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Research article ***RP-HPLC***

A new simple and specific RP-HPLC method development and validation for the simultaneous determination of linagliptin and metformin in bulk and tablet dosage form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Linagliptin and Metformin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (50:50) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 244nm. The retention time of the Linagliptin and Metformin was 2.0, 4.0±0.02min respectively. The method produce linear responses in the concentration range of 20-100µg/ml of Linagliptin and 40-200µg/ml of Metformin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Linagliptin, Metformin, RP-HPLC, Validation.

INTRODUCTION

Introduction to HPLC

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today.1In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production.2 HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.3

The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants. 4High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. 5 HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. (Figure-1) The technique of HPLC has following features.6

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase

HPLC Method Development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try &

separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.7

Steps involved in Method development are. 6,7

- Understanding the Physicochemical properties of drug molecule.
- Selection of chromatographic conditions.
- Developing the approach of analysis.
- Sample preparation
- Method optimization

Understanding the physicochemical properties of drug molecules

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase. 6 The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.7

Selection of chromatographic conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C18 column with UV detection. A decision on developing either an isocratic or a gradient method should be made at this point.

Selection of Column

A column is of course, the starting and central piece of a chromatograph. A appropriately selected column can produce a good chromatographic separation which provides an accurate and reliable analysis. An improperly used column can often generate confusion, inadequate, and poor separations which can lead to results that are invalid or complex to interpret.9The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Choosing the best column for application requires consideration of stationary phase chemistry, retention capacity, particle size, and column dimensions. The three main components of an HPLC column are the hardware, the matrix, and the stationary phase.

There are several types of matrices for support of the stationary phase, including silica, polymers, alumina, and zirconium. Silica is the most common matrix for HPLC columns. Silica matrices are robust, easily derivatized, manufactured to consistent sphere size, and does not tend to compress under pressure. Silica is chemically stable to most organic solvents and to low pH systems. One short coming of a silica solid support is that it will dissolve above pH 7. In recent years, silica supported columns have been developed for use at high pH. The nature, shape and particle size of the silica support effects separation. Smaller particle results in a greater number of theoretical plates, or increased. The nature of the stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography.

Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds. Commonly used reverse phase columns and their uses are listed below. Propyl (C3), Butyl (C4), and Pentyl (C5) phases are useful for ion-pairing chromatography (C4) and peptides with hydrophobic residues, and other large molecules. C3–C5 columns generally retain non-polar solutes more poorly when compared to C8 or C18 phases. Examples include Zorbax SB-C3, YMC-Pack C4, and Luna C5. These columns are generally less stable to hydrolysis than columns with longer alkyl chains. Octyl (C8, MOS) phases have wide applicability. This phase is less retentive than the C18 phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids.10Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high performance column. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible. The separation selectivity for certain components vary between the columns of different manufacturer as well as between column production batches from the same manufacturer. Column dimensions, silica substrate properties and bonded stationary phase characteristics are the main ones.

MATERIALS AND METHOD

HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector, pH meter- Lab India, Weighing machine- Sartorius, Volumetric flasks-Borosil, Pipettes and Burettes- Borosil, Beakers-Borosil, Digital ultra sonicator- Lab man.

Hplc method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the Linagliptin and o.4ml of the Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Methanol: TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 65:35 v/v respectively.

Optimization of Column

The method was performed with various columns like Symmetry and Phenomenex. Gemini C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used :Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Hypersil C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (50:50v/v)
Flow rate : 1ml/min
Wavelength : 235 nm
Injection volume : 10 µl
Run time : 10 min

Method validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 500 ml (50%) of Water, 500ml of Acetonitrile (50%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (50:50v/v)
Column : Hypersil C18 (4.6×250mm) 5µ
Column temperature : 40ºC
Wavelength : 235nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 8minutes

