma assay, these results agreed with the acceptance criteria set by ICH, 2005 (≤1% for intraday precision and ≤2% for Interday precision).

Table 4. Intraday and Interday precision data for estimation MET.

Ser. No.	Conc. (ng/mL)	Peaks areas on the same day	Peaks areas in six days
1	100	288.77	288.77
2		292.4	285.3
3		288.5	289.2
4		285.2	288
5		282	285.4
6		290.5	288
Mean		287.445	287.895
SD		1.687291	3.748
RSD%		0.586996	1.302

Table 5. Intraday and Interday precision data for estimation of DAP.

Ser. No.	Conc. (ng/mL)	Peaks areas in the same day	Peaks areas in six days
1	100	401.6	401.6
2		400	401.5
3		401	395.5
4		397.6	396
5		402.1	405.8
6		401.5	392.5
Mean		400.633	398.817
SD		1.648	4.951
RSD%		0.411	1.242

Table 6. Intraday and Interday precision data for estimation MET in plasma.

Ser. No.	Conc. (ng/mL)	Peaks areas on the same day	Peaks areas in six days
1	100	284.9183	284.9183
2		284.1	280.5
3		285	288.5
4		282.5	285.2
5		285.4	280.6
6		282.05	280.1
Mean		283.995	283.303
SD		1.405	3.425
RSD%		0.495	1.209

Table 7. Intraday and Interday precision data for estimation of dapagliflozin in plasma.

Ser. No.	Conc. (ng/mL)	Peaks areas on the same day	Peaks areas in six days
1	100	399.6112	399.6112
2		399	395.3
3		398.2	395.5
4		397.6	390.8
5		398.2	391.5
6		397.6	397.8
Mean		398.3685	395.085
SD		0.797614	3.443
RSD%		0.20022	0.872

3.2.5. Accuracy

Accuracy was expressed in terms of percentage recoveries of MET and DAP from the real samples as shown in Tables 7 and 8, and from the blank human plasma as shown in Tables S3 and S4. The found recovery from tablets was ranged (99.4-100.9 %) for MET and (100.9-101.7 %) for DAP, but the recovery from blank plasma was ranged (99.88-99.99 %) for MET and (100-100.1 %) for DAP. These results were within the acceptable range as the accuracy for drug assay was 98-102 % and for plasma assay was 85-110 %, therefore the present novel method is of high recovery and accuracy.

Table 8. The percentage recovery of MET standard addition.

Level Conc. ng/mL	Found conc. ng/mL	Mean	SD	RSD %	Recovery %	Accuracy
50	49.3	49.68	0.37	0.74	98.6	99.4 ± 0.74
	49.71				99.42	
	50.04				100.08	
100	99.1	99.7	0.66	0.66	99.1	99.7 ± 0.66
	100.4				100.4	
	99.6				99.6	
200	200.8	201.89	1.14	0.56	100.4	100.9 ± 0.57
	201.8				100.9	
	203.07				101.535	

Table 9. The percentage recovery of DAP standard addition

Level Conc. ng/mL	Found conc. ng/mL	Mean	SD	RSD %	Recovery %	Accuracy
50	51.1	51.2	0.75	1.47	102.2	101.7 ± 0.6
	50.5				101	
	51				102	
100	100.5	100.87	1.00	0.993	100.5	100.9 ± 1
	102				102	
	100.1				100.1	
200	203.4	202.67	2.87	1.42	101.7	101.3 ± 1.4
	205.1				102.55	
	199.5				99.75	

3.2.6. Determination of the LOD & LOQ.

In the drug assay, LOD for MET was 0.13 ng/mL and for DAP was 0.18 ng/mL. LOQ for MET was 0.39 ng/mL and for DAPA was 0.55 ng/mL, but for plasma assay, LOD for MET was 0.74 ng/mL and for DAP was 0.28 ng/mL. LOQ values for MET and DAP were 2.23 ng/mL and 0.83 ng/mL, respectively.

