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SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND METFORMIN IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Chromatography is a versatile laboratory technique that separates mixtures by exploiting differences in molecular interactions with a stationary phase while dissolved in a mobile phase. It serves both preparative and analytical purposes, with methods like column, ion-exchange, gel-permeation and more, each chosen based on specific separation needs. High-Pressure Liquid Chromatography (HPLC) is a powerful analytical tool utilizing pumps to push a sample mixture through a column packed with an adsorbent material. Components separate based on their interaction with the stationary phase. RP-HPLC, prominent in pharmaceuticals, employs a non-polar stationary phase and a moderately polar mobile phase, separating molecules via hydrophobic interactions. Retention time correlates with a molecule's hydrophobic surface area.

KEYWORDS

Chromatography, HPLC and RP-HPLC.

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INTRODUCTON

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid (gas or solvent) called the mobile phase, which car- ries it through a system (a column, a capillary tube, a plate, or a sheet) on which a material called the stationary phase is fixed. The different constituents of the mix- ture have different affinities for the stationary phase. The different molecules stay longer or shorter on the stationary phase, depending on their interactions with its surface sites. So, they travel at different apparent velocities in the mobile fluid, causing them to separate. The separation is based on the differential

partitioning between the mobile and the stationary phases. Subtle differences in a com- pound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Types of chromatography

Column chromatography
Ion-exchange chromatography
Gel-permeation (molecular sieve) chromatography
Affinity chromatography
Paper chromatography
Thin-layer chromatography
Gas chromatography
Dye-ligand chromatography
Hydrophobic interaction chromatography
Pseudo affinity chromatography
High-pressure liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

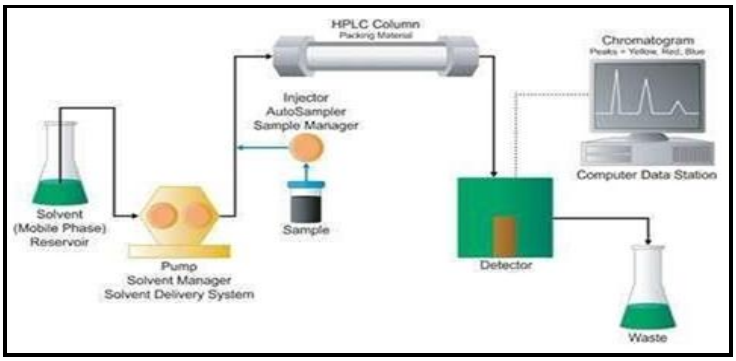


Figure No.1: High-performance liquid chromatography (HPLC)

Types of HPLC

Partition chromatography
Normal–phase chromatography
Displacement chromatography
Reversed-phase chromatography (RPC)
Size-exclusion chromatography
Ion-exchange chromatography
Bioaffinity chromatography
Aqueous normal-phase chromatography

Reversed phase HPLC (RP-HPLC)

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe2SiCl, where R is a straight chain alkyl group such as C18H37 or C8H17. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release.

Adsorption chromatography or Normal phase chromatography

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents.

The silica structure is saturated with silanol groups at the end. These OH group are statistically distributed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase.

These situations arise when the molecules hass one or several atoms with lone pair electron or a double bond. The adsorption strengths and hence K' value (elution values) increase in the following order. Saturated hydrocarbons < olefins < aromatics < organic halogen compounds < sulphides < ethers < esters < aldehydes and ketones < amines < sulphones < amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors.

SYSTEM COMPONENTS

Solvent delivery system

The mobile phases is pumped under pressure from one or more sever- al reservoir and flows through the column at a constant rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components.

Solvent degassing system

The constituents of the mobile phase should be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase.

Gradient elution devices

HPLC columns may be run isocratically, i.e., with constant eluent or they may be run in the gradient is a means in which the mobile phase composition varies during run. Gradient elution is a means of overcoming the problem of dealing with a complex mixture of solutes.

Column-packing materials

The heart of the system is the column. In order to achieve high efficiency of separation, the column material (micro-particles, 5-10µm size) packed in such a way that highest numbers of theoretical plates are possible.

Derivitization

In HPLC derivitazation is used to enhance the sensitivity and selectivity of detection when available detectors are not satisfactory for the underivatized compounds.

Gradient elution

Gradient elution or solvent programming is the change of solvent composition during a separation in which the solvent strength increases from the beginning to the end of the separation.

Method optimization

In the early days of high performance liquid chromatography (HPLC), the selection of column formats (particle size, type, and column diameters) was rather limited and thus, optimization often was done by adjusting operational variables such as eluent velocity, column temperature, and operating pressure.

METHOD VALIDATION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters:

Linearity

The linearity of an analytical method is its capability to elicit check consequences which might be at once, or with the aid of well described mathematical adjustments, proportional to the concentration of analytes in within a given range.

Precision

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.

Precision is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot. From the measured standard deviation (SD) and Mean values, precision as relative standard deviation (% rsd) is calculated.

%RSD or CV=SD/Mean×100%

The acceptable percent of relative standard deviation results for precision may be based on the Horwitz equation, an exponential relationship between the among- laboratory relative standard deviation (RSDR) and Concentration (C)

%RSDR=2(1-0.5logC)

Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Accuracy is measured by spiking the sample matrix of interest with a known concentration of analyte standard and analyzing the sample using the "method being validated." The procedure and calculation for Accuracy (as% recovery) will be varied from matrix to matrix and it will be given in respective study plan or amendment to the study plan.

Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as concentration, which gives rise to an instrument signal that is significantly different from the blank

LOD = 3 Sa/b

Limit of quantification (LOQ)

The LOQ is the concentration that can be quantitative reliably with a specified level of accuracy and precision. The LOQ represents the concentration of analyte that would yield a signal-to-noise ratio of 10.

LOQ = 10 Sa/b

Where, Sa- the estimate is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs. b – is slope of the corresponding calibration curve.

Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc., Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small changes in parameters such as pH of the mobile phase, temperatures, %organic solvent strength and buffer concentration etc., to determine the robustness of the method experimental conditions were purposely altered and chromatographic characters were evaluated.

System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

AIM

To estimate Metformin hydrochloride and Sitagliptin in tablet dosage forms by RP- HPLC method.

