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Development Of New HPLC Method and Stability Study of Voglibose and Metformin HCl

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Voglibose and Metformin in API form and its Marketed Tablet Dosage Form has been developed and validated as per ICH Guidelines. The separation was achieved by Develosil (250mm x 4.6mm) 5µm Particle size Column and Methanol: Acetate Buffer pH-5.6 (35:65 v/v) used as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 262 nm. Retention time of Voglibose and Metformin were found to be 2.345 min and 3.287 min, respectively. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, limits of detection and quantification for the impurities following the ICH guidelines. Linearity observed for Voglibose (10-30µg/ml) and for Metformin (30-70µg/ml). The percentage recoveries of Metformin and Canagliflozin were 100.06% and 100.373% respectively. The developed method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

KEYWORDS: *Voglibose and Metformin, RP-HPLC, Validation, Accuracy, Precision.*

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these

conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

1.1 DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

INTRODUCTION TO HPLC

HPLC is also called as high-pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important

since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

INSTRUMENTATION OF HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity. [8,24]

Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.

Sample introduction system

Injection ports are of two basic types,

1. The sample is injected directly into the column.
2. The sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000psi).They should also produce minimum band broadening and minimize possible flow disturbances.

Columns

Typical analytical columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 µm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered the best compromise among sample capacity, mobile phase consumption, speed and resolution.

Detectors

Optical detectors are most frequently used. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 mL) flow cell. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 460-600 nm, will be found suitable for the detection of the majority samples.

MATERIALS AND METHODS

Voglibose provided by Sura labs, Metformin provided bySura labs, Water and Methanol for HPLCLICHROSOLV (MERCK)m, Acetonitrile for HPLC Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Voglibose 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 Voglibose and 0.5ml of Metformin from the above stock solutions into a

10ml volumetric flask and dilute up to the mark with diluents.

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, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-5.6 in proportion 35:65 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Develosil (250mm x 4.6mm) 5µm Particle size Column 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: 40ºC
Column: Develosil (250mm x 4.6mm) 5µm Particle size Column
Mobile phase: Methanol: Acetate Buffer pH-5.6 (35:65 v/v)
Flow rate: 1ml/min
Wavelength: 262nm
Injection volume: 20 µl
Run time: 5 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Acetate Buffer (pH-5.6):

Prepare 800mL of distilled water in a suitable container. Add 3.859 g of Sodium Acetate (anhydrous) to the solution. Add 0.176 g of Acetic Acid to the solution. Adjust solution to final desired pH 5.6 using diluted orthophosphoric solution. Add distilled water until volume is 1 L. Filter and sonicate the solution by vaccum filtration and ultra sonication.

Preparation of mobile phase:

Accurately measured 350 ml (35%) of Methanol and 650 ml of Acetate Buffer (65%) a were mixed and degassed in digital ultra sonicater for 15 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: Methanol: Acetate Buffer pH-5.6 (35:65 v/v)
Column: Develosil (250mm x 4.6mm) 5µm Particle size Column
Flow rate: 1 ml/min
Wavelength: 262 nm
Column temp: 40ºC
Injection Volume: 20 µl
Run time: 5 minutes

