HPTLC Method For Simultaneous Estimation Of Empagliflozin, Linagliptin And Metformin In Combination

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ABSTRACT
A simple (HPTLC) technique was developed for Metformin, Linagliptin and Empagliflozin using N-Butanol:Water:Glacial Acetic Acid as a mobile phase in the ratio of 6:3:1 v/v/v. The method was validated as per guidelines ICH Q2(R1) in terms of all parameters of validation. The scanning is performed at 226nm. The developed method was found to be linear within range 250-1250ng/band, 100-500ng/band and 400-2000ng/band for Metformin, Linagliptin and Empagliflozin respectively. Rf values for Metformin, Linagliptin and Empgliflozin found to be 0.32 ± 0.03, 0.58 ± 0.03, 0.84 ± 0.03 respectively. The limits of detection (LOD) 19.45ng/band, 6.47ng/band and 26.12ng/band and quantification (LOQ) of the developed method were 58.9ng/band, 19.61ng/band and 79.17ng/band found respectively for Metformin, Linagliptin and Empaglifozin. Robustness tests confirmed the method's reliability under varying conditions. The validated method could be applied to the quality control and routine estimation of Metformin, Linagliptin and Empagliflozin in combination.

KEYWORDS: Metformin, Linagliptin, Empagliflozin, HPTLC

INTRODUCTION

Metformin(MET) is a drug prescribed for diabetic patients. It is chemically 3-(diaminomethylidene)-1,1-dimethylguanidine its chemical structure is shown in Fig 1[1]. Metformin is biguanide antihyperglycemic used in conjuction with diet and exercise control in type 2 diabetes mellitus[2]. It reduces the glucose and enhances the insulin sensitivity by increasing both peripheral glucose uptake and utilization[3,4].

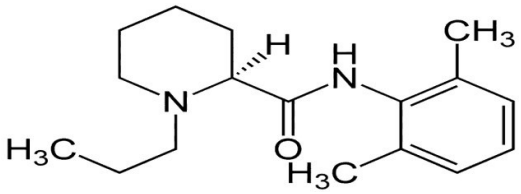

Fig. 1 Metformin

Linagliptin (LINA) is a competitive and reversible inhibitor of the DPP-4 receptor. molecular structure is depicted in Fig. 2[5]. The drug exerts its antidiabetic effect by preventing the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic

polypeptide (GIP). These incretins play a vital role in enhancing insulin secretion from pancreatic beta cells in response to glucose..

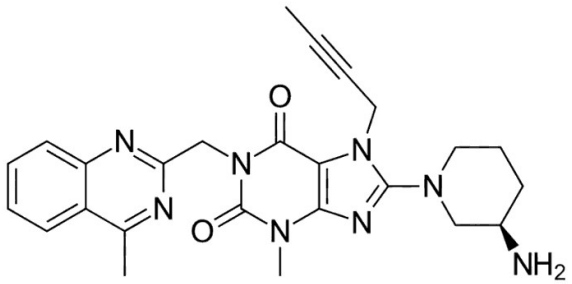

Fig. 2 Linagliptin

Empagliflozin(EMPA) is anti diabetic medication used for type 2 Diabetes Mellitus, that works by SGLT-2 Receptor Inhibition. its structure is shown in Fig 3[9] mpagliflozin is prescribed as an add-on to diet and exercise to enhance blood sugar control in individuals with type 2 diabetes, either as monotherapy or in combination with other antidiabetic agents such as metformin or linagliptin[10,11] . Additionally, it is approved for reducing the risk of cardiovascular death in adults who have both type 2 diabetes mellitus and established cardiovascular disease, either on its own or as part of a combination therapy with metformin[12].

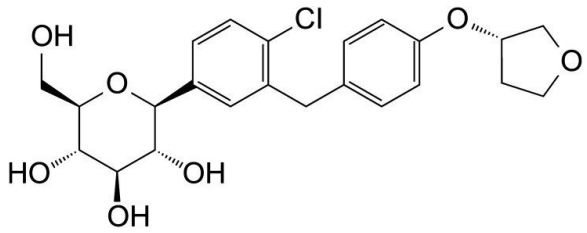

Fig. 3 Empagliflozin

A combination of Metformin (MET), Linagliptin (LINA) and Empagliflozin (EMPA) is available in market to treat Diabetes[13]. The label claim is 1000mg:2.5mg:12.5mg in TRIJARDY XR marketed tablet formulation.