To validate the method according to ICH guidelines.

OBJECTIVES

Selection of the solvent to be used as mobile phase:

Choosing the suitable solvent in which the drug is soluble and stable.

They must be easily available, economical and of the HPLC grade.

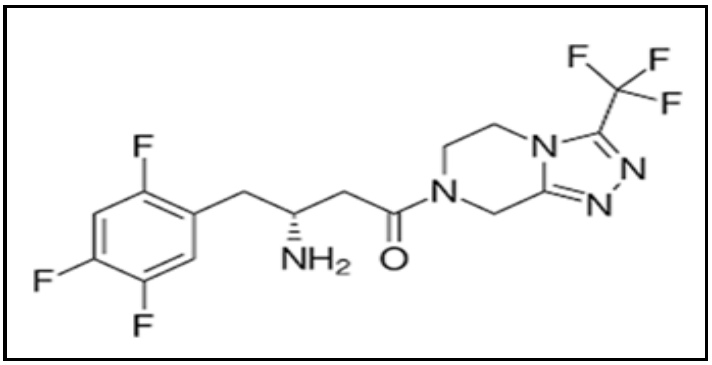
Selection of mobile phase

In order to select the wavelength to carry out the analysis, critical examination of the ultraviolet absorbance spectra of the drug should be done

A perfect study of the structure of drug and its physicochemical properties; to select the chromatographic parameters.

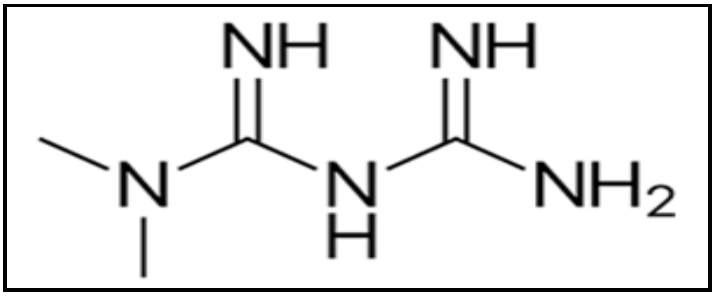
Selection of method for quantitative chromatographic analysis.
To validate the developed method as per ICH guidelines.

DRUG PROFILE
SITAGLIPTIN
Synonym: Sitagliptin phosphate
Chemical Structure



CHEMICAL STRUCTURE OF SITAGLIPTIN
IUPAC
(3R)-3-amino-1-[3-(trifluoromethyl)-6, 8-dihydro-5H-[1, 2, 4]triazolo[4, 3-a]pyrazin-7-yl]-4-(2, 4, 5-trifluorophenyl)butan-1-one; phosphoric acid
Molecular formula: C16H18F6N5O5P
Molecular weight: 505.31
Physico-chemical Properties Appearance: white to off-powder
Solubility: soluble in water and N, N-dimethyl formamide
Storage temp: 25°C
Melting Point: 216-219°C
pKa: strongest basic -8.78

METFORMIN
Synonym: Glucophage Chemical Structure
Chemical structure of metformin



IUPAC: 3-(diaminomethylidene)-1, 1-dimethylguanidine Molecular formula: C4H11N5
Molecular weight: 129.16
Physico Chemical Properties
Appearance: white to off-white crystalline powder
Solubility: soluble in DMSO and water
Melting Point: 223-226°C
Boiling point: 224.1ºC
pKa: Strongest Basic - 12.4

MATERIAL AND METHODS
Instruments: HPLC system with UV Detector, Sonicator
APPARATUS: Mortar and pestle,
250ml and 50ml volumetric flasks,
100ml beakers,
5ml pipettes.
Chemicals
The following chemicals are routinely used:
Acetonitrile, AR Grade
Potassium dihydrogen ortho phosphate, AR Grade
Orthophosphoric acid, HPLC Grade
Water, HPLC Grade
Metformin Hydrochloride Working Reference standard
Sitagliptin Phosphate Working Reference standard
Raw Materials
Sitagliptin Phosphate and Metformin Hydrochloride working reference standard.
Method development for HPLC
The objective of this experiment was to optimize the assay method for simultaneous estimation of Sitagliptin Phosphate and Metformin Hydrochloride on the literature survey made.so here the trails mentioned describes how to optimization was done.
Trail
Mobile Phase: Buffer and Methanol in the ratio of 80:20 V/V.
Buffer: 2.72g of potassium dihydrogen phosphate in 1000ml of water PH 3.5
Preparation of Standard Solution
Weigh accurately 25.0mg of Metformin Hydrochloride WS into a 50mL volumetric flask, dissolve in 10mL mobile phase, add 5 mL of the Sitagliptin stock solution and make up the volume with mobile phase.

Chromatographic Conditions

Flow rate: 0.8ml/min

Column: Nucleodur C18 250 × 4.6 mm Detector wavelength : 267nm

Column temp: Ambient

Injection volume: 20µl

Retention time: 2.64 min for MET and 10.34min for Sitagliptin

Observation: Sitagliptin got peak fronting and base line between 2 peaks is not straight. The trial 3 chromatogram result was shown in Figure No.2.

OPTIMIZED METHOD

Mobile Phase: Buffer: Acetonitrile (70:30)

Buffer: 2.72g of potassium dihydrogen phosphate in 1000ml of water PH 3.5

Preparation of Standard solution

Weigh accurately 25.0mg of Metformin Hydrochloride WS into a 50mL volumetric flask, dissolve in 10mL mobile phase, add 5mL of the Sitagliptin stock solution and make up the volume with mobile phase.

Preparation of Sample solution

Powder 20 tablets and weigh accurately 700.0mg of the sample into a 250mL volumetric flask, add 50mL water and sonicate for 30 minutes and make up the volume with water. Pipetteout 5 mL of the above solution into a 20mL volumetric flask and dilute to volume with mobilephase. Filter through 0.45µm membrane filter discarding first 5ml.

METHOD VALIDATION

Procedure

Inject 20µL of the standard preparation in 6 replicates and check the system suitability. If system suitability is found satisfactory, proceed with the injection of sample preparations.

