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ANALYTICAL METHOD VALIDATION OF METFORMIN HCL AND LINAGLIPTIN TABLET BY HPLC

Prakash Neupane, Sunita Arya, Gulbahar*, Pratishma pandey

Gyani Inder Singh Institute of Professional Studies, Dehradun, Uttarakhand, India.

*Corresponding Author

Email Id: prakashneupane0@gmail.com

Abstract:

An accurate, precise, economical, reproducible high-performance liquid Chromatographic method has been developed for the quantitative estimation of Metformin hydrochloride and Linagliptin simultaneously in the tablet dosage form. The instruments consisted of Agilent model G1310B 1260 infinity Iso pump equipped with a UV detector and Agilent model 1260 infinity autosampler equipped with a UV detector. The separation was carried out using Agilent column C18 150 cm 25 cm x 4.6 mm packed with Octadecylsilane bonded to porous silica (5µm) with a flow rate of 1.0 ml/min, column temperature ambient, and UV detector set at 265 and 295 nm. The mobile phase is comprised of phosphate Buffer 0.02M (2.7216 g Potassium Dihydrogen Orthophosphate dissolved in 1000 ml of water and, Acetonitrile in the ratio of 70:30 (v/v). The method was selective for Metformin HCL and Linagliptin with retention times 0.954 min and 2.226 min, respectively. The linearity was established over the 4000-6000 mcg/ml concentration range and 20-30 mcg/ml for Metformin HCL and Linagliptin, respectively. The developed method was validated by ICH harmonized tripartite guidelines: "Validation of analytical procedures: text and methodology Q2(R1)". The statistical result showed that the method was precise, accurate, reproducible and specific for the analysis of Metformin HCL and Linagliptin.

The marketed sample of Metformin HCL and Linagliptin combination immediate release tablet dosage form was analysed by the developed method. The proposed method is suitable for routine quality control analysis of Metformin Hydrochloride and Linagliptin in a Tablet dosage form.

Key Words: CGMP, GLP,HPLC

INTRODUCTION

Background

Analytical Chemistry is define as the Independent, chemical sub-discipline that provides appropriate methods and tools to obtain various information such as the composition and structure of matter, especially concerning type, number, energetic state and geometrical arrangement of atoms and molecules. Analytical chemistry splits into two main categories-qualitative analysis, which tells about the identification of chemical components in the sample, whereas quantitative chemical analysis estimates the amount of certain element or compound in the sample.

1. Qualitative inorganic analysis identifies the presence of the chemical compound in a sample.
2. Qualitative organic analysis identifies the presence of organic compound in a sample.
3. quantitative chemical analysis establishes the amount of a given element or compound in a sample.

The choice of analytical methodology is based on numerous considerations, like chemical properties of the analyte and its concentration, sample matrix, kind of measures i.e., quantitative or qualitative and the number of samples. A qualitative system provides information regarding the chemical identity of the species in the sample.

Analytical system development and validation are nonstop and connected conditioning throughout the medicine development. The way of system development and confirmation depends upon the type of system being developed. The way extensively used are as follows(1)

system development plan description.

High Performance Liquid Chromatography

Chromatography is defined as a biophysical technique for the separation, identification, and purification of the components by dynamic differential migration process in a system consisting of two or more mobile phases, one of which moves continuously in a given direction of stationary phase and the individual substances exhibit different mobilities. When mobile phase used is liquid the type of chromatography is called liquid chromatography.

High performance liquid chromatography (HPLC) is an advanced form of liquid chromatography that uses small particle columns through which the mobile phase is pumped at high pressure. It is basically a highly improved form of column chromatography which provides specific, sensitive and precise methods for analysis of different complicated samples [3,4]. The instrumentation of HPLC is given in Figure 1-1.

