

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

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Article Received on 17/03/2021

Article Revised on 07/04/2021

Article Accepted on 27/04/2021

**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.<sup>[1-2]</sup> It is official in Indian Pharmacopoeia.<sup>[3]</sup>

Linagliptine (LNG) is also oral antidiabetic drug used in treatment of type 2 diabetes mellitus.<sup>[4-5]</sup> It is also official Indian Pharmacopoeia.<sup>[3]</sup> 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 sensitivity to insulin (See Fig. 1 & 2).<sup>[6]</sup>

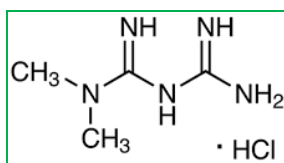


Fig. 1: Structure of metformin hydrochloride.

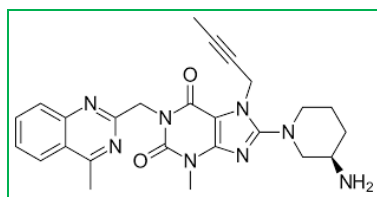


Fig. 2: Structure of linagliptine.

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.<sup>[7-46]</sup>

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.<sup>[47-57]</sup> 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<sup>®</sup>) (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.<sup>[58]</sup>

### 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 ( $\pm 0.1$  ml/min), mobile phase composition ( $\pm 1\%$ ), and change in wavelength ( $\pm 1$  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.<sup>[59-60]</sup>

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					
	Parameter		80%	100%	120%	Range (µg/ml)	R <sup>2</sup>	LOD	LOQ		
Result	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 <sup>#</sup>					
	Parameter	Ret. time	Theor. plates	Tail. factor		FR <sup>#</sup> (ml/min)		MP composition (a: b) <sup>**</sup>		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 ( $\pm 0.1$ ), mobile phase composition ( $\pm 1$ ), and wavelength ( $\pm 1$ ), 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.

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