As per literature search methods like, UV[14], RP-HPLC[15,16], HPTLC[17] have been reported on the individual study of these three drugs. Many analytical methods are reported to estimate MET, LINA and EMPA in combination with other drugs and some of them are RP-HPLC[18-25,] RP-UPLC[26], HPTLC[27-34]. HPTLC work reported for only the single drug or combination of two drug. There is no any estimation method developed for simultaneous estimation of MET, LINA and EMPA using HPTLC in their combination.

MATERIALS AND METHODS

Chemicals and Reagents

Metformin was received from Emcure Pharmaceuticals, Pune. Empagliflozin was received as gift sample from Alkem Laboratories, Mumbai. Methanol (HPLC and AR Grade), Butanol, Glacial acetic acid .HPLC grade water from water purifier system.

Chromatographic condition

The samples were analysed on CAMAG HPTLC system, with CAMAG TLC scanner 3, sample applied on plate with Linomat 5 applicator, on Merck TLC plates. Application done with Hamilton microliter syringe (100 µl). Development of silica plate was performed glass chamber (10*10 cm). Saturation lasted for 20 minutes at room temperature, developed plate dried by hot air dryer and results obtained by TLC scanner 3 on winCATS software V-1.4.3. The study involved other instruments like UV Cabinet (Bio medica) and Lablink Xtrapure water purification system and PRAMA SM-12 Sonicator.

Optimisation of Mobile phase

In order to achieve optimal system suitability parameters, various proportion of mobile phase solvents were tried out such as, Ethanol:Ethylacetate:Water, N-Butanol:Water:GAA, Chloroform:Methanol:Ammonia, Initially Ethanol:EthylAcetate:Water (3.5:3:1) was tried which showed broad peaks and the no peak separation, Ethanol:Ethyl acetate: Water (4:2:1) showed improper peak separation and high Rf value for EMPA. Significant resolution and desired system suitability parameters were obtained by using n-Butanol:Water:GAA as mobile phase in the ratio 6:3:1v/v/v.

Preparation of Solution:

MET, LINA and EMPA all three drugs were found soluble in methanol AR grade.

Standard stock solution preparation

A precisely weighed 10 mg of MET was transferred to a 10 mL volumetric flask, and 8 mL Methanol was added. It was shaken well till it gets dissolved. After that volume built up with methanol, to get separate standard stock solutions of MET 1000 μgmL^{-1}. Similarly prepared stock solutions of LINA and EMPA.

Preparation of standard solution is selected as per the ratio of marketed formulation, which contain 400:1:5 proportion of MET, LINA and EMPA respectively. Now Pipetted 0.5mL of MET standard stock solution (1000 μgmL^{-1}), Pipetted 0.2mL of LINA standard stock solution (1000 μgmL^{-1}) and add Pipetted 0.8mL of EMPA standard stock solution (1000 μgmL^{-1}) and transferred to separate volumetric flasks and volume was built up with methanol to 10 mL to obtain standard solutions. The resultant solutions becomes of MET 50μgmL^{-1}, LINA 20μgmL^{-1} and 80μgmL^{-1} EMPA. Spotted 10µl of resultant mixture and developed under optimized chromatographic conditions. Representative densitogram are shown in Fig.3.

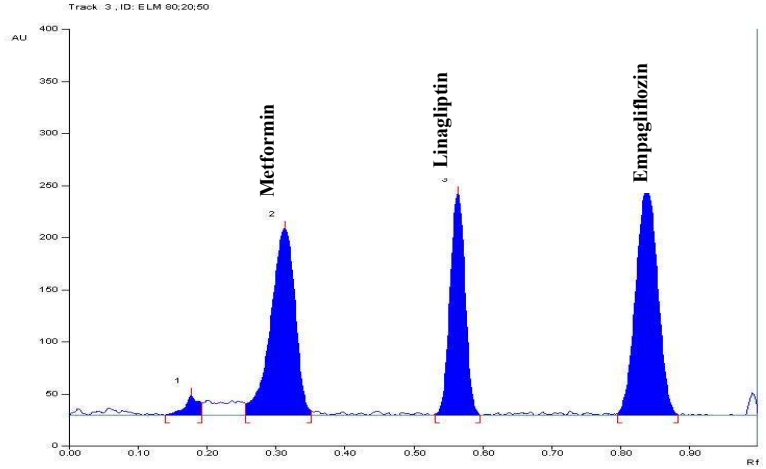

Fig.3. Representative Densitogram of MET, LINA and EMPA (500ng/band, 200ng/band and 800ng/band . Rf values 0.32±0.03, 0.58±0.03 and 0.84±0.03 respy.)