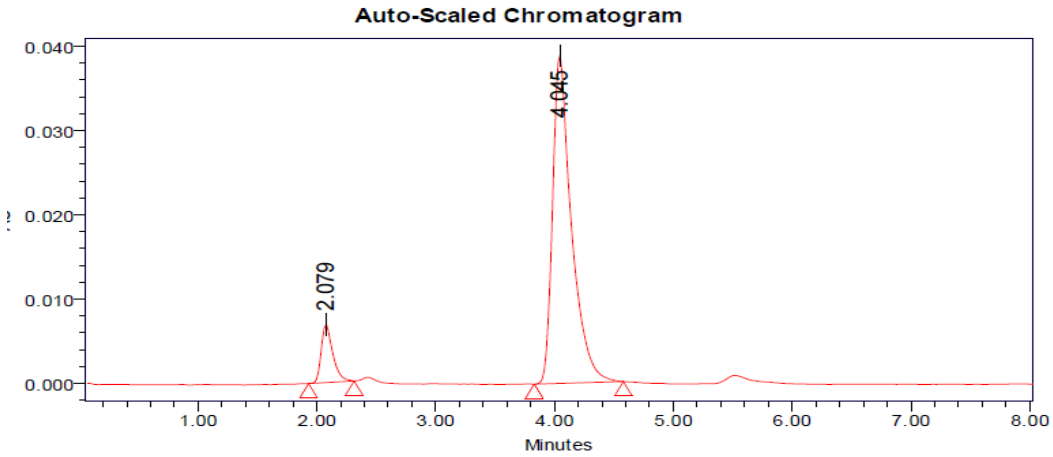

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Linagliptin	2.079	46168	6841	1.33	4251
2	Metformin	4.045	429069	38885	1.59	5224

Optimized Chromatogram (Sample)

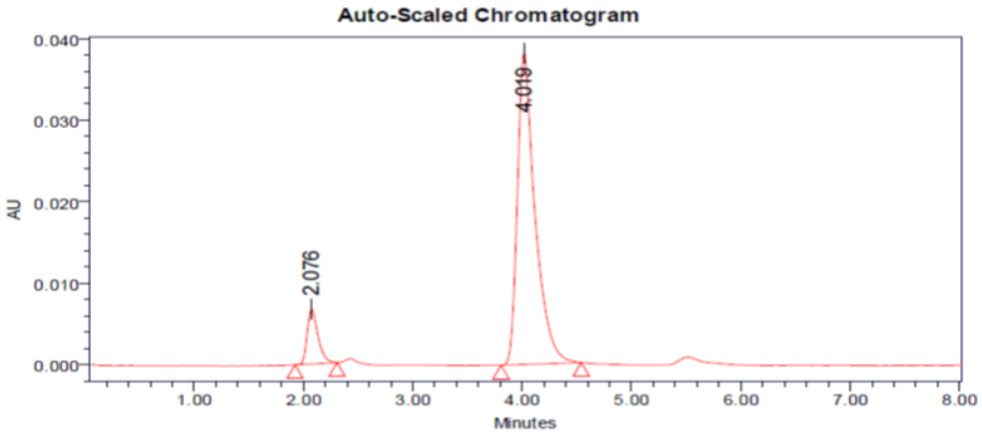

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Linagliptin	2.076	46150	6766	1.36	5152
2	Metformin	4.019	427826	38246	1.58	6071

- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay standard

Table 3: Peak results for assay standard of Linagliptin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Linagliptin	2.078	49569	6811	6945	1.51
2	Linagliptin	2.080	49649	6999	6149	1.57
3	Linagliptin	2.078	49731	6972	6473	1.49
4	Linagliptin	2.079	49479	6971	6190	1.49
5	Linagliptin	2.082	49684	6841	6294	1.49
Mean			49607			
Std. Dev.			107.963			
% RSD			0.217637			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Peak results for assay standard of Metformin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	4.041	423328	44147	7672	1.35
2	Metformin	4.033	423805	44538	7786	1.13
3	Metformin	4.050	423229	44964	5772	1.34
4	Metformin	4.045	423876	44959	5191	1.35
5	Metformin	4.032	423575	38885	5137	1.35
Mean			423559.5			
Std. Dev.			328.2606			
% RSD			0.0775			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay

Table 5: Sample Peak results for Assay sample of Linagliptin

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Linagliptin	2.078	46684	6918	1.34	5217	1
2	Linagliptin	2.079	46168	6841	1.33	5251	2
3	Linagliptin	2.077	46088	6851	1.37	7127	3

Table 6: Peak results for Assay sample of Metformin

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Metformin	4.050	430575	39127	1.60	6197
2	Metformin	4.045	429069	38885	1.59	6224
3	Metformin	4.037	429543	38892	1.58	8203

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Linagliptin and Metformin in pharmaceutical dosage form was found to be 98.2%

Linearity

Chromatographic data for linearity study for linagliptin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	20	15065
66.6	40	31009
100	60	46166
133.3	80	60569
166.6	100	76862