3.2.7. Robustness

After each alteration, the sample solution was injected and % RSD was checked and tabulated as shown in Tables S5 and S6, and those of plasma assay were summarized in Tables S7 – S9. All obtained pooled RSD% were ≤ 2% as mentioned by ICH (2005).

3.2.8. System suitability

The theoretical plate count, symmetry, and tailing factor were determined for 6 replicates as shown in Figures S6-S9. Results are shown in Tables S10-S13.

3.2.9. Application to AGREEprep tool

AGREE prep was used in the present study to assess the ecological impacts of various sample preparation methods. Through the AGREEprep approach, evaluation is integrated with ecologically responsible sample preparation principles to simplify the procedure. It consists of ten distinct stages evaluating each individual's ability, with a score of 1 denoting the ideal level of performance [41, 42]. As shown in Figure S10, each of the ten sectors has its graphic representation. Figure S10 illustrates our methodology's ecological effectiveness with a value of 0.55. These figures allowed us to determine the method's effectiveness and assess its success fairly and accurately. As a result, the proposed method proved to be a safe and efficient one of preparing samples for analysis.

4. Conclusion

This technique can be utilized for the simultaneous determination of Metformin and Dapagliflozin in the tablet dosage form and human plasma for further pharmacokinetic study. The findings indicate that the suggested methodology is more eco-friendly than traditional techniques. This improvement was accomplished by reducing the operational time to 5 minutes, employing a shorter column size of 150 mm, and replacing hazardous substances. The data show that the proposed technique is faster and more dependable than earlier documented methods. The technique is validated, verified, shown to be precise and accurate. The validation studies results showed that the developed RP-HPLC technique is eco-friendly, accurate, sensitive robust, and specific without any interference from the excipients and plasma. The developed method was successful in the quantitative analysis of MET and DAPA in tablets and plasma. The method may also be utilized for routine analysis, and quality control studies of the pharmaceutical tablets containing these analytes and in human plasma.

Declaration of Competing Interest

The authors declare no conflict of interest.

5. References

[1] Kawalec, P., Mikrut, A., Łopuch, S. The safety of dipeptidyl peptidase-4 (DPP-4) inhibitors or sodium-glucose cotransporter 2 (SGLT-2) inhibitors added to metformin background therapy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes/metabolism research and reviews, 30(4), 269-283(2014). doi: 10.1002/dmrr.2494

[2] Carlson, C. J., Santamarina, M. L. Update review of the safety of sodium-glucose cotransporter 2 inhibitors for the treatment of patients with type 2 diabetes mellitus. Expert Opinion on Drug Safety,

15(10), 1401-1412(2016). https://doi.org/10.1080/14740338.2016.1216100
[3] Glovaci, D., Fan, W., Wong, N. D. Epidemiology of diabetes mellitus and cardiovascular disease. Current cardiology reports, 21, 1-8(2019). DOI: 10.1007/s11886-019-1107-y
[4] Rawshani, A., Rawshani, A., Franzén, S., Eliasson, B., Svensson, A. M., Miftaraj, M., Gudbjörnsdottir, S. Mortality and cardiovascular disease in type 1 and type 2 diabetes. New England journal of medicine, 376(15), 1407-1418(2017). DOI: 10.1056/NEJMoa1608664
[5] Antakli,S., Kabbani,R. and Labban,R. Analytical Spectrometric Study For Determining Dapagliflozin Propanediol Monohydrate Individually Or In Presence Of Metformin Hydrochloride In Tablets Formulation. Journal of Advances in Chemistry, 17, (2020) ISSN: 2321-807X. DOI: https://doi.org/10.24297/jac.v17i.8812
[6] Sen, D.B., Jatu, S., Maheshwari, R.A., Zanwar, A.S., Velmurugan, R., Sen, A.K. New Eco-friendly UV-spectroscopic Methods for Simultaneous Assessment of Dapagliflozin, Saxagliptin and Metformin in Ternary Mixture. Indian J of Pharmaceutical Education and Research,57(2):559-569(2023). doi:10.5530/ijper.57.2.69
[7] Suman, S. S. and Chaubey, R. Simultaneous Spectrophotometric Estimation of Metformin, Saxagliptin and Dapagliflozin in Marketed Formulations. Journal of Pharmaceutical Research International, 33(61B), 167–173(2021). doi: 10.9734/jpri/2021/v33i61B35495.
[8] Farid, N.F., N. Ali, A. Taha, and M. Farid, N.F., N. Ali, A. Taha, and Magdy, M. Development And Evaluation Of Simple Spectrophotometric Methods For The Simultaneous Determination Of Alogliptin Benzoate Metformin Hydrochloride And Melamine. Egyptian Journal of Chemistry, 2023. 66(7): p. 119-129. Doi. 10.21608/EJCHEM.2022.150683.6527