Preparation of sample solution

Prepared the spiked blend by spiking of MET, LINA and EMPA in proportion 1000:2.5:12.5 due to unavailability of its marketed preparation. We have considered the weight of tablet as 1300mg/tab of which the drug constituents covered 1015mg and the remaining part was 285mg which is of excipients. Mixed the 1gm of MET, 2.5mg LINA and EMPA 12.5mg with blank blend by geometric mixing. We have blended eq to 1 tablets, weighed the amount equivalent to 1 tablets of marketed drug content and transferred to a10 mL volumetric flask, add 5 mL Methanol, sonicated for 10 min and filtered through the whatman filter paper. Made up the volume with methanol, to get 100000 μgmL^{-1} sample stock solution for MET, 250µg mL^{-1} of LINA And 1250 µg mL^{-1} of EMPA leading to sample stock soln (A). Quantity spotted in ng/band is adjusted as per the sample by dilution factor and application volume.

For assay of Metformin 0.5ml of sample stock sol^{n} (A) is added to 10 ml volumetric flask and built up with methanol resultant solution is 5000 μgmL^{-1}. It was suitably diluted with methanol to get 50 μgmL^{-1} solution which was applied on the plate for assay analysis.

For analysis of LINA and EMPA 0.5 ml of sample solution (A) is added to 10ml volumetric flask

and built up with methanol to get the desired solution of 12.5 µgmL^{-1} and 62 µgmL^{-1} for LINA and EMPA respectively. Appropriate volume was spotted to get amount spotted within linear range.

Selection of Analytical wavelength:
Analysed the formulated standard solution using UV-visible spectrophotometer (Jasco V-730) in spectrometric mode (400-200 nm). Maxima of MET drug (λ max) found at 237nm , LINA (λ max) found at 226nm and 293nm, (λ max) for EMPA found at 225nm. For scanning of all drugs at same wavelength we have compared the the UV Spectral overlay of all drugs and choosen the single wavelength for detection scanning which is 226nm. Overlay is shown in Fig.4.

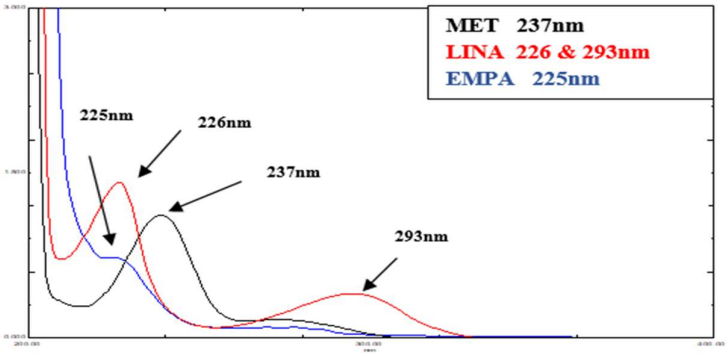

Fig. 4 UV Spectrum overlay of Metformin, Linagliptin and Empagliflozin (10 µg/mL each)

METHOD VALIDATION:
The method for combination of MET, LINA and EMPA was validated as per the ICH guidelines ICH Q2(R1) in terms of all parameters of validation.

Linearity and range:
A standard mixture solution of MET (50µg/ml), LINA (20µg/ml), and EMPA of (80µg/ml), was applied on the TLC plate of the volume 5-25µl, thus leading to spotted amounts in the range of 250-1250 ng/band, 100-500ng/band and 400-2000ng/band respy. Rf values found to be 0.32, 0.58 and 0.84 respectively. The plate was developed and this procedure was repeated 5 times. The correlation coefficient was found 0.995 with equation of y = 6.980x + 1017 for MET, for LINA 0.996 with equation of y = 10.84x + 538.6 and EMPA 0.994 with equation y = 4.343x + 623.9. The 3D densitogram is shown in the Fig.5 for linearity.