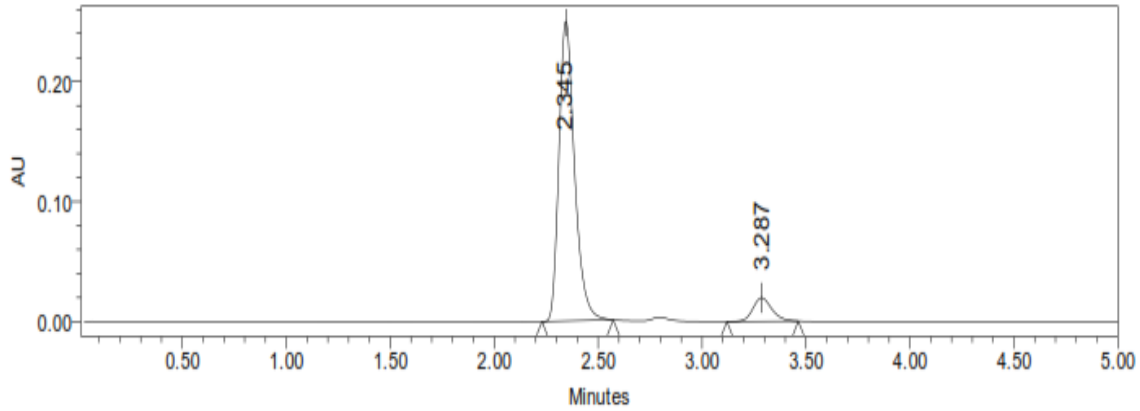

Figure 1: Optimized Chromatogram (Standard)

Table: - peak results for optimized

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Voglibose	2.345	1268547	325854		1.48	6587
2	Metformin	3.287	65842	7583	6.48	1.76	8524

Observation: Fom the above chromatogram it was observed that the Voglibose and Metformin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

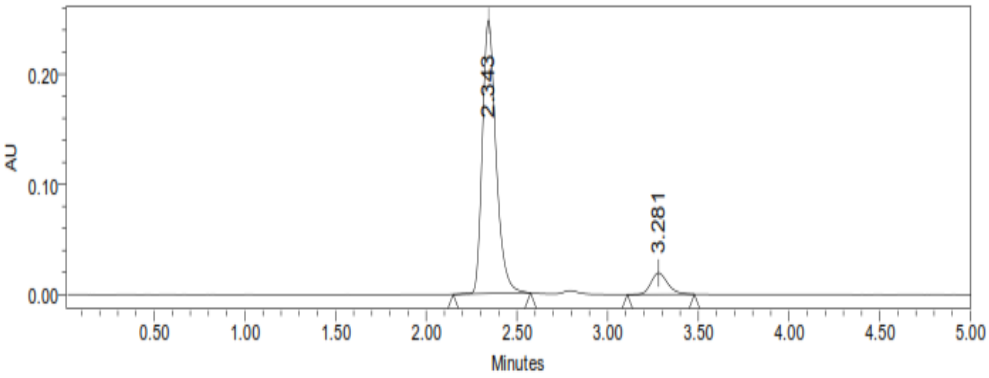

Figure 2: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Voglibose	2.343	1357485	336854		1.49	6685
2	Metformin	3.281	66584	7685	6.49	1.77	8657

Assay (Standard):

Table-: Result of system suitability for Voglibose

S No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Voglibose	2.343	1268598	326586	6598	1.48
2	Voglibose	2.343	1269854	325874	6523	1.49
3	Voglibose	2.342	1268547	326598	6584	1.48
4	Voglibose	2.344	1269856	324857	6597	1.49
5	Voglibose	2.342	1263584s	325864	6542	1.48
Mean			1268088			
Std. Dev			2598.142			
% RSD			0.204887			

Acceptance Criteria:

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

Table: Result of system suitability for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.281	65898	7658	8569	1.76	6.48
2	Metformin	3.285	65974	7596	8574	1.77	6.49
3	Metformin	3.282	65835	7652	8596	1.76	6.48
4	Metformin	3.282	65982	7856	8641	1.76	6.49
5	Metformin	3.282	65368	7729	8499	1.77	6.48
Mean			65811.4				
Std. Dev			255.0506				
% RSD			0.387548				

Acceptance Criteria:

- %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.

Assay (Sample):

Table-: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Voglibose	2.344	1365845	336582		1.50	6685	1
2	Metformin	3.282	66854	7584	6.50	1.78	8569	1
3	Voglibose	2.342	1354282	336895		1.51	6675	2
4	Metformin	3.282	66239	7485	6.49	1.80	8652	2
5	Voglibose	2.342	1325485	335242		1.50	6624	3
6	Metformin	3.282	66875	7534	6.50	1.79	8628	3

%ASSAY =

Sample area Weight of standard Dilution of sample Purity Weight of tablet

_____ × _____ × _____×_____×_____×100

Standard area Dilution of standard Weight of sample 100 Label claim

The % purity of Voglibose and Metformin in pharmaceutical dosage form was found to be 100.14 %.

