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RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE AND LINAGLIPTINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

RP-HPLC method was developed for simultaneous determination of metformin hydrochloride (MTL) and linagliptine (LNG) in pharmaceutical dosage form. Mobile phase used for separation consisting of mixture of methanol and water (0.05% O-phosphoric acid) in the ratio 50:50 v/v at flow rate of 0.9 mL/min using C8 Primesile column (250mmX 4.6mm) at 238 nm. Injection volume used was 20 µL and temperature used was ambient. The retention time of MTL and LNG was found to be 2.85 min and 8.49 min, respectively. The linearity range for MTL and LNG observed was 200-1000 µg/ml and 1-5 µg/ml, respectively. Method was validated as per ICH guidelines. Validation parameters studied were linearity and range, recovery study, precision, LOD, LOQ and robustness. Data obtained was found to statistically satisfactorily.

KEYWORDS: RP-HPLC, Metformin hydrochloride, Linagliptine, Method Validation.

1. INTRODUCTION

Metformin hydrochloride (MTL) is an oral anti-diabetic drug. It acts directly or indirectly on the liver to lower glucose production, and acts on the gut to increase glucose utilisation, increase GLP-1 and alter the microbiome.[1-2] It is official in Indian Pharmacopoeia.[3] Linagliptine (LNG) is also oral antidiabetic drug used in treatment of type 2 diabetes mellitus.[4-5] It is also official Indian Pharmacopoeia.[3] The combination of MET and LNG is antidiabetic medication works by lowering the glucose production in the liver, delaying glucose absorption from the intestine and increasing the body's sensetivity to insulin (See Fig. 1 & 2).[6]

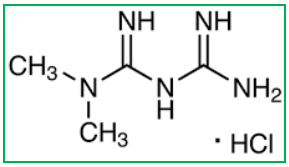

Fig. 1: Structure of metformin hydrochloride.

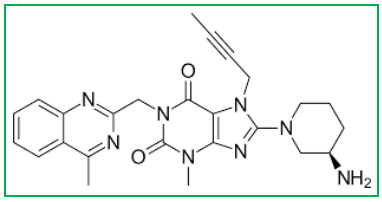

Fig. 2: Structure of linagliptine.

Analytical methods are used for qualitative and quantitative analysis of various pharmaceuticals products including tablets, capsules, injections, etc. Such methods includes Uv-spectroscopy, gas chromatography, high performance liquid chromatography, high performance thin layer chromatography, etc.[7-46]

Literature survey revealed various analytical methods have been reported for estimation of MTL alone and in combination with other drugs. Likewise, in literature there is one UV-spectroscopic method and one RP-HPLC and one HPTLC method available for simultaneous analysis of MTL and LNG in pharmaceutical dosage form.[47-57] However, nobody has enclosed the complete validation as per ICH guidelines. Therefore, attempts were made to develop new RP-HPLC method for simultaneous determination of MTL with LNG in pharmaceutical dosage form.

2. MATERIALS AND METHODS

2.1 Instrumentation & chemicals

Chromatography was performed with Youngline ACME 9000 (Autochro-3000 software) system coupled with Primesile column (4.6 mm I.D x 250 mm) C8 column and UV 730D detector. A Rheodyne injector (manual loading) with a 20 µL external loop was used. All chemicals and reagents used in method were of HPLC grade. Standard drugs were obtained as gift samples from Lupin Pvt. Ltd, Mumbai and tablet formulations (Ondero

Met®) (Metformin hydrochloride- 500mg & Linagliptine- 2.5mg) were purchased from local medical shop.

2.2 Wavelength & conditions

Wavelength for analysis of both the drugs was selected by scanning the individual drug's standard solutions in methanol (i.e. MTL 1000 µg/ml, LNG 5 µg/ml). From overlain spectra, wavelength 238 nm was selected for further experimental work. Mobile phase for separation of drugs from mixed standard solution (containing MTL 500 µg/ml & LNG- 2.5 µg/ml) was consists of mixture of methanol and water (0.05% O-phosphoric acid (0.05% OPA)) in the ratio 50:50 v/v in isocratic mode with flow rate 1 ml/min using 20 µl injection volume.

2.3 System suitability parameters

The system suitability test was performed by collecting data from five replicate injections (20 µl) of mixed standard solution (containing MTL 500 µg/ml, LNG 2.5 µg/ml in methanol) at selected chromatographic conditions. The studied parameters includes retention time, HETP and tailing factor.