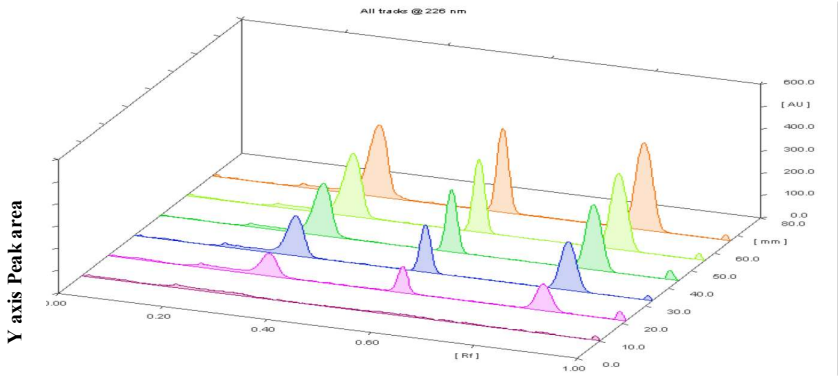

Fig.5: Representative 3D Densitogram of Linearity for Met, Lina and Empa (Met250-1250ng/band, Lina 100- 500ng/band and Empa 400- 2000ng/band). Rf values 0.32±0.03, 0.58±0.03 and 0.84±0.03 respy.

Assay:
For MET 2 spots of the sample solution (50 µg mL-1) applied on the plate with band size of 250ng/band. On the other plate 2 spots of the sample stock solution (12.5 µg mL-1) for LINA with 125ng/band and 187.5 ng/band. The 2 spots of the sample solution of EMPA (62 µg mL-1)

applied of with 625ng/band and 937ng/band. This plate developed and scanned over 226nm after development amount recovered is calculated. Results obtained are summarized in Table no.1

Table .1 Assay Studies

Drug	Amount Spotted (ng/band)	Amount Recovered (ng/band)	%Drug Content	Mean± %RSD
Metformin	250	248.13	99.25	100.54±1.81
	250	254.58	101.83	
Linagliptin	125	123.65	98.92	99.77±1.21
	187.5	188.69	100.63	
Empagliflozin	625	623.32	99.73	100.66±1.30
	937	951.89	101.59	

Accuracy :

Accuracy of the method was determined by standard addition method. the drugs to be analyzed was added to the sample solution. In sample solution pure drug was spiked at 80%, 100% and 120% level. The standard drugs of MET, LINA and EMPA was added to pre analysed sample solution at 3 level. The sample application considered for MET, LINA and Empa to perform accuracy are 500ng/band, 125ng/band and 625ng/band. Then standard stock of drug added in it. The results obtained are summarized in Table.2

Table.2 Accuracy Studies

Drug	%Addition	Amount from marketed formulation (ng/band)	Amount of standard added	Total amount of the drug (ng/band)	Amount recovered	% recovery
Metformin	80%	500	400	900	883.52	98.16
	100%	500	500	1000	993.55	99.35
	120%	500	600	1100	1100.43	100.03
Linagliptin	80%	125	100	225	223.09	99.15
	100%	125	125	250	251.69	100.67
	120%	125	150	275	280.11	101.85
Empagliflozin	80%	625	500	1125	1104.55	98.18
	100%	625	625	1250	1255.83	100.46
	120%	625	750	1375	1388.92	101.01

Precision :

Precision study was performed as intraday (repeatability) precision and inter day (intermediate) precision. Intraday precision was performed by analysing 250ng/band of MET, 100ng/band of LINA and 400ng/band of EMPA as 6 replicates in same day. For interday precision, the procedure was repeated on three consecutive days, results are given in Table.no.3

Table.3 Peak area in precision studies

Sr. No	Metformin		Linagliptin		Empagliflozin	
	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	2489	2557	1562	1564	2224	2278
2	2575	2635	1603	1545	2155	2191
3	2559	2591	1549	1489	2125	2258
4	2517	2512	1588	1566	2203	2189
5	2603	2623	1539	1542	2137	2230
6	2495	2578	1537	1553	2144	2197
Mean	2539.66	2582.66	1563	1543.16	2164.66	2223.83
SD	46.28	44.96	27.10	28.25	39.62	37.81
%RSD	1.82	1.74	1.73	1.83	1.83	1.7

(n=3, for interday precision 6 replicates for consecutive 3 days)

(LOD) and (LOQ)

From the regression equation the limits were calculated. Both LOD and LOQ were determined using formulae 3.3σ/S and 10σ/S respectively. The results obtained are summarized in Table.no.4.