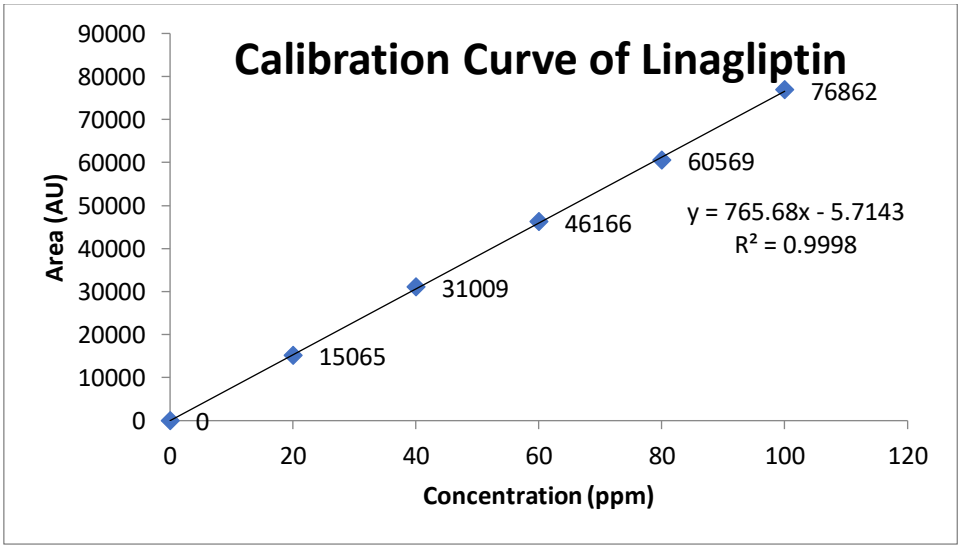

Fig 3: Chromatogram showing linearity level

Chromatographic data for linearity study for Metformin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	40	131289
66.6	80	284775
100	120	427559
133.3	160	555861
166.6	200	712514

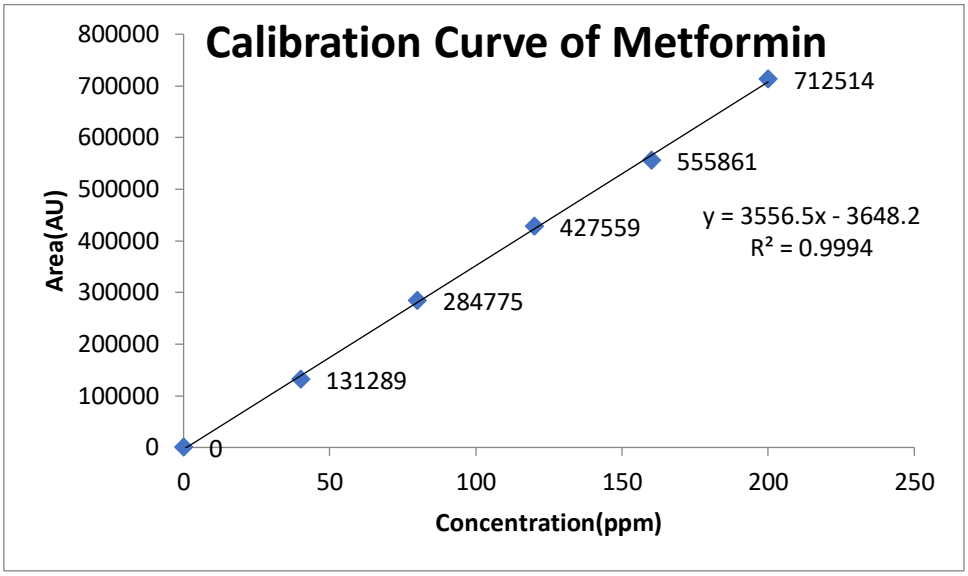

Fig 4: Chromatogram showing linearity level

Repeatability

Table 7: Results of repeatability for Linagliptin

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Linagliptin	2.077	46054	6784	4208	1.32
2	Linagliptin	2.076	46803	6867	6088	1.34
3	Linagliptin	2.076	46150	6766	4152	1.36
4	Linagliptin	2.077	46056	6715	4184	1.32
5	Linagliptin	2.074	46247	6746	4065	1.33
Mean			46262			
Std.dev			312.7099			
%RSD			0.675954			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of repeatability for Metformin

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	4.031	427962	38634	5158	1.57
2	Metformin	4.024	429623	38673	5092	1.58
3	Metformin	4.019	427826	38246	5071	1.58
4	Metformin	4.016	427829	38310	5046	1.58
5	Metformin	4.014	429559	38181	5036	1.58
Mean			428559.8			
Std.dev			943.2246			
%RSD			0.220092			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 9: Results of Intermediate precision day1 for Linagliptin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Linagliptin	2.075	46204	6673	5117	1.33
2	Linagliptin	2.074	46300	6735	5043	1.36
3	Linagliptin	2.075	46259	6652	5087	1.28

4	Linagliptin	2.075	46223	6667	5134	1.31
5	Linagliptin	2.075	46205	6674	5151	1.32
6	Linagliptin	2.074	46189	6703	5157	1.33
Mean			46230			
Std. Dev.			41.88556			
% RSD			0.090603			

%RSD of Six different sample solutions should not more than 2.