2.4 Assay of pharmaceutical dosage form

Average weight of 20 tablets was determined and were then crushed to fine powder. Average power equivalent to 500 mg of MTL (also contain 2.5 mg of LNG) was weighed accurately and was transferred to 100 ml volumetric flask. To this 20 ml of methanol was added and shaken for 30 min and sonicated for 10 min. Final volume was added up to 100 ml with same solvent. The solution was filtered the Whatman filter paper. About 10 ml of above solution was diluted to 100 ml with methanol. The contained 500 µg/ml of MTL and 2.5 µg/ml of LNG. About 20µl sample solution was injected into the system and concentration of each drug was calculation from respective regression equation prepared for individual drug using AUC.

2.5 Validation of method

Studied validation parameters includes accuracy and precision, linearity & range, LOD (limit of detection) & LOQ (limit of quantitation) and robustness.[58]

2.5.1. Accuracy & precision

To study the accuracy and precision, recovery study was carried out by addition of standard drugs solutions to preanalysed sample. Recovery study was undertaken at three levels i.e. 80%, 100% and 120%.

2.5.2. Linearity & range

Linearity was studied by injecting a series of dilutions of mixed standard stock solution in the concentration range 200-1000 µg/ml (MTL) and 1-5 µg/ml (LNG) into the HPLC system using 20µl volume. Calibration graph was plotted as concentration versus AUC.

2.5.3. LOD & LOQ

The LOD & LOQ were confirmed by diluting known concentrations of drug until the average AUC were approximately 3 or 10 times the standard deviation of AUC of the blank for five replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively.

2.5.4. Robustness

Robustness was studied by making changes in the chromatographic conditions, such as slight change in change in mobile phase flow rate (±0.1 ml/min), mobile phase composition ((±1%), and change in wavelength (±1 nm). Percent contents of drugs were measured in preanalysed tablet formulation.

3. RESULTS AND DISCUSSION

Combination of metformin hydrochloride and linagliptine was selected for RP-HPLC method development for simultaneous estimation of both from pharmaceutical dosage form. Solvent methanol was used to prepare standard and sample solutions as it dissolved both the drugs at selected concentration. Wavelength for detection selected was 238 nm because at this wavelength both the drug showed better sensitivity. Concentration selected were 500 µg/ml for MTL and 2.5 µg/ml for LNG. At selected chromatographic conditions i.e. mobile phase consisting of mixture of methanol and water (0.05% O-phosphoric acid) in the ratio 50:50 v/v at a flow rate of 0.9 mL/min with Primesile C8(4.6 mm I.D x 250 mm) column at ambient temperature, retention time obtained for MTL and LNG was 2.85 and 8.49 min, respectively. 0.05% O-phosphoric acid was used to correct the pH so as to get sharp peak with minimum tailing and fronting. Herein, MTL elutes first as it is more polar in nature followed by less polar LNG.[59-60]

The validation study was performed as per ICH guidelines. Linearity and range was studied by using the series of dilution of each drug solution. Both the drugs shows linear response over the studied range. From this, concentration for MTL and LNG were selected. The LOD & LOQ were checked by diluting known concentration of standard drug until the mean responses were approximately 3 or 10 times the standard deviation of the responses of the blank for five replicate measurements. The signal/noise ratios 3:1 and 10:1 were considered as the LOD and LOQ, respectively. LOD and LOQ values obtained are given in **Table 1.** Precision of the method was checked by measuring system suitability parameter by replicate injection of mixed standard solution. The results are expressed % RSD.

Recovery study was performed to determine the recovery of pure drugs from sample solution. Recovery study by standard addition method at three levels i.e. 80,100 and 120 %. The percentage recovery for both the drug was closed to 100% w/w for both drugs. The percent contents of drugs were measured in preanalysed tablet formulation. Precision was determined by studying

system suitability parameters by injecting standard solution (Table 1).

Table 1: Results of the validation of developed method.

Study	L. Claim		% Recovery at*			Linearity & range			
Result	Parameter		80%	100%	120%	Range (µg/ml)	R^2	LOD	LOQ
	MTL	500mg	99.18	99.61	101.13	200-1000	0.999	0.32	0.94
	LNG	2.5mg	98.85	98.91	100.08	1-5	0.996	0.006	0.016
	Study	System suitability parameter				Robustness#			
	Parameter	Ret. time	Theor. plates	Tail. factor		FR # (ml/min)		MP composition (a: b)**	Variation
						0.8	1.0	49:51 51:49	Intraday Interday
	MTL	2.85	3118	1.31		2.89	3.12	2.87 2.84	100.21 99.13
	LNG	8.49	5386	1.46		9.01	9.33	8.48 8.49	102.00 100.50

* Contents of drugs were measured in preanalysed tablet formulation; ** a- methanol, b- water (0.05% O-phosphoric acid); # measured effect on ret. time.

