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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND GLIPIZIDE 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 Glipizide and Metformin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Glipizide and Metformin was 2.061, 2.462 ±0.02min respectively. The method produce linear responses in the concentration range of 1-5µg/ml of Glipizide and 100-500µg/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: *Glipizide, Metformin, RP-HPLC, validation.*

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INTRODUCTION:

Analytical chemistry [1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;

- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available – methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.[16]

Table: Analytical techniques and principal applications

Technique	Property measured	Principal areas of application
Gravimetry	Weight of pure analyte or compound of known as stoichiometry	Quantitative for major or minor components
Titrimetry	Volume of standard reagent solution reacting with the analyte	Quantitative for major or minor Component
Atomic molecular spectrometry	Wavelength and intensity of electromagnetic radiation emitted/ absorbed by the analyte	Qualitative, quantitative or structural or for major down to trace level components
Mass spectrometry	Mass of analyte or fragments of it	Qualitative or structural for major down to trace level components isotope ratios
Chromatography and electrophoresis	Various physicochemical properties of separated analytes	Qualitative and quantitative separations of mixtures at major to trace levels
Thermal analysis	Chemical/physical changes in the analyte when heated or cooled	Characterization of single or mixed major/minor compounds
Electrochemical analysis	Electrical properties of the analyte in solution	Qualitative and quantitative for major to trace level components
Radiochemical analysis	Characteristic ionizing nuclear radiation emitted by the analyte	Qualitative and quantitative at major to trace levels

Geological assays The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

MATERIALS AND METHODS:

Glipizide from Sura labs, Metformin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

Hplc method development:
Trails
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Glipizide 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 0.03ml of Glipizide and 3.0ml 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 with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and X-Bridge. Zorbax 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 : Zorbax C18 (4.6×150mm, 5µ)
Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Flow rate : 1ml/min
Wavelength : 255nm
Injection volume : 10 µl
Run time : 8 min

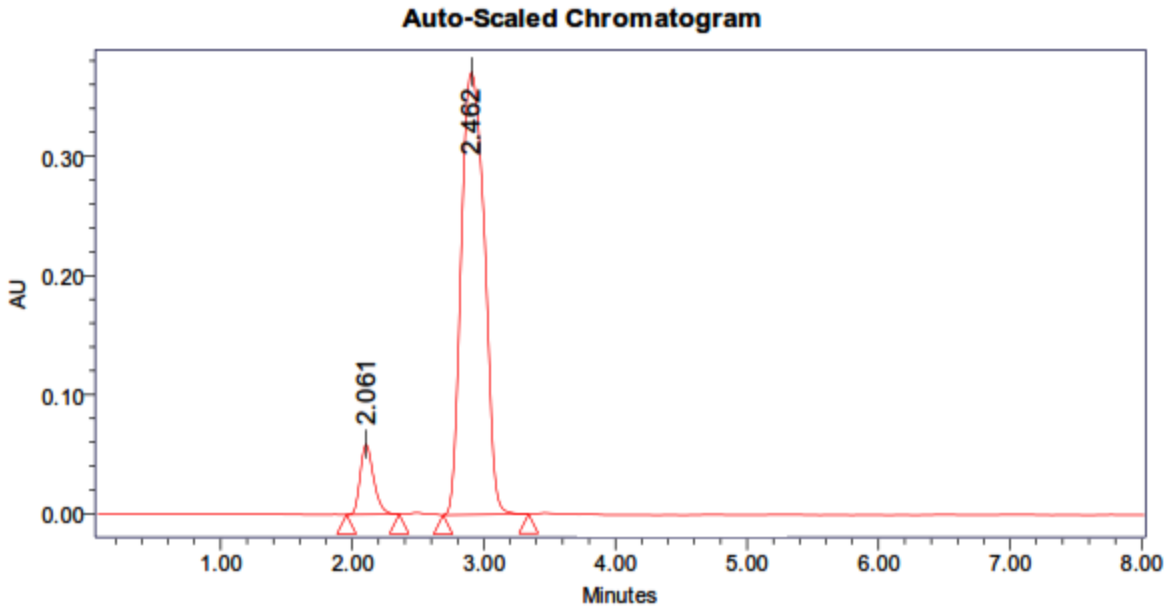
Validation
Preparation of buffer and mobile phase:
Preparation of Phosphate buffer pH 3.9:
Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase:
Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) 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 : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Column : Zorbax C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 255 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 8minutes