Table 10: Results of Intermediate precision day1 for Metformin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metformin	4.013	428922	38004	7038	1.58
2	Metformin	4.011	428524	37935	7999	1.57
3	Metformin	4.010	427239	37850	7003	1.57
4	Metformin	4.008	427667	37780	7982	1.57
5	Metformin	4.006	427826	37824	7983	1.57
6	Metformin	4.006	427093	37970	7042	1.58
Mean			427878.5			
Std. Dev.			718.1952			
% RSD			0.16785			

- *%RSD of Six different sample solutions should not more than*

Table 11: Results of Intermediate precision Day 2 for Linagliptin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Linagliptin	2.076	46803	6867	5149	1.57
2	Linagliptin	2.076	46056	6715	5190	1.13
3	Linagliptin	2.077	46252	6652	6088	1.58
4	Linagliptin	2.075	46205	6674	5184	1.58
5	Linagliptin	2.075	46940	7249	5087	1.57
6	Linagliptin	2.072	46727	6983	5151	1.57
Mean			46497.17			
Std. Dev.			369.4739			
% RSD			0.794616			

%RSD of Six different sample solutions should not more than 2 Table

Table 12: Results of Intermediate precision Day 2 for Metformin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metformin	4.024	429623	38673	6789	1.49
2	Metformin	4.024	427829	38310	5772	1.34
3	Metformin	4.016	427263	37850	5092	1.32
4	Metformin	4.010	427826	37824	6046	1.28
5	Metformin	4.006	421284	40752	6003	1.32
6	Metformin	4.008	421832	40281	6983	1.33
Mean			425942.8			
Std. Dev.			3492.681			
% RSD			0.819988			

- *%RSD of Six different sample solutions should not more than 2.*

Accuracy

Table 13: The accuracy results for Linagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	22938.33	30	29.9655	99.88	100.166
100%	45426	60	59.33511	98.89	
150%	70096.67	90	91.55572	101.7285	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 14: The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	60	59.8	99%	99%
100%	420697.7	120	119.8	99%	
150%	631550.7	180	179.8	99%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness –Linagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	46168	2.079	4251	1.33
Less Flow rate of 0.8mL/min	51177	2.29	5269	1.38
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	42190	1.890	5126	1.32
Less organic phase (about 5 % decrease in organic phase)	42402	1.885	5126	1.19
More organic phase (about 5 % Increase in organic phase)	42112	1.908	5854	1.36

Table 16 : Results for Robustness-Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	4.045	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.450	6328	1.58
More Flow rate of 1.0mL/min	392497	3.660	6217	1.54
Less organic phase (about 5 % decrease in organic phase)	391379	4.251	6996	1.61
More organic phase (about 5 % Increase in organic phase)	391703	3.239	6120	1.50

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Summary

- The analytical method was developed by studying different parameters.
- First of all, maximum absorbance was found to be at 235 nm and the peak purity was excellent.
- Injection volume was selected to be 10µl which gave a good peak area.
- The column used for study was Hypersil C18 (4.6×250mm) 5µ because it was giving good peak.
- 35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.
- Mobile phase is Water and Acetonitrile (50:50) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

- Run time was selected to be 8min because analyze gave peak around 2.0, 4.0 ±0.02min respectively and also to reduce the total run time.
- The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.
- The analytical method was found linearity over the range 20-100µg/ml of Linagliptin and 40-200 µg/ml of Metformin of the target concentration.
- The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Linagliptin and Metformin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Linagliptin was found to be very slightly soluble in water (0.9 mg/mL). Linagliptin is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol (ca. 10 mg/mL), very slightly soluble in isopropanol (<1 mg/mL), and very slightly soluble in acetone. Metformin was found to be freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride, freely-soluble in water, slightly soluble in ethanol, but almost insoluble in acetone, ether, or chloroform. Water and Acetonitrile (50:50) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Linagliptin and Metformin in bulk drug and in Pharmaceutical dosage forms.

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