The order of elution will be as follows

Metformin Hydrochloride

Sitagliptin phosphate monohydrate

System suitability

A Standard solution was prepared by using Metformin Hydrochloride and Sitagliptin working standards as per test method and was injected five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Metformin Hydrochloride and Sitagliptin phosphate retention times and peak areas.

Acceptance criteria

The % RSD for the peak area responses of principal peak from 6 replicate injections of each standard Solution should be not more than 2.0%. The Tailing factor (T) for the peaks is NMT 2.0%

Observation

The %RSD for retention times and peak areas were found to be within the limit.

Accuracy (recovery)

Acceptance Criteria

A study of Accuracy was conducted. Drug Assay was performed as per test method with equivalent amount of Metformin Hydrochloride and Sitagliptin. The recovery at various levels should between 98.0% and 102.0%. The average % recovery of Metformin Hydrochloride and Sitagliptin were calculated.

Acceptance criteria

The % relative standard deviation of Metformin Hydrochloride and Sitagliptin of the prepared sample solution should not be more than 2.0%.

Observation

$$\%\text{Recovery} = \frac{\text{Obtained value}}{\text{Amount added}} \times 100$$

Accuracy was assessed using the above determinations of recoveries. The details are tabulated. Refer Table No.6 and Table No.7.

PRECISION

Method Precision

Prepared six sample preparations individually using single as per test method and injected each solution.

Intermediate Precision: Analyst, instrument and day variability

Separately inject standard solution in 6 replicates and the sample solutions and record the peak area for major peaks. Calculate the content of Metformin

Hydrochloride and Sitagliptin phosphate per tablet in all the six preparations.

ACCEPTANCE CRITERIA

The % RSD for the six assay determinations shall be NMT 2.0%.

The overall % RSD for the two sets (Intermediate Precision and Precision) is NMT 2.0%.

OBSERVATION

Test results are showing that the test method is precise. Refer Table No.9.

SPECIFICITY

A placebo was prepared which contained all the ingredients except Metformin Hydrochloride and Sitagliptin in the same proportion as present in the formulation. A sample solution from this preparation was injected into the system.

LINEARITY OF TEST METHOD

Concentrations ranging from 80% to 120% of operating concentration were prepared and injected into the HPLC system and the areas were noted.

Acceptance criteria

Correlation Coefficient should be not less than 0.995.

% of y- Intercept should not be more than 2.0%.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation

The linear fit of the system was illustrated graphically. The result are presented. Refer Table No.3.

ROBUSTNESS

System suitability and assay values were checked. Stability of the sample solution was also checked. A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rate 1 ml/min. The system suitability parameters were evaluated and found to be within the limits for 1 ml/min flow. Metformin Hydrochloride and Sitagliptin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates

0.1 ml/min.

Acceptance criteria

The % Relative standards areas of both the peaks should not more than 2.0%. Tailing factor of losartan peaks is not more than 2.5.

Observation

The tailing factor for Metformin Hydrochloride and Sitagliptin was found to be within the limits. As shown in Table No.12.

RESULTS AND DISCUSSION

Method development

Inference

Sitagliptin got peak fronting and base line between 2 peaks is not straight.

Validation data

Specificity

Optimized chromatographic conditions

Table No.1: Optimized chromatographic conditions

S.No	Parameters	Method
1	Stationary phase (column)	Nucleodur – C18
2	Mobile Phase	Buffer: Acetonitrile (70:30)
3	Flow rate (ml/min)	0.8ml/min
4	Column temperature (°C)	Ambient
5	Volume of injection loop (µl)	20µL
6	Detection wavelength (nm)	267nm

Table No.2: Optimized method

S.No	Name of the peak	Retention time (miniutes)
1	Metformin hydrochloride	2.64
2	Sitagliptin phosphate	10.34

Table No.3: Data for specificity

S.No	Injection No	Response of the peak with Retention time	Influence of placebo
1	Blank	No peaks observed	-
2	Placebo	No peaks observed	No influence
3	Standard solution	**Metformin Hydrochloride** Area=166253, Retention time= 2.751 **Sitagliptin phosphate** Area=328540, Retention time= 7.150	-
4	Test solution	Two peaks observed at 2.751 minutes with an area 161540 and at 7.156 minutes with an area 323532 which corresponds to Metformin Hydrochloride and Sitagliptin Phosphate respectively	No influence due to placebo

Linearity and Range

Table No.4: Data for linearity

S.No	Sample ID	Metformin Hydrochloride		Sitagliptin	
		Concentration, in mcg/mL	Area	Concentration, in mcg/mL	Area
1	50% of operating concentration	248.80	81616	24.56	157751
2	80% of operating concentration	398.08	133723	39.29	256940
3	100% of operating concentration	497.59*	160489	49.12*	316497
4	120% of operating concentration	597.11	187209	58.94	375469
5	150% of operating concentration	746.39	243897	73.68	472672
6	Correlation coefficient (r >0.995)	0.9957		0.9996	
7	y- intercept (NMT±2.0%)	+1.90%		+1.11%	

Range

Table No.5: Data for range

S.No	Metformin Hydrochloride			Sitagliptin phosphate	
		50% Range	150% Range	50% Range	150% Range
1	Average	89396	229392	156988	471763
2	RSD (NMT 2.0%)	0.24%	0.11%	0.24%	0.15%

Accuracy

Metformin Hydrochloride

Table No.6: Data for accuracy of metformin hydrochloride

S.No	Sample ID		Amount added (mg)	Amount recovered (mg)	Recovery (98.0% to 102.0%)	Mean and RSD
1	50	A1	25.5295	25.5365	100.03%	Mean = 99.11% RSD (NMT 2.0%) =0.86. %
		A2	25.9316	25.6611	98.96%	
		A3	26.0321	25.6016	98.35%	
2	100	A1	46.9382	46.9217	99.96%	Mean = 99.64% RSD (NMT 2.0%) =0.34%
		A2	47.1392	46.9812	99.66%	
		A3	47.2397	46.9019	99.29%	
3	150	A1	46.9382	67.4332	98.52%	Mean = 98.58% RSD (NMT 2.0%) =0.07%
		A2	47.1392	67.6234	98.65%	
		A3	47.2397	67.3777	98.58%	