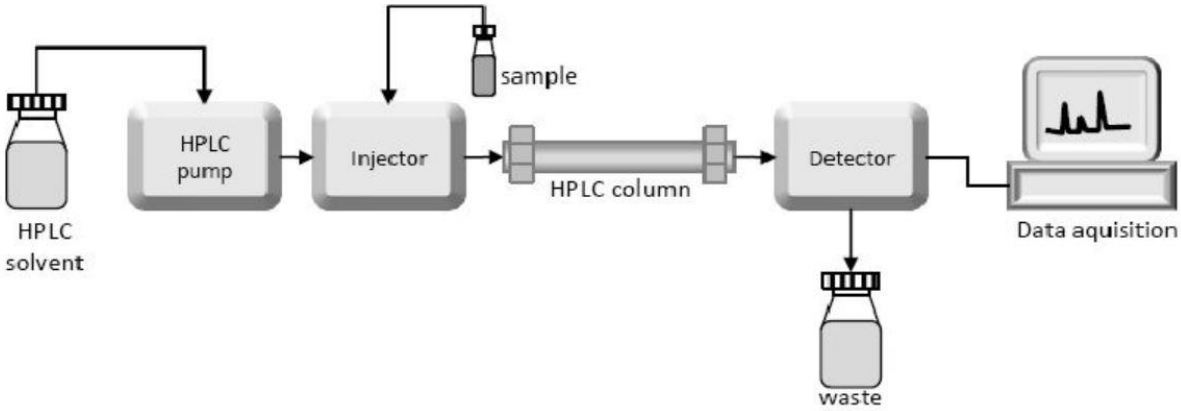

Figure Error! No text of specified style in document.**-1: HPLC instrumentation (source: Laboratoryinfo.com)**

Types of HPLC

A. Normal Phase HPLC :

This method separates analytes on the basis of polarity. It uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether and mixtures of these. Polar samples retain on the polar surface of the column packing longer than less polar materials.[4] The Normal phase chromatography is given in Figure 1-2.

Figure Error! No text of specified style in document.**-2: Normal-Phase Chromatography (sources: https://www.waters.com)**

B. Reverse Phase HPLC:

The stationary phase is non polar (Hydrophobic) in nature ,while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more non polar the materials is, the longer it will be retained.[4] The reverse phase HPLC is given in Figure 1-3.

Figure Error! No text of specified style in document.**-3: Reverse-Phase Chromatography (sources: https://www.waters.com)**

Key Parameters of the Analytical Method Validation

The Various Performance Parameters that should be considered are listed below

i. Accuracy
ii. Precision
    a. Repeatability
    b. Intermediate Precision
iii. Specificity
iv. Detection Limit
v. Quantitation Limit
vi. Linearity
vii. Range

The validation parameters required for testing identification, impurities and assay as per ICH guidelines.

2.1 HPLC Method development and Optimization

For establishing the optimum condition for the assay, different chromatographic components such as mobile phase and wavelength was varied one at a time, keeping the others fixed and observing the effect produced on the peak parameters (height, tailing factor, and theoretical plate), retention time, and resolution of Metformin and Linagliptin.

2.1.1 Selection of mobile phase

Different composition mobile phase were used by keeping all other chromatographic components constant. The buffer used in mobile phase is 2.7216 g Potassium Dihydrogen Orthophosphate dissolved in 1000 ml of water i.e 0.02 M Potassium dihydrogen orthophosphate. The effect of different mobile phase on chromatographic parameters is given in Table 6-1.

Table 2.1: Effect of mobile phase composition on peak parameters

Mobile Phase composition	RT		Theoretical plate		Resolution
	Met	Lina	Met	Lina	

Buffer:ACN (65:35)	**1.141**	**1.768**	**911.1397**	**1336.641**	**3.64687**
Buffer:ACN (75:25)	**1.175**	**4.051**	**2946.157**	**1840.482**	**10.83491**
Buffer:ACN (70:30)	**0.954**	**2.226**	**2755.986**	**2493.137**	**8.90936**

The observation shows the low baseline separation of Metformin HCL and Linagliptin peak in the ratio (Buffer: ACN) 65:35. To overcome this the buffer ratio was increased for the separation of components. At the ratio 75:25 the separation was observed but theoretical plate was less than 2000. Trails were performed and the best combination of Buffer: ACN was found to be in the ratio 70:30 with good resolution of 8.9 and good theoretical plate counts of 2755 for Metformin HCL and 2493 for Linagliptin.