[9] Fendrych, K.; Górska-Ratusznik, A.; Smajdor, J. Electrochemical Assays for the Determination of Antidiabetic Drugs—A Review. Micromachines, 15, 10(2024). https://doi.org/ 10.3390/mi15010010
[10] Abdelrahman, A.E., Maher, H.M., Alzoman, N.Z. HPTLC Method for the Determination of Metformin Hydrochloride, Saxagliptin Hydrochloride, and Dapagliflozin in Pharmaceuticals. Current Analytical Chem,16(5), 609 – 619(2020). DOI: 10.2174/1573407215666190131123029
[11] Fawzy, M.G., Said, M.A. Valuation of environmental influence of recently invented high-performance liquid chromatographic method for hypoglycemic mixtures of gliflozins and metformin in the presence of melamine impurities: Application of molecular modeling simulation approach, Journal of Separation Science ,46,19(2023).DOI:10.1002/jssc.202300267.
[12] Abdelwahab, N.S., Morsi, A., Ahmed, Y.M., Hassan, H.M., AboulMagd, A.M. Ecological HPLC method for analyzing an antidiabetic drug in real rat plasma samples and studying the effects of concurrently administered fenugreek extract on its pharmacokinetics. RSC Adv., 11, 4740-4750(2021). DOI: 10.1039/D0RA08836F.
[13] Gundala, A., Prasad, K., Koganti, B. Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form. Braz. J. Pharm. Sci, 55 , (2019) • https://doi.org/10.1590/s2175-97902019000218129
[14] Dighe, N. S., Varade, P. R., Shinde, G. S., Rao, P. S. Quantitative Estimation and Validation of Dapagliflozin and Metformin Hydrochloride in Pharmaceutical Dosage form by RP-HPLC. Asian Journal of Research in Chemistry, 12(3), 136-142(2019). DOI: 10.5958/0974-4150.2019.00028.2
[15] Bhavyasri, K., Surekha, T., Begum, S., Sumakanth, M. RP-HPLC Method for Dapagliflozin and Metformin HCL in Bulk and Combined Formulation. Arch Pharm Pract,12(4),106-110(2021). https://doi.org/10.51847/Czxl0wYrYr
[16] Venkatesh, P., Harisudhan, T., Choudhury, H., Mullangi, R., Srinivas, N. R. Simultaneous estimation of six anti-diabetic drugs—glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. Biomedical Chromatography, 20(10), 1043-1048(2006). DOI: 10.1002/bmc.635
[17] Lakshmi, K. S., Rajesh, T. Separation and quantification of eight antidiabetic drugs on a high-performance liquid chromatography: its application to human plasma assay. International Scholarly Research Notices, 2011(2011). DOI: 10.5402/2011/521353
[18] Bahgat,E.A., Hafez,H.M., El-Sayed,H.M., Khabil, N.A.-S. Development of a solvent-free micellar HPLC method for determination of five antidiabetic drugs using response surface methodology, Microchemical Journal, 179, 107446(2022). https://doi.org/10.1016/j.microc.2022.107446
[19] Saravanan,V.S., Tamilselvan, N. , Hemalatha, P. V. , Vikneshwaran, D. , Dinesh, K. S. APPLICATION OF DOE IN DEVELOPING A VALIDATED RPHPLC METHOD TO EVALUATE DAPAGLIFLOZIN AND MENTFORMIN IN COMBINED TABLET DOSAGE FORM. European Chemical Bulletin,12(10),(2023) .doi: 10.48047/ecb/2023.12.si10.00189
[20] Chaitali R Dhale and Rao J R. Stability indicating HPLC method development and validation for the simultaneous estimation of metformin hydrochloride and dapagliflozin in API and pharmaceutical dosage form. Int. Res. J. Pharm,12(8),52-57(2021). http://dx.doi.org/10.7897/2230-8407.1208157
[21] Bhavyasri K, Surekha T, Begum S, Sumakanth M. RP-HPLC Method for Dapagliflozin and Metformin HCl in Bulk and Combined Formulation. Arch Pharm Pract,12(4),106-110(2022). https://doi.org/10.51847/Czxl0wYrYr
[22] Sebaiy, M.M., El-Adl,S.M. Baraka,M.M. and Hassan,A.A. Rapid RP-HPLC method for simultaneous estimation of some antidiabetics; Metformin, Gliclazide and Glimepiride in Tablets. Egyptian journal of chemistry, 2019. 62(3): p. 429-440. DOI. 10.21608/EJCHEM.2018.4394.1388
[23] Amin, A.S., Mohamed, S.F. and Abo-Taleb, M.M. Stability Indicating Assay Method of Metformin, Linagliptin and Empagliflozinin