Sitagliptin

Table No.7: Data for accuracy of sitagliptin

S.No	Sample ID		Amount added (mg)	Amount recovered (mg)	Recovery (98% to 102%)	Mean and RSD
1	50	A1	24.7078	24.3680	98.62%	Mean = 98.74% RSD (NMT 2.0%) = 0.30%
		A2	24.7541	24.3850	98.51%	
		A3	24.6152	24.3867	99.07%	
2	100	A1	49.4156	49.1620	99.49%	Mean = 99.68% RSD (NMT 2.0%) = 0.23%
		A2	49.5082	49.3128	99.61%	
		A3	49.2303	49.1980	99.93%	
3	150	A1	74.1234	73.9913	99.82%	Mean = 99.88% RSD (NMT 2.0%) = 0.14%
		A2	74.2623	74.0904	99.77%	
		A3	73.8455	73.8762	100.04%	

Precision

System Precision

Table No.8: Data for system precission

SET	Metformin Hydrochloride		Sitagliptin phosphate		
	(RSD NMT 2.0%)	Number of theoretical plates NLT 1500	(RSD NMT 2.0%)	Number of theoretical plates NLT 1500	Resolution NLT4.0
1	0.19%	3003	0.13%	8127	17.20
2	0.28%	3026	0.11%	7869	17.13
3	0.16%	3013	0.17%	7703	17.24
4	0.13%	3019	0.12%	7741	17.46
5	0.10%	3446	0.06%	8679	19.27
6	0.14%	3411	0.44%	8384	19.12

Method Precision

Table No.9: Data for method precision

S.No	Metformin Hydrochloride		Sitagliptin
	Sample ID	Content (% of Label claim)	Content (% of Label claim)
1	Sample-1	494.46mg (98.89%)	50.53mg (101.06%)
2	Sample-2	500.10mg (100.02%)	50.34mg (100.68%)
3	Sample-3	491.13mg (98.23%)	50.18mg (100.36%)
4	Sample-4	499.23mg (99.85%)	50.30mg (100.60%)
5	Sample-5	498.11mg (99.62%)	50.37mg (100.74%)
6	Sample-6	497.43mg (99.49%)	49.36mg (98.72%)
7	Mean	496.74mg (99.35%)	50.18mg (100.36%)
8	RSD (NMT 2.0%)	0.68%	0.83%

Intermediate Precision

Metformin hydrochloride

Table No.10: data for intermediate precision of metformin hydrochloride

S.No	Intermediate Precision		Precision	
	Content /tablet	% of label claim	Content/tablet	% of label claim
1	493.29mg	98.66%	494.46mg	98.89%
2	492.26mg	98.45%	500.10mg	100.02%
3	499.48mg	99.90%	491.13mg	98.23%
4	496.85mg	99.37%	499.23mg	99.85%
5	498.18mg	99.64%	498.11mg	99.62%
6	491.59mg	98.32%	497.43mg	99.49%
Average	495.28mg	99.06%	496.74mg	99.35%
RSD (NMT 2.0%)	0.68%	0.68%%	0.68%	0.68%

Overall RSD =0.93%

Sitagliptin

Table No.11: Data for intermediate precision of sitagliptin

S.No	Intermediate Precision		Precision	
	Content /tablet	% of label claim	Content /tablet	% of label claim
1	50.09mg	100.18%	50.53mg	101.06%
2	49.82mg	99.64%	50.34mg	100.68%
3	50.36mg	100.72%	50.18mg	100.36%
4	50 .36mg	100.72%	50.30mg	100.60%
5	50.04mg	100.08%	50.37mg	100.74%
6	50.22mg	100.44%	49.36mg	98.72%
Average	381.31mg	101.68%	50.18mg	100.36%
RSD (NMT 2.0%)	1.10%	1.105%	0.83%	0.83%

Overall RSD = 0.97%

Robustness

Table No.12: Robustness for metformin hcl and sitagliptin

S.No	Sitagliptin Phosphate				Sitagliptin Phosphate		Resolution NLT 4.0
	Parameters	Variation	RSD NMT 2.0%	Theoretical plate NLT 1500	RSD NMT 2.0%	Theoretical plate NLT 1500	
1	Change in wavelength	265 nm	0.37%	4056 - 4221	0.26%	8740 - 8839	18.17-18.37
		269 nm	0.56%	3529 - 3797	0.31%	8822 - 8969	17.59-17.91
2	Flow rate	0.7mL/min	0.32%	4129 - 4202	0.18%	9271 - 9450	18.58-18.67
		0.9mL/min	0.91%	4887- 5119	0.69%	9385 - 9608	23.49-23.92
3	pH of buffer	pH 4.2	0.32%	3552 - 3639	0.43%	8592 - 8663	17.80-18.05
		pH 4.4	1.02%	3582- 3709	0.83%	8578 - 8772	17.77-18.02
4	Mobile phase composition	Buffer: Acetonitrile (58:42)	0.49%	4721 – 4782	0.36%	9004 -9157	17.65-17.91
		Buffer: Acetonitrile (62:38)	0.50%	4484 – 4652	1.06%	8944 -9114	20.31-20.87

Filter Integrity

Metformin Hydrochloride

Table No.13: Filter integrity of metformin hydrochloride

S.No	Filtration	Standard Area	% Deviation in area	Test Area	% Deviation in area
1	Centrifuge	164507	-	163835	-
2	PVDF	162758	1.06%	164163	0.21%
3	Nylon	167475	1.80%	163575	0.57%
4	Teflon	163846	0.40%	164248	0.16%

Sitagliptin phosphate

Table No.14: Filter integrity of sitagliptin phospahte

S.No	Filtration	Standard Area	% Deviation in area	Test Area	% Deviation in area
1	Centrifuge	326100	-	325484	-
2	PVDF	324319	0.55%	325307	0.24%
3	Nylon	324419	0.52%	324264	0.56%
4	Teflon	325208	0.27%	325456	0.20%

Solution stability

Metformin Hydrochloride

Table No.15: Solution stability of metformin hydrochloride

S.No	Time (Hour)	Standard Area	% Deviation from the initial absorbance	Test Area	% Deviation from the initial absorbance
1	Initial	161322	-	161747	-
2	After 2 hours	162597	1.16%	162929	0.96%
3	After 8 hours	162946	0.95%	163458	0.64%
4	After 12 hours	164306	0.12%	166477	1.20%
5	After 24 hours	162833	1.02%	168680	2.54%