2.1.2 Selection of wavelength

In UV Spectrophotometer Metformin HCL and Linagliptin showed absorption maxima at 265 nm and 295 nm respectively. The cut off value of acetonitrile is 190 nm and phosphate buffer is 210 nm, so the wavelength should be set above 210 nm. The standard was run in Agilent with UV detector and the peak area in different wavelength was observed and analysed. The effect of different wavelength in area is given in Table

Table 2.1: Effect of wavelength on peak area

Wavelength	**RT**		**Peak Area**		**T plate**		**Resolution**
	Met	**Lina**	**Met**	**Lina**	**Met**	**Lina**	
250/280	1.068	2.233	2484.682	331.931	273.412	490.870	14.269
255/285	1.067	2.241	955.417	391.005	253.329	495.8943	14.796
260/290	1.068	2.240	309.466	435.171	243.682	496.798	14.826
265/295	1.067	2.240	99.790	451.917	232.961	497.339	14.480
270/300	1.067	2.240	37.111	428.112	219.641	496.465	14.480

The number of theoretical plate and resolution were more than 2000 and more than 2.5 except on 255 nm where no Linagliptin peak was observed . But the Peak area varies with wavelength. From the above table, 265 and 295 nm was chosen as a maximum wavelength based on peak area, theoretical plate count and resolution.

2.2 Method Validation

Method of validation, ICH guidelines were followed and following parameters were studied:

Linearity

Metformin HCL

The result of Linearity of Metformin HCL is shown in Figure

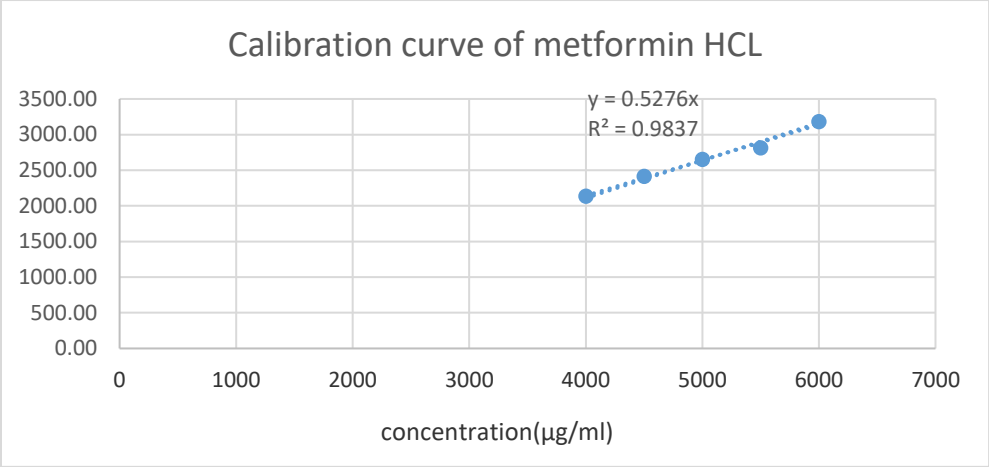

Figure 2-2 : Calibration curve of Metformin HCL

The calibration curve shows that there is linear relationship between Metformin HCL concentration and its corresponding area. The linear equation was found to be y = 57.06x + 783.76 with R^2 value 0.9986. The p-value for the intercept is 0.294 that is greater than 0.05 which indicates the intercept is insignificant.