Pharmaceutical Dosage Form by HPLC Method. Egyptian Journal of Chemistry, 67(7): p. 443-449 (2024). DOI. 10.21608/EJCHEM.2024.161798.6953

[24] Babel, R. A., Dandekar, M. P. A review on cellular and molecular mechanisms linked to the development of diabetes complications. Current Diabetes Reviews, 17(4), 457-473(2021). DOI: 10.2174/1573399816666201103143818

[25] BADIKELA RAMAKRISHNA, SUMANTA MONDAL A REVIEW OF ANALYTICAL METHODS FOR DETERMINATION OF TYPE- II ANTIDIABETIC DRUGS IN PHARMACEUTICALS AND BIOLOGICAL MATRICES,Asian J Pharm Clin Res,14(1),69-76(2021). DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i1.40049.

[26] Golder S, Bach M, O'Connor K, Gross R, Hennessy S, Gonzalez Hernandez G Public Perspectives on Anti-Diabetic Drugs: Exploratory Analysis of Twitter Posts JMIR Diabetes,6(1),e24681(2021), doi: 10.2196/24681

[27] Dahlén, A.D., Dashi, G., Maslov, I., Attwood, M.M., Jonsson, J., Trukhan, V., Schiöth, H.B. Trends in Antidiabetic Drug Discovery: FDA Approved Drugs, New Drugs in Clinical Trials and Global Sales. Front. Pharmacol, 12,807548(2022). doi: 10.3389/fphar.2021.807548

[28] Nagulancha, B.R., Vandavasi,K.R. Stability-indicating method development and validation for quantitative estimation of organic impurities of the antidiabetic drug glipizide in drug substance and pharmaceutical dosage form using HPLC. Biomedical Chromatography,11,e5727(2023) . https://doi.org/10.1002/bmc.5727

[29] Piragine, E.,Petri, D., Martelli, A., Calderone, V., Lucenteforte, E. Adherence to Oral Antidiabetic Drugs in Patients with Type 2 Diabetes: Systematic Review and Meta-Analysis. J. Clin. Med, 12(5), 1981(2023). https://doi.org/10.3390/jcm12051981

[30] Rakusanova, S., Cajka,T. Current analytical methods to monitor type 2 diabetes medication in biological samples. TrAC Trends in Analytical Chemistry, 158, 116831(2023). https://doi.org/10.1016/j.trac.2022.116831.