LINEARITY

Voglibose:

Concentration µg/ml	Average Peak Area
10	652874
15	964575
20	1268589
25	1585745
30	1895472

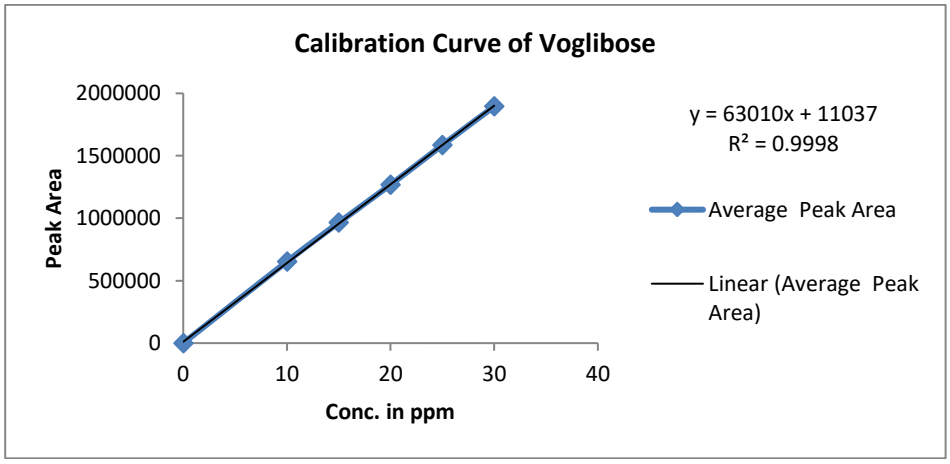

Figure 3: Calibration Grach for Voglibose

Metformin

Concentration µg/ml	Average Peak Area
30	44482
40	58856
50	72584
60	85789
70	99583

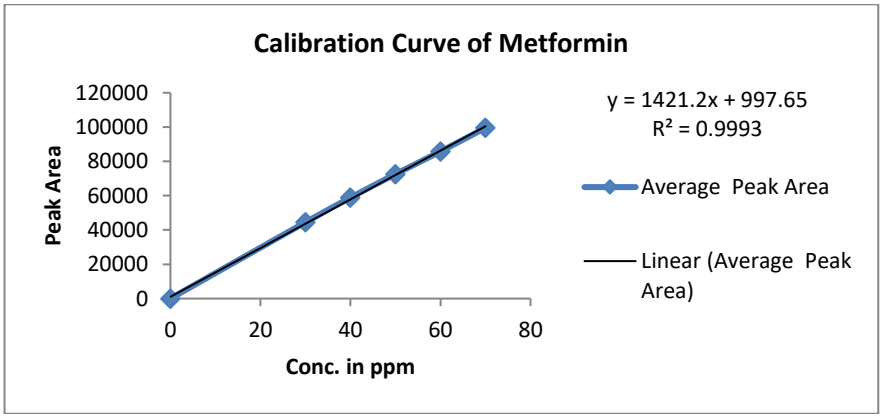

Figure 4: calibration graph for Metformin

REPEATABILITY

Table-: Results of Method Precision for Voglibose:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Voglibose	2.345	1265784	326854	6589	1.48
2	Voglibose	2.344	1269853	326523	6587	1.48
3	Voglibose	2.343	1265875	326547	6593	1.49
4	Voglibose	2.344	1265846	326584	6582	1.49
5	Voglibose	2.345	1269852	326985	6598	1.48
Mean			1267442			
Std. Dev			2200.721			
% RSD			0.173635			

Acceptance criteria:

- %RSD for sample should be NMT 2

Table: Results of method precision for Metformin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.287	65874	7569	8569	1.76	6.49
2	Metformin	3.287	65879	7625	8547	1.77	6.50
3	Metformin	3.288	65982	7598	8596	1.76	6.49
4	Metformin	3.285	65289	7581	8621	1.77	6.50
5	Metformin	3.288	65878	7563	8595	1.76	6.49
Mean			65780.4				
Std. Dev			278.4444				
% RSD			0.423294				

Acceptance Criteria:

- %RSD for sample should be NMT 2.