Sitaglitpin phosphate

Table No.16: Solution stability of sitagliptin phospahte

S.No	Time (Hour)	Standard Area	% Deviation from the initial absorbance	Test Area	% Deviation from the initial absorbance
1	Initial	323107	-	323757	-
2	After 2 hours	323887	0.68%	323790	0.71%
3	After 8 hours	325032	0.33%	326325	0.07%
4	After 12 hours	324044	0.63%	328620	0.77%
5	After 24 hours	326442	0.10%	335858	2.99%

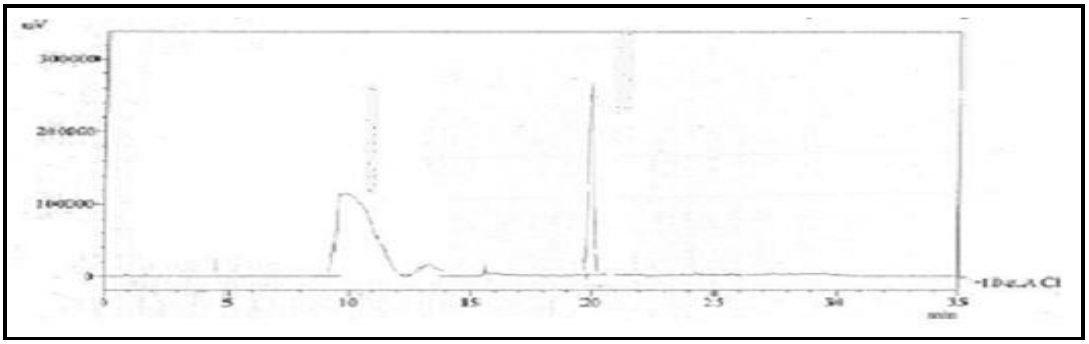


Figure No.2: Trial graph 3