[31] Dalal, D., Kant, R., Badhwar,R. A Review on Analytical Method of Dapagliflozin and Empagliflozin by QBD Approach. Mathews J Pharma Sci, 7(1),17(2023). https://doi.org/10.30654/MJPS.10017

[32] Nimra ,M., Usman,H.M., Daniah,R., Zamurad,K.M., Olatunde,O.M., Binish,J., Chinonso,U.S., Aymar,A. Dapagliflozin-metformin combination therapy; a novel solution for obese women with gestational diabetes in Pakistan. International Journal of Surgery: Global Health,6(5),e0316(2023). DOI: 10.1097/GH9.0000000000000316

[33] Alkhanferi, S. , Alhuraiz, K. , Alyami, H. , Alenazi, S. , Aldhaban, M. , Alrowedann, N. , Albedaiwi, Y. , Alnazawi, N. , Bokhamseen, A. and Talaqof, F. The Safety and Efficacy of Combination Therapy of Dapagliflozin and Metformin in Patient with Type 2 Diabetes Mellitus: A Review Study. Journal of Diabetes Mellitus,12, 271-283(2022). doi: 10.4236/jdm.2022.124022.

[34] Xu X, Xu W, Zhuo Q, Yan Y. The efficacy and safety of dapagliflozin combined with oral hypoglycemic agents in patients with type 2 diabetes: a systematic review and meta-analysis. Ann Palliat Med,11(3):1028-1037(2022). doi: 10.21037/apm-22-121

[35] Ferreira-Hermosillo A, Molina-Ayala MA, Molina-Guerrero D, Garrido-Mendoza AP, Ramírez-Rentería C, Mendoza-Zubieta V, Espinosa E, Mercado M. Efficacy of the treatment with dapagliflozin and metformin compared to metformin monotherapy for weight loss in patients with class III obesity: a randomized controlled trial. Trials,21(1):186(2020). doi: 10.1186/s13063-020-4121-x

[36] Zhao,X. , Ning,R. , Hui,A. , Boulton,D.W. , Tang,W. Pharmacokinetic Variables of Dapagliflozin/Metformin Extended-release Fixed-dose Combination in Healthy Chinese Volunteers and Regional Comparison. Clinical Therapeutics, 45(8), 762-769(2023). https://doi.org/10.1016/j.clinthera.2023.06.012

[37] United States Pharmacopeia 27—National Formulary 22, General Chapter Validation of Compendial Methods, U.S. Pharmacopeial Convention Inc., Rockville, 2622–2625(2004).

[38] United States Pharmacopeia 27—National Formulary 22, General Chapter Chromatography, U.S. Pharmacopeial Convention Inc., Rockville, 2272–2284(2004).

[39] Stringer, Janet L. , Thomas, John A.. "antidiabetic drug". Encyclopedia Britannica, 5 Jan. 2024, https://www.britannica.com/science/antidiabetic-drug. Accessed 27 January 2024.

[40] ICH. Stability Testing of New Drug Substances and Products. Current step, Q1A (R2), 4, 1-2(2003).

[41] Mahgoub, S.M., Elsherief, A.H., Mahmoud, R., Mahmoud, M.R., Mohamed, M.A. Validated Stability Indicating Eco-friendly RP-HPLC Method for the Concurrent Quantification of Gabapentin and Diclofenac K in Wastewater and Pharmaceutical Formulations. Egypt. J. Chem,67(1):411-422 (2024). DOI: 10.21608/EJCHEM.2023.213243.8018

[42] Mohamed, M.A., Hassouna, M.E.M. Ecofriendly Spectrophotometric and Chromatographic Methods for Simultaneous Analysis of a Quaternary Mixture of Cephalexin, Sodium Benzoate, Methylparaben and Propylparaben with Application of the Holding Time Study in Bulk and Pharmaceutical Dosage Forms. Pharmaceutical Chemistry Journal,57(1),146-159(2023). https://doi.org/10.1007/s11094-023-02862-9.