Optimized Chromatogram

Table: - peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Glipizide	2.061	247392	58952	1.2	7243
2	Metformin	2.462	3530866	371748	1.1	3389

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

Optimized Chromatogram (Sample)

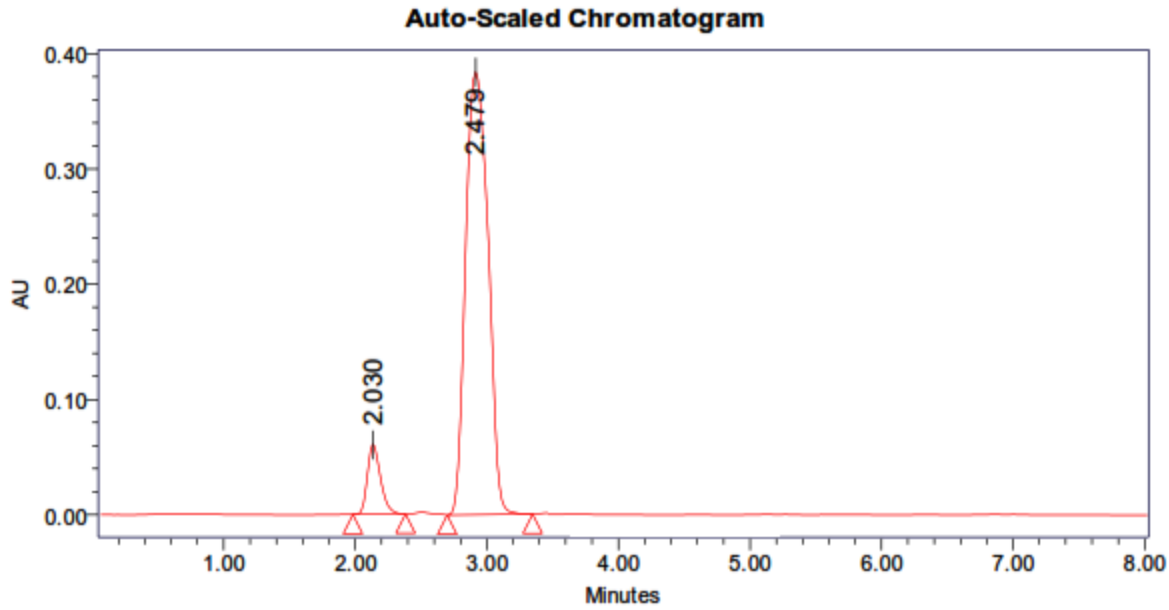


Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Glipizide	2.030	240019	60878	1.2	7246
2	Metformin	2.479	3544380	384304	1.1	3375

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not more 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: Results of system suitability for Glipizide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.048	246713	73455	11318	1.1
2	Glipizide	2.074	245617	78152	7105	1.2
3	Glipizide	2.071	245830	78146	8974	1.2
4	Glipizide	2.069	240552	78242	7087	1.2
5	Glipizide	2.070	245725	77705	5124	1.2
Mean			244887.4			
Std. Dev			2462.26			
% RSD			1.005466			

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 4: Results of system suitability for Metformin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.446	3363754	636862	8484	1.1
2	Metformin	2.490	3326434	641486	7889	1.0
3	Metformin	2.489	3345949	638081	7846	0.9
4	Metformin	2.488	3336621	617725	6772	0.9
5	Metformin	2.490	3355244	631710	6884	0.9
Mean			3345600			
Std. Dev			14753.43			
% RSD			0.44098			