The capacity of developed method was checked by performed robustness study. The conditions changed deliberately were change in flow rate (± 0.1), mobile phase composition (± 1), and wavelength (± 1), Intraday and inter-day variation and percent contents in formulation were estimated. The result showed develop method remain unaffected.

CONCLUSION

Novel RP-HPLC method for simultaneous analysis of metformin hydrochloride and linagliptine from pharmaceutical dosage form is simple, accurate and precise. It does not get affected upon smaller variation in experimental condition. Thus, It be used for routine quality control analysis of bulk drugs and pharmaceutical formulation.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia, 2017; 60(9): 1577-1585. doi:10.1007/s00125-017-4342-z.
2. Thakur S, Daley B, Klubo-Gwiezdzinska J. The role of an anti-diabetic drug metformin in the treatment of endocrine tumors. J Mol Endocrinol, 2019; 63(2): R17-R35. doi:10.1530/JME-19-0083
3. Indian Pharmacopoeia. Indian Pharmacopoeia Commission, Ghaziabad; 8th Ed. Government of India, Ministry of Health and Family Welfare. New Delhi, 2018.
4. McGill JB. Linagliptin for type 2 diabetes mellitus: a review of the pivotal clinical trials. Ther Adv Endocrinol Metab., 2012; 3(4): 113-124. doi:10.1177/2042018812449406.
5. Ceriello A, Inagaki N. Pharmacokinetic and pharmacodynamic evaluation of linagliptin for the treatment of type 2 diabetes mellitus, with consideration of Asian patient populations. J Diabetes Investig, 2017; 8(1): 19-28. doi:10.1111/jdi.12528.
6. Haak T. Combination of linagliptin and metformin for the treatment of patients with type 2 diabetes. Clin Med Insights Endocrinol Diabetes, 2015; 8: 1-6. doi:10.4137/CMED.S10360.
7. Vaidya VM, Manwar JV, Mahajan NM, Sakarkar DM. Design and *in vitro* evaluation of mucoadhesive buccal tablets of terbutaline sulphate. Int J PharmTech Res., 2009; 1(3): 588-597.
8. Dhamankar AK, Manwar JV, Kumbhar DD. The novel formulation design of O/of ketoprofen for improving transdermal absorption. Int J of Pharm Tech Res., 2009; 4(1Suppl): 1449-1457.
9. Manwar J, Kumbhar DD, Bakal RL, Baviskar S, Manmode R. Response surface based co-optimization of release kinetics and mucoadhesive strength for an oral mucoadhesive tablet of cefixime trihydrate. Bulletin of Faculty of Pharmacy, Cairo University, 2016; 54: 227–235.
10. Manwar JV, Patil SS, Patil B, Jadhao RG, Kumbhar DD, Bakal R. Diclofenac Sodium Loaded Nanosized Ethosomes: An Investigation on Z-Average, Polydispersity and Stability. J Pharm Res., 2017; 1(3): 000115.
11. Patil SS, Kumbhar DD, Manwar JV, Jadhao RG, Bakal RL, Wakode S. Ultrasound-Assisted Facile Synthesis of Nanostructured Hybrid Vesicle for the Nasal Delivery of Indomethacin: Response Surface Optimization, Microstructure, and Stability. AAPS PharmSciTech, 2019; 20(3): 97.
12. Nimbalwar MG, Upadhye K, Dixit G. Fabrication and evaluation of ritonavir proniosomal transdermal gel as a vesicular drug delivery system. Pharmacophore, 2016; 7(2): 82-95.
13. Pophalkar PB, Wakade RB, Hole SU, Kadam CY, Suroshe RS. Panchale WA. Development and evaluation of ondansetron medicated jelly. World