Linagliptin

The result of linearity of Linagliptin is shown in figure 2-3

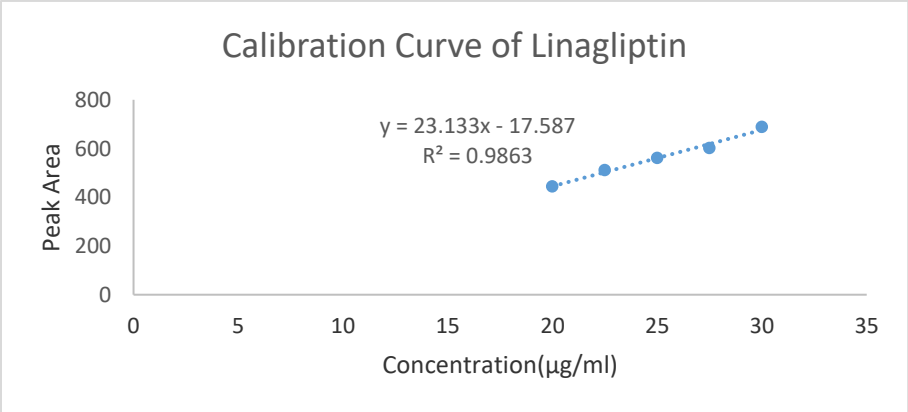

Figure 2-3 : Calibration curve of Linagliptin.

The calibration curve shows the linear relationship between Linagliptin concentration and its corresponding area. The linear equation was found y = 23.133x – 17.587 and R^2 value 0.9863. The p-value of the intercept was 0.228 which is greater than 0.05 It indicates that the intercept is insignificant.

According to the data of calibration curve, the range was found 4000-6000 mcg/ml for Metformin HCL, 20-30 mcg/ml for Linagliptin (80-120%).

3.1.1 System suitability Test

To ensure that the analytical system is working properly each time when it is used, system suitability test was performed. Five replicate injections of standard solutions was carried out. The result of system suitability is given in Table 6.3.

Table 3.1.1: Observed data for system suitability test

Parameters	Limit	Observation	
		Metformin HCL	Linagliptin
Injection Precision	RSD≤ 2%	0.221%	0.320%
Resolution	NLT 2	-	8.953
Tailing factor	NMT 2	1.228	1.409
Theoretical plate	NLT 2000	2847.381	2519.826

The observation from the table shows that the method complies with the system suitability parameters. Therefore system suitability test meets the requirement of method validation.

4.1 Summary and Conclusion

A simple and reproducible reversed-phase high-performance liquid chromatography with UV detection has been developed for simultaneous estimation of Metformin Hydrochloride and Linagliptin for tablet dosage form with lower solvent consumption and greater feasibility. During method development, the initial chromatographic conditions were optimized to obtain an adequate separation of eluted compounds and to meet the required system suitability condition. Different peak parameters such as retention time, theoretical plate, tailing factor, resolution, peak height were observed and the mobile phase, flow rate, solvent mixture, wavelength and column temperature were selected. The simple isocratic elution with Mobile phase: Buffer (2.7216 g Potassium Dihydrogen Orthophosphate and dissolved in 1000 ml of water and Acetonitrile in the ratio of 70:30 (v/v) with 1.0 ml/min flow rate was found to be optimum for the separation of compounds. The optimum wavelength for detection was 265 nm for metformin HCl and 295 for Linagliptin at 25°C. The retention times for Metformin HCL and Linagliptin was 0.992 min and 2.902 min, respectively.The

method was validated according to ICH harmonized tripartite guideline: Validation of analytical procedures: text and methodology Q2 (R1).

System suitability test was also performed to verify the reproducibility of the chromatographic system. In specificity study, the peaks of Metformin HCL and Linagliptin was detected separately from blank values demonstrating that the method is specific for the active ingredient. The method was found to be accurate and precise. The stability studies showed that the solutions of Metformin HCL and Linagliptin was stable for up to 24 hours when stored at 2 to 8°C. The method was found to be linear with R^2 value of 0.9986 and 0.9938 for Metformin HCL and Linagliptin respectively. The method was found to be accurate and precise. Limit of detection and Limit of quantification for Metformin HCL was found to be 1.12 mcg/ml and 3.38 mcg/ml, respectively, and for Linagliptin, it was found to be 1.25 mcg/ml and 3.77 mcg/ml, respectively. Robustness of the method was determined by varying the chromatographic parameters such as pH ,Flow rate and acetonitrile ratio in mobile phase composition. The influence of changes of chromatographic parameters on peak parameters was found to significant in Retention Time, Tailing Factor , number of Theoretical Plate and peak area but all the parameters were found to be within the limit. No significant difference was observed in the developed method and existing Inhouse R&D method.

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