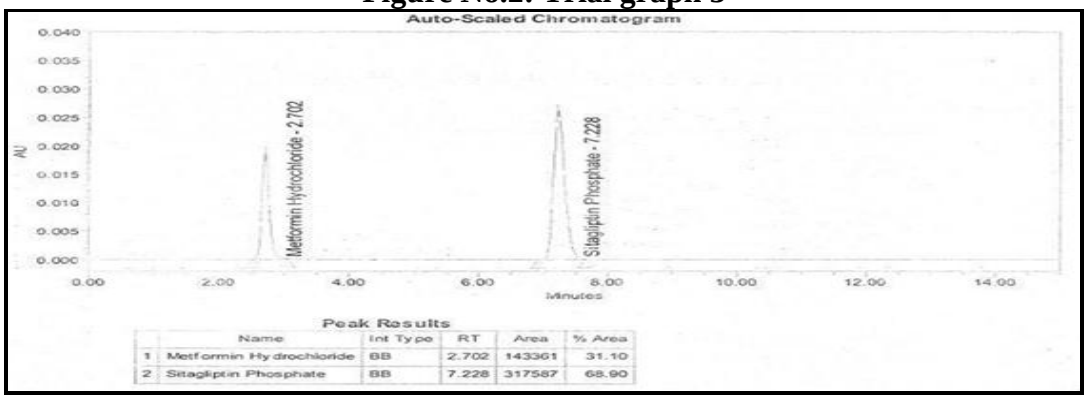


Figure No.3: Chromatogram of standard

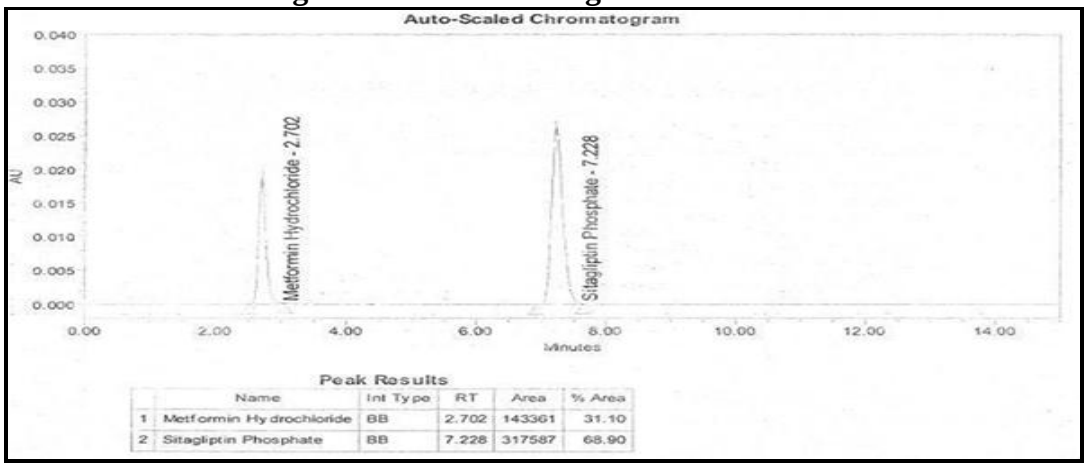


Figure No.4: Chromatogram of test

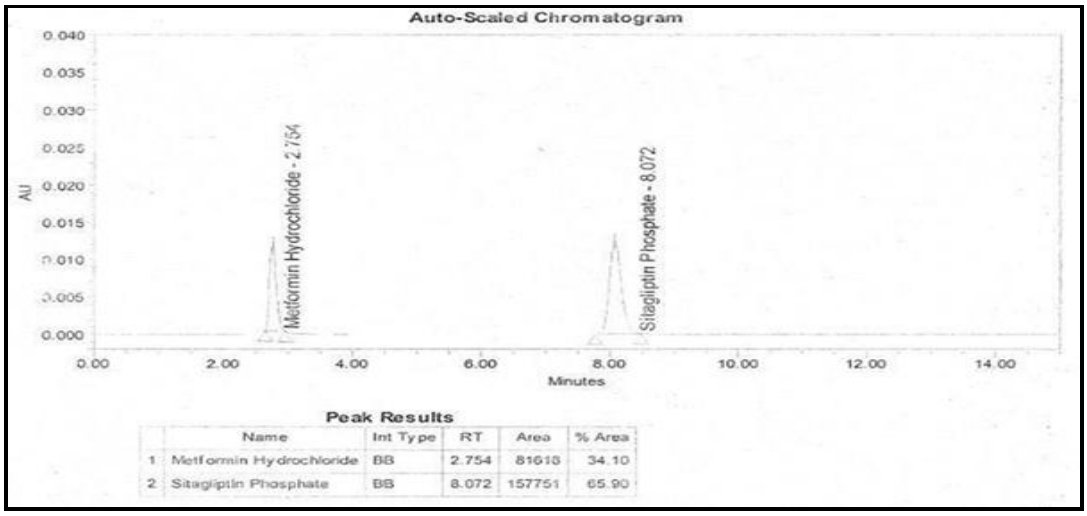


Figure No.5: Chromatogram of 50% linearity

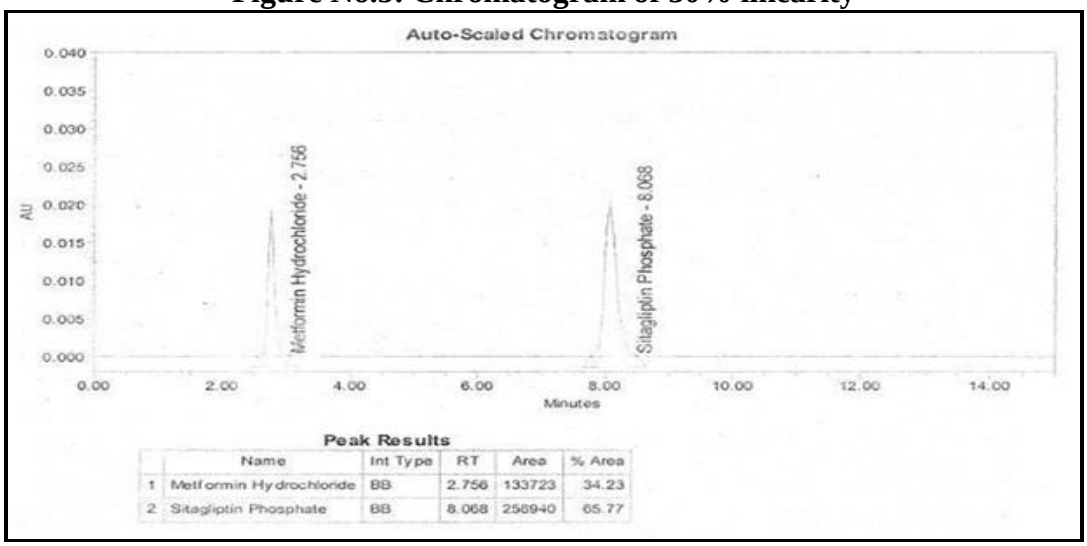


Figure No.6: Chromatogram of 80% linearity

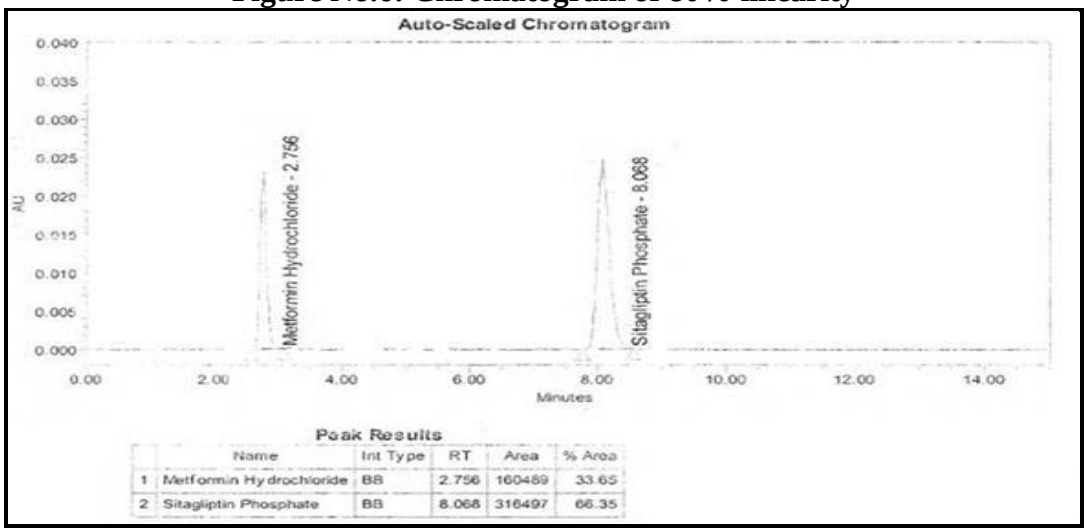


Figure No.7: Chromatogram of 100% linearity

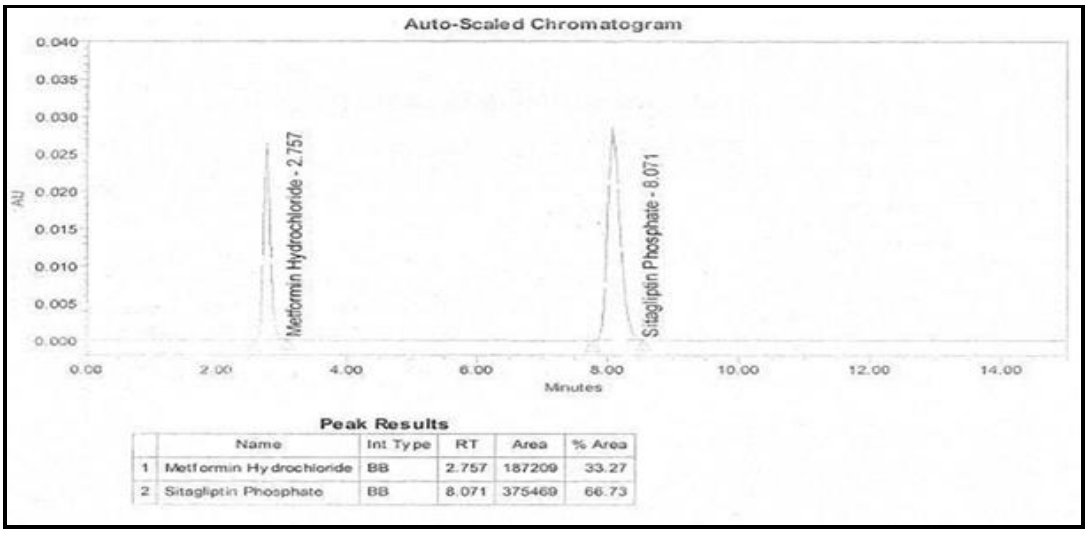