Journal of Pharmaceutical Research, 2018; 7(19): 1252-1263.
14. Suroshe RS, Wakade RB, Panchale WA, Sakhare AD, Rathod RR, Pophalkar PB. Development and characterization of osmotic drug delivery system of model drug. World Journal of Pharmaceutical Research, 2018; 7(18): 1158-1171.
15. Kadam CY, Bobade NN, Pophalkar PB, Hole SU, Suroshe RS, Panchale WA. Design and In vitro characterization of phase transition system using rivastigmine tartrate for nasal drug delivery system. World Journal of Pharmaceutical Research, 2018; 8(1): 815-829.
16. Panchale WA, et al. Chromatographic analysis of famotidine, paracetamol and ibuprofen from tablet formulation. Research Journal of Pharmacy and Technology, 2019; 12: 231-263.
17. Panchale WA, et al. Concurrent analysis of ambroxol HCl and salbutamol sulphate from tablet formulation by RP-HPLC. GSC Biological and Pharmaceutical Sciences, 2020; 13(03): 197-202.
18. Sabhadinde AF, et al. Novel RP-HPLC method for simultaneous analysis of chlorthalidone and telmisartan from combined dosage form. Ijppr.Human, 2020; 20(1): 491-502.
19. Panchale WA, et al. RP-HPLC method for simultaneous determination of escitalopram oxalate and flupentixol HCl in tablet dosage form. GSC Biological and Pharmaceutical Sciences, 2021; 14(01): 169-174.
20. Nimbokar SW, et al. Development and validation of RP-HPLC method for determination of zonisamide from tablet formulation. World Journal of Pharmaceutical and Medical Research. 2021;7(2):196-200.
21. Manwar JV, et al. Development of newer RP-HPLC method for simultaneous estimation of cefixime and linezolide in bulk drugs and combined dosage form. International Journal of Pharmacy and Life Sciences, 2021; 12(1): 26-31.
22. Panchale WA, Gulhane CA, Manwar JV, Bakal RL. Simultaneous estimation of salbutamol sulphate and ambroxol HCl from their combined dosage form by UV-Vis spectroscopy using simultaneous equation method. GSC Biological and Pharmaceutical Sciences, 2020; 13(03): 127-134.
23. Panchale WA, Bakal RL. First-order derivative spectrophotometric estimation of gemifloxacin mesylate and ambroxol HCl in tablet dosage form. GSC Biological and Pharmaceutical Sciences, 2021; 14(2): 029-036.
24. Bakal RL, et al. Spectrophotometric estimation of amitriptyline HCL and chlordiazepoxide in tablet dosage form. International Journal of Chemical Sciences, 2007; 5(1): 360–364.
25. Manwar JV, et al. Application of simultaneous equation method for the determination of azithromycin and cefixime trihydrate in tablet formulation. Research Journal of Pharmacy and Technology, 2017; 10(1): 108-112.
26. Manwar JV, et al. Response surface based optimization of system variables for liquid chromatographic analysis of candesartan cilexetil. Journal of Taibah University for Science, 2017; 11: 159–172.
27. Gulhane CA, et al. Liquid chromatographic method for simultaneous estimation of thiocolchicoside and etoricoxib from tablet formulation. Asian Journal of Pharmaceutical Analysis, 2021; 11(3).
28. Manwar J, Mahadik K, Paradkar A, Patil S, Sathiyanarayanan L, Manmode R. Gas chromatography method for the determination of non-ethanol volatile compounds in herbal formulation. International Journal of Analytical and Bioanalytical Chemistry. 2013; 3(1): 12-17.
29. Badukale NA, et al. Phytochemistry, pharmacology and botanical aspects of *Madhuca indica*: A review. Journal of Pharmacognosy and Phytochemistry, 2021; 10(2): 1280-1286.
30. Khadatkar SN, Manwar JV, Sahare AY. Preparations and evaluation of microcapsules of capsaicin. International Journal of Chemical Sciences, 2007; 5(5): 2333-2341.
31. Sahare AY, et al. Hypericum perforatum: A Medicinal plant. Plant Archives, 2007; 7(2): 463-468.
32. Manmode R, Manwar J, Vohra M, Padgilwar S, Bhajipale N. Effect of preparation method on antioxidant activity of ayurvedic formulation kumaryasava. J Homeop Ayurv Med. 2012;1:114. doi:10.4172/2167-1206.1000114.
33. Padgilwar S, et al. Traditional Uses, phytochemistry and pharmacology of *Oroxylum Indicum*: A Review. International Journal of Pharmaceutical and Phytopharmacological Research, 2014; 3 (6): 483-486.
34. Manwar J, et al. Isolation, biochemical and genetic characterizations of alcohol-producing yeasts from the flowers of *Woodfordia fruticosa*. J Young Pharm, 2013; 5(4): 191-194.
35. Wadekar AB, et al. Morphology, phytochemistry and pharmacological aspects of *Carica papaya*, an review. GSC Biological and Pharmaceutical Sciences, 2020; 14(03): 234-248.
36. Khadatkar SN, Manwar JV, Bhajipale NS. *In-vitro* anthelmintic activity of root of *Clitoria ternatea* linn, 2008; 4(13): 148-150.
37. Sahare AY, et al. Antimicrobial activity of *Pseudarthria viscida* roots. Asian Journal of Microbiology Biotechnology & Environmental Sciences, 2008; 10(1): 135-136.
38. Gudalwar BR, et al. Allium sativum, a potential phytopharmacological source of natural medicine for better health. GSC Advanced Research and Reviews, 2021; 06(03): 220–232.
39. Manwar JV, et al. Experimental design approach for chromatographic determination of ketorolac tromethamine from bulk drug and tablet formulation. Global Journal of Pharmacy & Pharmaceutical Sciences, 2017; 3(2): 38-47.