Table.4 LOD and LOQ

Drug	LOD(ng/band)	LOQ(ng/band)
Metformin	19.45	58.9
Linagliptin	6.47	19.61
Empagliflozin	26.12	79.17

Robustness:

Robustness of developed method was analysed by small deliberate changes in mobile phase ratio, saturation time and detection wavelength. Mobile phase volume was changed by ± 0.2 ml, saturation time was varied by ±5 min i.e., 15 min and 25 min. Detection wavelength were varied by ±2 nm, one factor at a time was varied for analysis. Conc. of 250ng/band of MET, 100ng/band of LINA and 400ng/band of EMPA applied to study effect of all factors on peak area of drug which is shown in Table no.5.

Table .5 Robustness Studies

Parameter	Condition	%RSD		
		Metformin	Linagliptin	Empagliflozin
Saturation Time	15 min	1.56	1.73	1.64
	25 min	1.38	1.85	1.82
Mobile Phase Ratio	5.8:3.2:1 v/v/v	1.84	1.42	1.83
	6.2:2.8:1 v/v/v	1.80	1.48	1.85

Wavelength	224 nm	1.80	1.86	1.82
	228 nm	1.67	1.78	1.21

RESULT

For a combination of Metformin, Linagliptin and Empagliflozin a High-performance thin layer chromatographic technique has been developed using n-Butanol:Water:Glacial Acetic Acid as a mobile phase in the ratio of 6:3:1 v/v/v. On the basis of mobile phase trials we noticed that the development time was 70 min by our analyis. Rf was confirmed for MET, LINA and EMPA i.e. 0.32 ± 0.03, 0.58 ± 0.03, 0.84 ± 0.03 respectively. The wavelength of detection was 226 nm. Range selected for MET, LINA and EMPA was 250-1250ng/band, 100-500ng/band and 400-2000ng/band respectively. The correlation coefficient were found 0.995 with equation of y =6.980x + 1017 for MET. LINA shows equation of y = 10.84x + 538.6 and correlation coefficient was found to be 0.996. The correlation coefficient of EMPA found to be 0.994 and equation y = 4.343x + 623.9. Recovery studies were used to determine the method's accuracy. A simple, precise, sensitive validation method have been developed. Summary of validation parameters is shown in Table no.6

Table.6 Summary of validation parameters

Sr. No	Validation Parameter	Result		
		Metformin	Linagliptin	Empagliflozin
2	Linearity	y = 6.980x + 1017 R^2 = 0.995	y = 10.84x + 538.6 R^2 = 0.996	y = 4.343x + 623.9 R^2 = 0.994
	Range	250-1250 ng/band	100-500 ng/band	400-2000 ng/band
3	Intraday Precision% Interday Precision%	2539.66±1.82 2582.66±1.74	1563.09±1.73 1543.16±1.83	2164.66±1.83 2223.83±1.70
4	Assay%	100.54±1.81	99.77±1.21	100.66±1.30
5	Accuracy Level 80% Level 100% Level 120%	 98.16 99.35 100.03	 99.15 100.67 101.85	 98.18 100.46 101.01
6	LOD LOQ	19.45 ng/band 58.9 ng/band	6.47 ng/band 19.61 ng/band	26.12 ng/band 79.17 ng/band
7	Robustness	Robust	Robust	Robust

CONCLUSION

The developed HPTLC method for Metformin, Linagliptin, and Empagliflozin was validated as per ICH guidelines. This method was found to be effective for the study of this drug combination. Previously reported methods are for evaluation of a single and combination of any two drugs, but our method is better with respect to the peak separation and peak shape achieved by this method. As per the literature survey very few methods have been reported, usually for single and combination of two drugs but simultaneous estimation of these three drugs was not reported, so considered this and worked on it. There is further scope for researchers to work on development of stability-indicating method for this combination. The developed, validated method could be applied to the quality control and routine monitoring of simultaneous analysis of these drugs.