Assay (Sample):

Table: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Glipizide	2.068	244102	89282	1.2	5949
2	Metformin	2.489	3357566	576562	1.0	6866
3	Glipizide	2.070	240052	88021	1.2	5861
4	Metformin	2.491	3371663	576999	1.0	6808
5	Glipizide	2.067	243230	88882	1.2	5879
6	Metformin	2.489	3364001	570315	1.0	6823

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

=3364410/3345600×10/300×300/0.0253×99.6/100×1.2661/500×100

= 100.2%

The % purity of Glipizide and Metformin in pharmaceutical dosage form was found to be 100.2 %.

Linearity

Chromatographic data for linearity study:

Glipizide:

Concentration Level (%)	**Concentration µg/ml**	**Average Peak Area**
33.3	1	88442
66.6	2	165724
100	3	242754
133.3	4	315906
166.6	5	396371

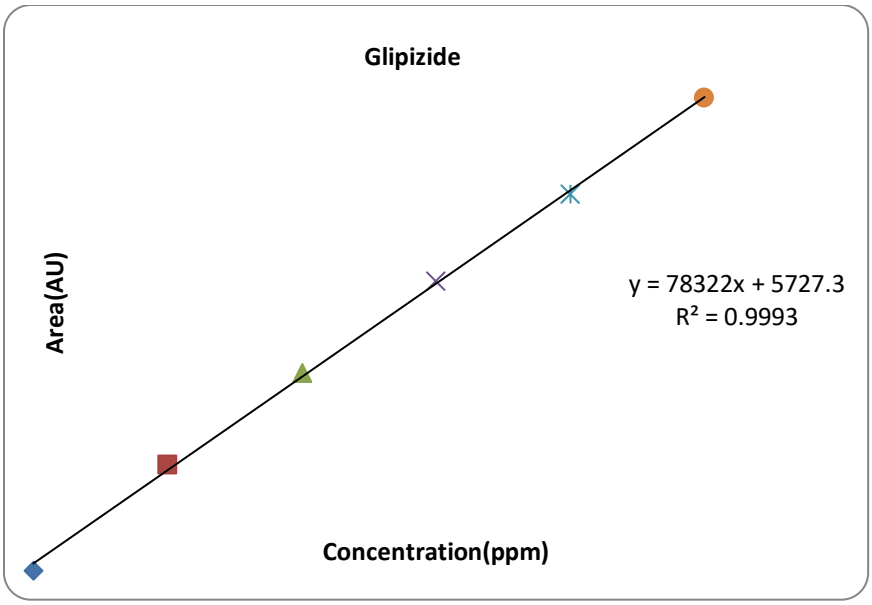


Figure 6.3.4 calibration graph for Glipizide

Table 7: Chromatographic Data for Linearity Study Metformin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	100	1131032
66	200	2345302
100	300	3355282
133	400	4429382
166	500	5623754