Figure No.8: Chromatogram of 120% linearity

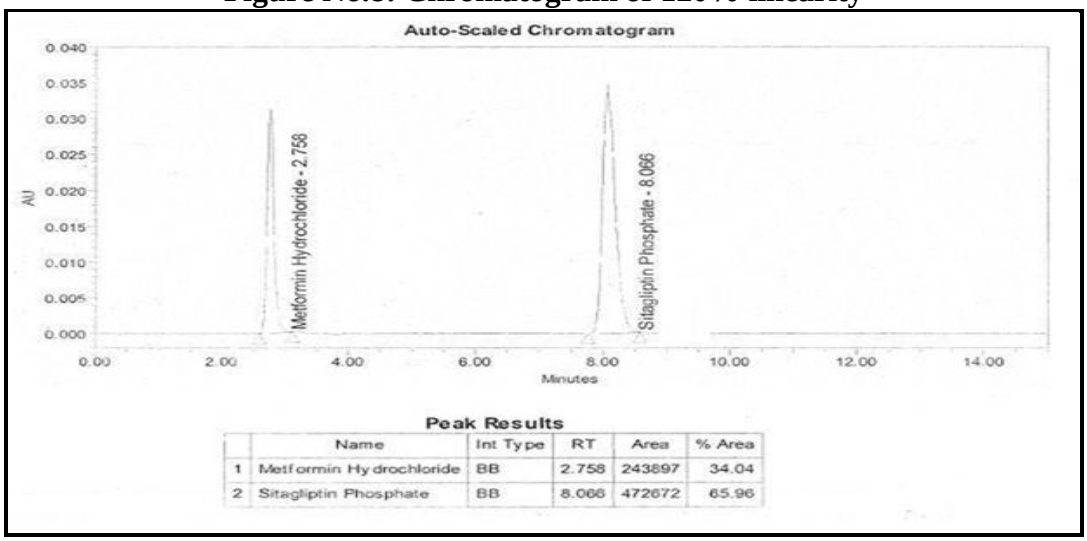


Figure No.9: Chromatogram of 150% linearity

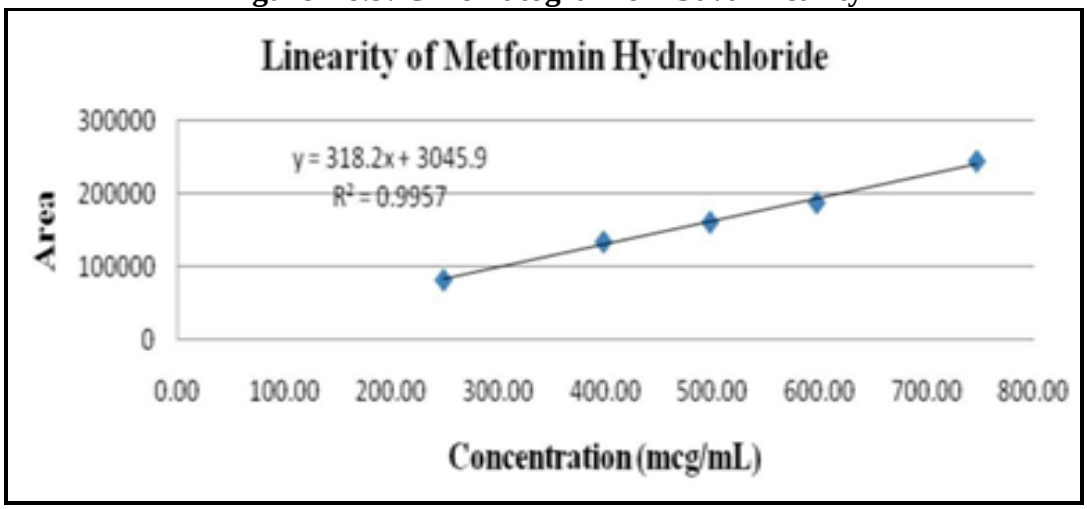


Figure No.10: Linearity of metformin hydrochloride

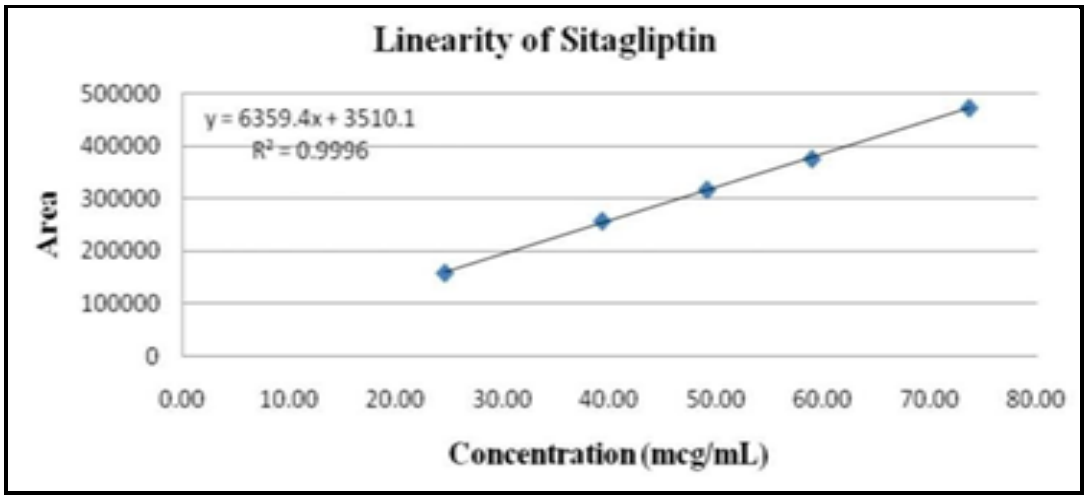


Figure No.11: Linearity of sitagliptin

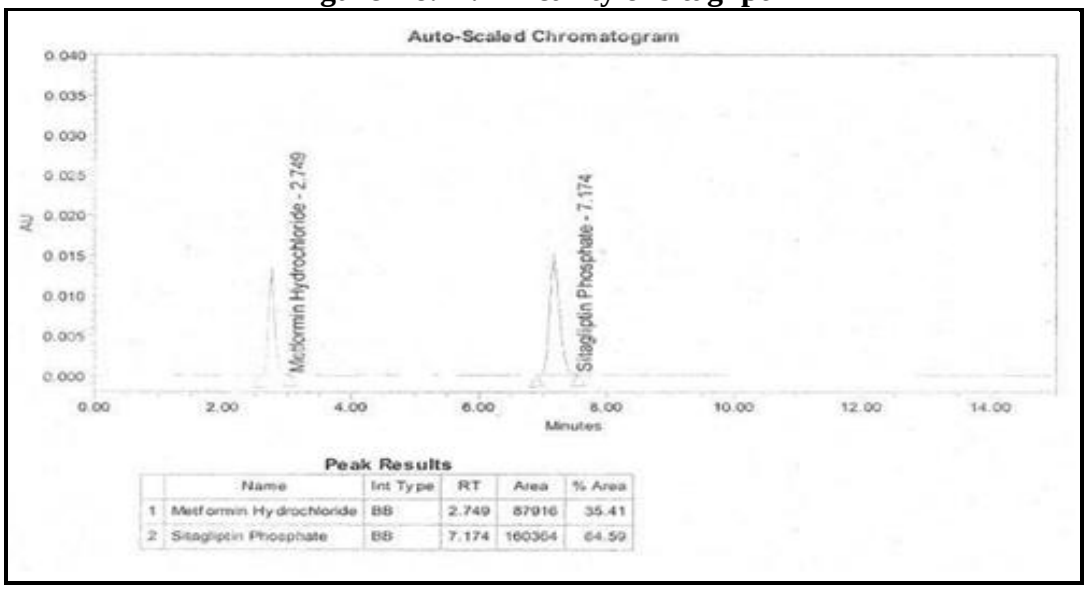


Figure No.12: Chromatogram of 50% recovery

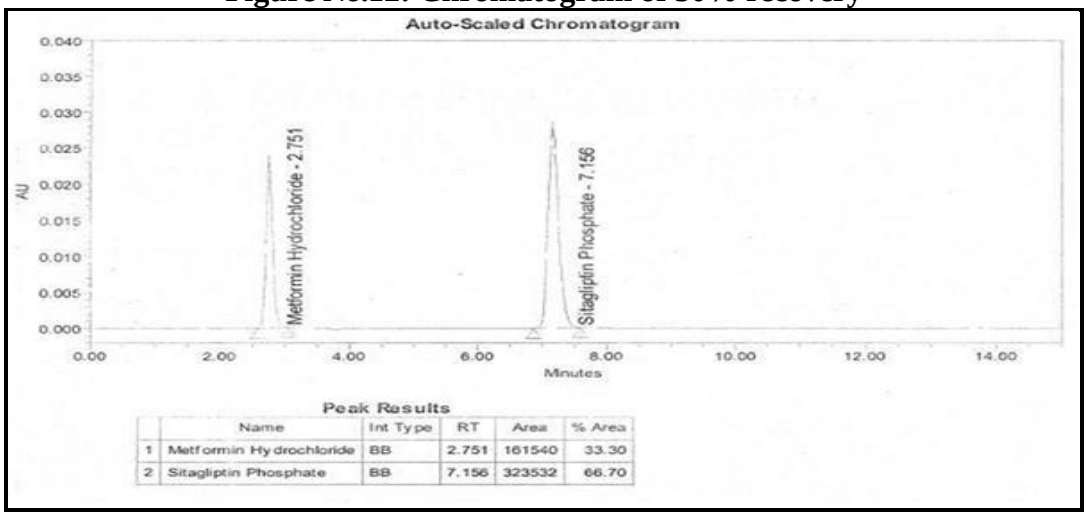


Figure No.13: Chromatogram of 100% recovery