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REFERENCE

1. Drugbank “metformin” https://go.drugbank.com/drugs/DB00331 (Accessed on: 26 July 2024)
2. National library of medicine pubchem “metformin” https://pubchem.ncbi.nlm.nih.gov/compound/Metformin (Accessed on: 26 July 2024)
3. WebMD “metformin” https://www.webmd.com/drugs/2/drug-11285-7061/metformin-oral/metformin-oral/details (Accessed on: 26 July 2024)
4. Mayoclinic, drugs and suppliments oral rout metformin https://www.mayoclinic.org/drugs-supplements/metformin-oral-route/proper-use/drg-20067074 (Accessed on: 26 July 2024)
5. Drugbank “ linagliptin” https://go.drugbank.com/drugs/DB08882 (Accessed on: 26 July 2024)
6. National library of medicine , pubchem “ linagliptin” https://pubchem.ncbi.nlm.nih.gov/compound/Linagliptin (Accessed on: 26 July 2024)
7. WebMD “linagliptin” https://www.webmd.com/drugs/2/drug-155816/linagliptin-oral/details (Accessed on: 29 July 2024)
8. Medlineplus “ linagliptin” https://medlineplus.gov/druginfo/meds/a611036 (Accessed on: 29 July 2024)
9. PubChem. Empagliflozin.Nih.gov. https://pubchem.ncbi.nlm.nih.gov/compound/Empagliflozin. (Accessed on: 29 July 2024)
10. Empagliflozin. go.drugbank.com. https://go.drugbank.com/drugs/DB09038. (Accessed on: 29 July 2024)
11. https://www.researchgate.net/figure/Chemical-structure-ofempagliflozin_fig2_261610278 (Accessed on: 29 July 2024)
12. Empagliflozin: MedlinePlus Drug Information. medlineplus.gov. https://medlineplus.gov/druginfo/meds/a614043.html. (Accessed on: 29 July 2024)
13. https://patient.boehringer-ingelheim.com/us/trijardy/ (Accessed on: 30 July 2024)
14. Dwivedi A, Sharma R. Development and validation of simultaneous equation method for estimation of sitagliptin phosphate and empagliflozin in bulk form by UV spectroscopy. Research Journal of Pharmacy and Technology. 2023;16(8):3714-8.
15. Patil SD, Amurutkar SV, Upasani CD. Development and validation of stability indicating RP-HPLC method for empagliflozin. Asian Journal of Pharmaceutical Analysis. 2016;6(4):201-6.
16. Ali SI, Kumar P. Stability indicating simultaneous estimation of metformin and empagliflozin in pharmaceutical tablet dosage form by RP-HPLC. Asian Journal of Research in Chemistry. 2017;10(6):783-8.
17. Nannaware PS, Siddheshwar SS, Kolhe MH. A review on analytical methods for estimation of linagliptin in bulk and tablet dosage form. Research Journal of Science and Technology. 2021;13(2):127-32.
18. Pathan MA, Kshirsagar A. Stability indicating hplc method development and validation for

simultaneous estimation of metformin and empagliflozin in bulk and pharmaceutical dosage form. Research Journal of Pharmacy and Technology. 2022;15(2):830-6.

19. Dighe NS, Shinde GS, Vikhe JJ. Simultaneous estimation, validation and force degradation study of metformin hydrochloride and empagliflozin by RP-HPLC method. Research Journal of Science and Technology. 2019;11(2):135-47.

20. Nandre DS, Ahmed AE, Khan GJ. Stability indicating HPLC method development and validation for simultaneous estimation of dapagliflozin and metformin tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research. 2022 Oct 8;15(10):109-14.

21. Patel K, Shah UA, Joshi HV, Patel JK, Patel CN. Qbd stressed development and validation of stability-indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin HCl in pharmaceutical dosage form. Research Journal of Pharmacy and Technology. 2022;15(5):1917-23.

22. Kagarana CS, Patel KN, Patel AB. Stability indicating RP-HPLC method development and validation for simultaneous estimation of metformin hydrochloride and remogliflozin etabonate in bulk and tablet formulation. Research Journal of Pharmacy and Technology. 2024;17(5):2025-30.