40. Malode GP, et al. Phytochemistry, pharmacology and botanical aspects of *Murraya Koenigii* in the search for molecules with bioactive potential - A review. GSC Advanced Research and Reviews, 2021; 06(03): 143–155.
41. Shubham Garibe, et al. Bioequivalence study of test formulations T1 and T2 Nadolol tablets USP with reference formulation in healthy adult, human subjects under fed conditions. Ijppr.Human, 2021; 20(2): 20-28.
42. Chaudhari KD, et al. Comprehensive review on characterizations and application of gastro-retentive floating drug delivery system. GSC Advanced Research and Reviews, 2021; 07(01): 035-044.
43. Manmode RS, Dhamankar AK, Manwar JV, Laddha SS. Stability indicating HPLC method for simultaneous determination of methocarbamol and nimesulide from tablet matrix. Der Chemica Sinica, 2011; 2(4): 81-85.
44. Padgilwar SS, Manwar SS. Relative influence of adrenergic β-agonist and antagonist on the inflammation and their interaction with aspirin. European Journal of Experimental Biology, 2013; 3(1): 467-472.
45. Vohra MH, Manwar JV, Manmode RS, Padgilwar SS, Patil SV. Bioethanol production: Feedstock and current technologies. Journal of Environmental Chemical Engineering, 2014; 2: 573-584.
46. Bagade SB, Meshram DB, Manwar JV, Tajne MR. Simultaneous high performance thin layer chromatographic estimation of methocarbamol and nimesulide in combined dose tablet. Journal of Pharmaceutical Research, 2006; 5(4): 137-140.
47. Vemula P, Dodda D, Balekari U, Panga S, Veeresham C. Simultaneous determination of linagliptin and metformin by reverse phase-high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms. J Adv Pharm Technol Res., 2015; 6(1): 25-28. doi:10.4103/2231-4040.150368.
48. Borole, T. et al. Simultaneous estimation of metformin hydrochloride and repaglinide by HPLC method, 2012; 49: 54-60.
49. Ahir KB, Patelia EM, Shah A. Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC-Densitometry Method. J Chromat Separation Techniq, 2013; 4: 166. doi:10.4172/2157-7064.1000166.
50. Madhukar A, et al. Simple And Sensitive Analytical Method Development And Validation Of Metformin Hydrochloride By RP-HPLC. Int Journal Pharm Pharm Sci., 2011; 3: 117-120.
51. Lakshmi KS, Rajesh T, Sharma S. simultaneous determination of metformin and pioglitazone by reversed phase HPLC In pharmaceutical dosage forms. International journal of pharmacy and pharmaceutical sciences, 2009; 1: 162-166.
52. Bhamare PC, Bari SB, Natarajan S, Patil AA, Patil SH, et al. development and validation of a precise single stability indicating HPLC method for determinations of metformin hydrochloride and fenofibrate in pure form and in pharmaceutical tablets. International Journal of PharmTech Research, 2011; 3: 505-515.
53. Srivani J, Umamahesh B, Veeresham C. development and validation of stability indicating HPTLC method for simultaneous determination of linagliptin and metformin. International Journal of Pharmacy and Pharmaceutical Sciences, 2016; 8(1): 112-115.
54. Kavitha RR, Arivukkarasu R. Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in a pharmaceutical formulation. World J Pharm Sci., 2014; 2: 317-327.
55. Gajare S, Zalte A, Saudagar RB. Simultaneous Spectrophotometric determination of linagliptin and metformin hydrochloride in combined tablet dosage form. Asian J. Pharm. Ana, 2017; 7(2): 79-83. doi: 10.5958/2231-5675.2017.00013.8.
56. Varaprasad C, Asif M, Karipeddi R. RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form, 2015; 8: 426-432.
57. Eman I. El-Kimary, Dalia A. Hamdy, Sara S. Mourad, Magda A. Barary. HPTLC determination of three gliptins in binary mixtures with metformin. *Journal of Chromatographic Science*, 2016; 54(1): 79-87. https://doi.org/10.1093 /chromsci/bmv106
58. ICH validation of analytical procedures: text and, methodology Q2(R1), 2005.
59. Kirkland JJ, Snyder LR. Practical HPLC Method Development, Wiley Inter Science Publication, New York, 1997.
60. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development, 2nd ed., Wiley-Interscience, New York, 1997.