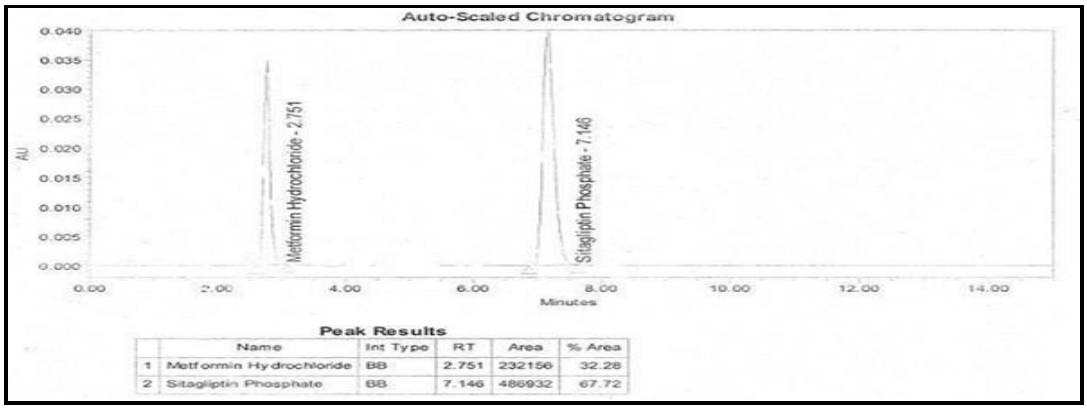


Figure No.14: Chromatogram of 150% recovery

CONCLUSION

On evaluating the various parameters, it is concluded that the results obtained meets the pre-established acceptance criteria. Hence the method adopted for estimating the assay in Sitagliptin phosphate 50mg and Metformin Hydrochloride 500mg tablets is validated.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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