23. Kiran T, Parvathi P, Kumar JN. Development and validation of rp-hplc method for the simultaneous estimation of linagliptin, empagliflozin and metformin in solid dosage forms. Asian Journal of Pharmaceutical Analysis. 2020;10(3):117-24.

24. Sri KV, Anusha M, Reddy SR. A rapid RP-HPLC method development and validation for the analysis of linagliptinin bulk and pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis. 2015;5(1):16-20.

25. Kiran T, Parvathi P, Kumar JN. Development and validation of rp-hplc method for the simultaneous estimation of linagliptin, empagliflozin and metformin in solid dosage forms. Asian Journal of Pharmaceutical Analysis. 2020;10(3):117-24.

26. Padmaja N, Veerabhadram G. A novel stability indicating RP-UPLC-DAD method for determination of metformin and empagliflozin in bulk and tablet dosage form. Oriental Journal of Chemistry. 2017;33(4):1949.

27. Bhole RP, Wankhede SB, pandey M. Stability indicating HPTLC method for simultaneous estimation of empagliflozin and linagliptin in pharmaceutical formulation. Analytical Chemistry Letters. 2017 Jan 2;7(1):76-85.

28. Munde MK, Kulkarni NS, Sen AK, Sen DB. A novel validated stability indicating analytical method for simultaneous quantification of metformin hydrochloride and empagliflozin in bulk and marketed formulation by HPTLC using box-wilson experimental design approach.

29. El-Desouky EA, Abdel-Raoof AM, Abdel-Fattah A, Abdel-Zaher A, Osman AO, Abdel-Monem AH, Morshedy S. Determination of linagliptin and empagliflozin by UPLC and HPTLC techniques aided by lean six sigma approach. Biomedical Chromatography. 2021 Jul;35(7):e5102.

30. Rizk M, Attia AK, Mohamed HY, Elshahed MS. Development and validation of thin-layer chromatography and high-performance thin-layer chromatography methods for the simultaneous determination of linagliptin and empagliflozin in their co-formulated dosage form. JPC–Journal of Planar Chromatography–Modern TLC. 2020 Dec;33(6):647-61.

31. Vichare V, Choudhari V, Tambe V, Dhole S. Inherent stability testing of empagliflozin in the presence of metformin HCl by HPTLC and characterization of degradation products of empagliflozin by LC–ESI–QTOF–MS/MS. JPC–Journal of Planar Chromatography–Modern TLC. 2022 Feb;35(1):61-71.

32. Dedhiya PP, Singh RS, Shah PJ, Shah SA. Development and validation of high performance thin layer chromatographic method for estimation of metformin hydrochloride and empagliflozin in combined tablet dosage form using quality by design approach. Pharma Science Monitor. 2019 Apr;10(2):83-100.

33. Thakor NS, Amrutkar SV, Chaudhari PD. Simultaneous estimation of empagliflozin and metformin by high-performance thin-layer chromatography using quality-by-design approach. JPC–Journal of Planar Chromatography–Modern TLC. 2019 Aug;32:295-307.

34. Hosny NM. Insights into the lipophilicity of four commonly prescribed antidiabetic drugs and their simultaneous analysis using a simple TLC-spectrodensitometric method: application to fixed-dose combination tablets and human plasma. Journal of Chromatography B. 2022 Aug 15;1206:123341.

35. El Sheikh R, Hassan WS, Youssef E, Hamdi AY, Badahdah NA, Alzuhiri ME, Gouda AA. Development and validation of rapid stability-indicating high-performance liquid chromatography method for the determination of linagliptin and empagliflozin in pure and dosage forms. DEVELOPMENT. 2020;13(4).

36. Joshi SS, Nahire RR, Shastri NR, Surendranath KV, Satish J. Validated stability-indicating RP-HPLC UV method for simultaneous determination of metformin and repaglinide. Acta Chromatographica. 2012 Sep 1;24(3):419-32.

37. Abbas NS, Derayea SM, Omar MA, Saleh GA. Innovative TLC-densitometric method with fluorescent detection for simultaneous determination of ternary anti-diabetic mixture in pharmaceutical formulations and human plasma. Microchemical Journal. 2021 Jun 1;165:106131.

38. Sahu LK, Si S, Patro SK. Stability indicating assay of empaglifozin and metformin. Research Journal of Pharmacy and Technology. 2024;1(3).