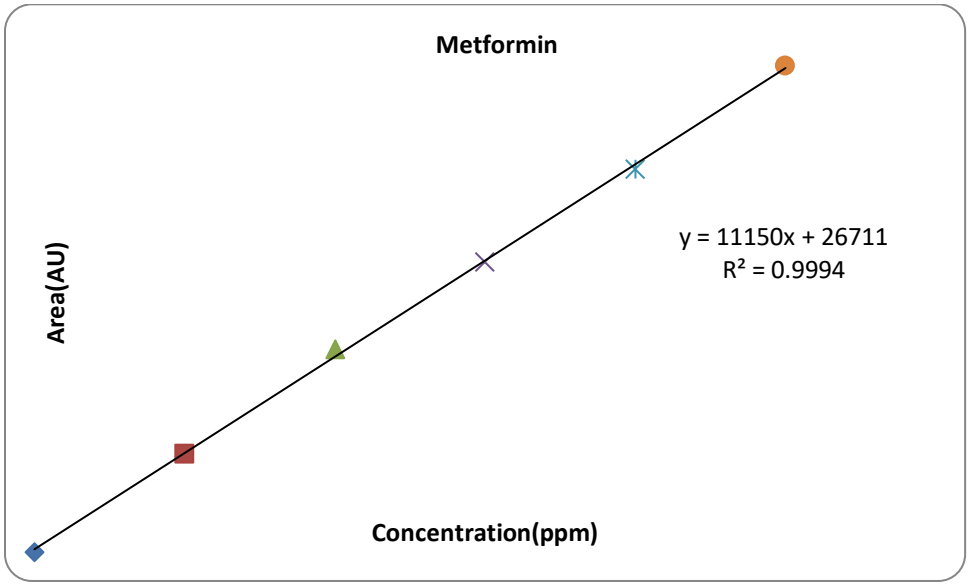


Figure 6.3.4 calibration graph for Metformin

REPEATABILITY

Table: Results of repeatability for Glipizide:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.065	249684	12079	5343	1.0
2	Glipizide	2.064	249696	12068	5473	1.2
3	Glipizide	2.064	246325	11949	5473	1.1
4	Glipizide	2.065	249816	11811	5389	1.1
5	Glipizide	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Acceptance criteria:

- %RSD for sample should be NMT 2

Table: Results of method precession for Metformin:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.486	3233700	59095	6654	1.2
2	Metformin	2.484	3241323	57552	6524	1.3
3	Metformin	2.482	3245927	57213	6440	1.3
4	Metformin	2.483	3245927	57096	6411	1.4
5	Metformin	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Acceptance criteria:

- %RSD for sample should be NMT 2

Intermediate precision:

Table: Results of Intermediate precision Day 1 for Glipizide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.066	242721	11323	5272	1.21
2	Glipizide	2.066	240155	11564	5168	1.16
3	Glipizide	2.066	240945	11887	5310	1.14
4	Glipizide	2.065	240385	11938	5275	1.19
5	Glipizide	2.069	249920	11652	5078	1.10
6	Glipizide	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

Acceptance criteria:

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

Table: Results of Intermediate precision Day 1for Metformin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.477	3325309	54143	6149	1.25
2	Metformin	2.478	3323780	53740	6127	1.21
3	Metformin	2.483	3328190	54791	6607	1.28
4	Metformin	2.486	3329035	55098	6769	1.28
5	Metformin	2.489	3325968	52379	6709	1.30
6	Metformin	2.483	3327725	54779	6756	1.36
Mean			3326668			
Std. Dev			1985.641			
% RSD			0.059689			

Acceptance criteria:

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

Table: Results of Intermediate precision Day 2 for Glipizide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.067	249499	11594	5240	1.2
2	Glipizide	2.069	240991	11357	5130	1.2
3	Glipizide	2.068	240431	11878	5136	1.2
4	Glipizide	2.069	241330	11748	5267	1.2
5	Glipizide	2.067	240519	11830	5222	1.2
6	Glipizide	2.067	240470	11475	5982	1.2
Mean			242206.7			
Std. Dev			3590.034			
% RSD			1.48222			

Acceptance criteria:

- %RSD of six 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
1	Metformin	2.485	3426979	53353	6700	1.3
2	Metformin	2.484	3446641	54454	6563	1.3
3	Metformin	2.496	3430606	53532	6855	1.3
4	Metformin	2.484	3430952	55157	6864	1.3
5	Metformin	2.490	3431676	56223	6942	1.3
6	Metformin	2.490	3429187	58578	6644	1.3

Mean			3433812			
Std. Dev			7041.409			
% RSD			0.205061			

Acceptance criteria:

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

Accuracy:

The accuracy results for Glipizide

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

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

Table: results for robustness
Glipizide:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

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	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

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 Glipizide 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.

Glipizide and Metformin was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: Phosphate Buffer pH 3.9 (55:45v/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 Glipizide and Metformin in bulk drug and in pharmaceutical dosage forms.

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