Intermediate precision:

Table: Results of Intermediate precision for Voglibose

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Voglibose	2.344	1358754	336582	6485	1.47
2	Voglibose	2.343	1365825	335879	6496	1.47
3	Voglibose	2.345	1374582	335682	6478	1.46
4	Voglibose	2.344	1385754	334254	6489	1.47
5	Voglibose	2.342	1369856	336241	6493	1.47
6	Voglibose	2.343	1358542	335898	6524	1.46
Mean			1368886			
Std. Dev			10362.82			
% RSD			0.757026			

Acceptance criteria:

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

Table: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.281	66985	7689	8659	1.77	6.50
2	Metformin	3.281	66258	7692	8647	1.78	6.51
3	Metformin	3.283	66479	7625	8625	1.78	6.50
4	Metformin	3.281	66358	7648	8692	1.77	6.51
5	Metformin	3.278	66259	7693	8675	1.78	6.51
6	Metformin	3.281	66986	7645	8692	1.78	6.50
Mean			66554.17				
Std. Dev			343.8217				
% RSD			0.516604				

Acceptance criteria:

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

Table: Results of Intermediate precision Day 2 for Voglibose

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Voglibose	2.343	1245125	315268	6425	1.46
2	Voglibose	2.343	1236589	316895	6398	1.45
3	Voglibose	2.342	1241542	315872	6487	1.46
4	Voglibose	2.344	1226898	316524	6485	1.45
5	Voglibose	2.343	1236524	316598	6426	1.45
6	Voglibose	2.344	1245853	314857	6489	1.46
Mean			1238755			
Std. Dev			7056.674			
% RSD			0.569658			

Acceptance Criteria:

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

Table: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.281	64859	7458	8453	1.75	6.45
2	Metformin	3.285	64985	7496	8365	1.74	6.46
3	Metformin	3.282	64258	7465	8496	1.75	6.45
4	Metformin	3.286	64598	7425	8357	1.73	6.47
5	Metformin	3.283	64535	7463	8345	1.74	6.45
6	Metformin	3.287	63258	7524	8485	1.75	6.47
Mean			64415.5				
Std. Dev			621.8073				
% RSD			0.965307				

Acceptance Criteria:

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

ACCURACY:

Table: The accuracy results for Voglibose

% Concentration (at specification Lev	Area	Amount Add (ppm)	Amount Foun (ppm)	% Recovery	Mean Recovery
50%	641242	10	10.001	100.01%	100.06%
100%	1269095	20	19.966	99.83%	
150%	1907722	30	30.101	100.336%	

Table: The accuracy results for Metformin

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	36760.67	25	25.167	100.668	100.373%
100%	72369.67	50	50.226	100.452	
150%	107580.3	75	75.005	100.00	

Acceptance Criteria:

- 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

Voglibose:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	1268547	2.345	6587	1.48
Less Flow rate of 0.9 mL/min	1465895	2.911	6452	1.47
More Flow rate of 1.1 mL/min	1234576	2.014	6385	1.46
Less organic phase	1186595	2.361	6458	1.45
More organic phase	1247587	2.038	6268	1.47

Acceptance Criteria:

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

Metformin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	65842	3.287	8524	1.76
Less Flow rate of 0.9 mL/min	72568	4.075	8825	1.75
More Flow rate of 1.1 mL/min	64258	3.089	8667	1.74
Less organic phase	63597	4.422	8765	1.75
More organic phase	62857	3.015	8396	1.73

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

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Voglibose 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.

Voglibose is soluble in the organic solvent DMSO, easily soluble in water, acetic acid, but not easily soluble in methanol, and ethanol, and. almost insoluble in ether. Metformin was found to be freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.

Methanol: Acetate Buffer pH-5.6 (35:65 v/v) 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 Voglibose and Metformin in bulk drug and in pharmaceutical dosage forms.

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