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Analytical method development and validation for the estimation of Metformin and Canagliflozin using RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of Metformin and Canagliflozin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of k2hpo4+NaHPO4 buffer,40 volumes of acetonitrile and 20 volumes of Methanol with detection of 251 nm. Linearity was observed in the range 75-175 µg/ml for Metformin (r^2 =0.997) and 24-56 µg /ml for Canagliflozin (r^2 =0.993) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Liquid chromatography (LC), RSD Relative standard deviation, R^2 Correlation coefficient.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books.[1]

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis.

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the estimation of **METFORMIN AND CANAGLIFLOZIN** in pharmaceutical dosage form.

Plan of Work

- Solubility determination of **METFORMIN AND CANAGLIFLOZIN** various solvents and buffers.

- Determine the absorption maxima of the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of K_2HPO_4+$NAHPO_4$: ACN:MEOH were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of METFORMIN

10 mg of METFORMINwas weighed and transferred in to 10ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 125 µg /ml of solution by diluting 1.25ml to 10ml with water.

Preparation of standard stock solution of CANAGLIFLOZIN

5 mg of CANAGLIFLOZIN was weighed in to 10ml volumetric flask and dissolved in water and then dilute up to the mark with water and prepare 40 µg /ml of solution by diluting 0.8ml to 10ml with water.

RESULTS AND DISCUSSIONS

Solubility Studies

These studies are carried out at 25 ^{0}C

METFORMIN

Freely soluble in methanol,water and mixed phosphate buffer.

CANAGLIFLOZIN

Freely soluble in ethanol and methanol, and slightly soluble in acetone and very slightly soluble in water.

Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of METFORMIN

10 mg of METFORMIN was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, wavelength is found to be 232nm.

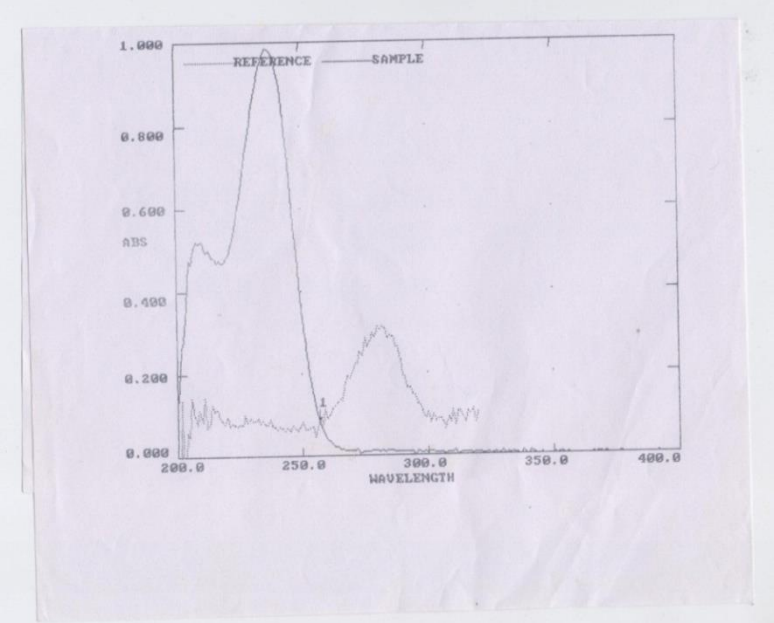
Preparation of standard stock solution of CANAGLIFLOZIN

10 mg of CANAGLIFLOZIN was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, wavelength is found to be 290nm.

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra and the absorption curve shows the isobestic point was found to be 251 nm for the combination.

Isobestic point of METFORMIN AND CANAGLIFLOZIN



METHOD DEVELOPMENT OF METFORMIN AND CANAGLIFLOZIN

Trial- 1

Preparation of mixed standard solution

Weigh accurately 10 mg of METFORMIN and CANAGLIFLOZIN in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of METFORMIN and CANAGLIFLOZIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

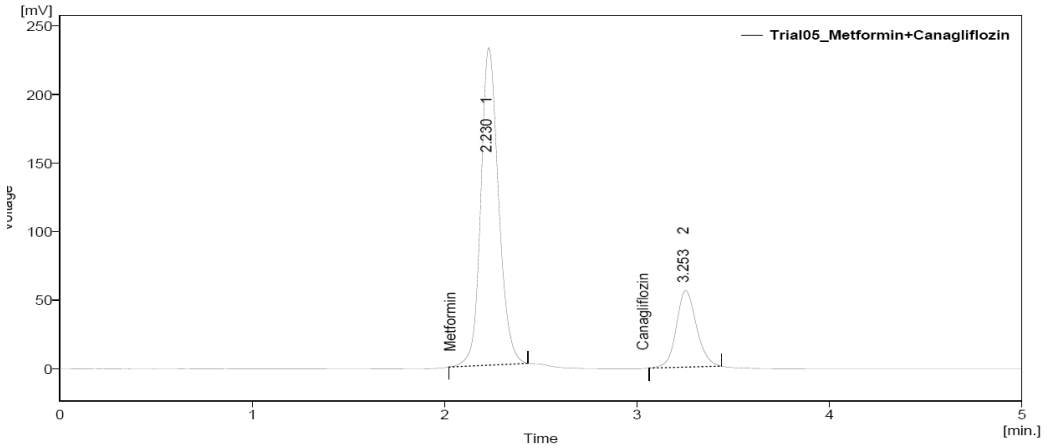


Fig. 8.3.5: Chromatogram of CANAGLIFLOZIN and METFORMIN by using mobile phase

Observation

- Peak Asymmetry factor for CANAGLIFLOZIN and METFORMIN meet the system suitability requirements.
- The run time is very correct.
- Theoretical plates were more than 2000.
- Hence it is taken for optimization.

Table 1: Optimized chromatographic conditions

Mobile phase	k2hpo4+NaHPO4 Buffer:ACN:MEOH (40:40:20)
Ph	5.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	251
Injection volume	20 µl
Run time	6 min
Retention time	About 2.237 min for METFORMIN and 3.263 min for CANAGLIFLOZIN.

ASSAY

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of METFORMIN and 5 mg of CANAGLIFLOZIN in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of METFORMIN and CANAGLIFLOZIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains CANAGLIFLOZIN-50 mg METFORMIN-500 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of CANAGLIFLOZIN and METFORMIN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of CANAGLIFLOZIN and METFORMIN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of CANAGLIFLOZIN and METFORMINwas made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of CANAGLIFLOZIN and METFORMINpresent in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of CANAGLIFLOZIN /METFORMINin mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

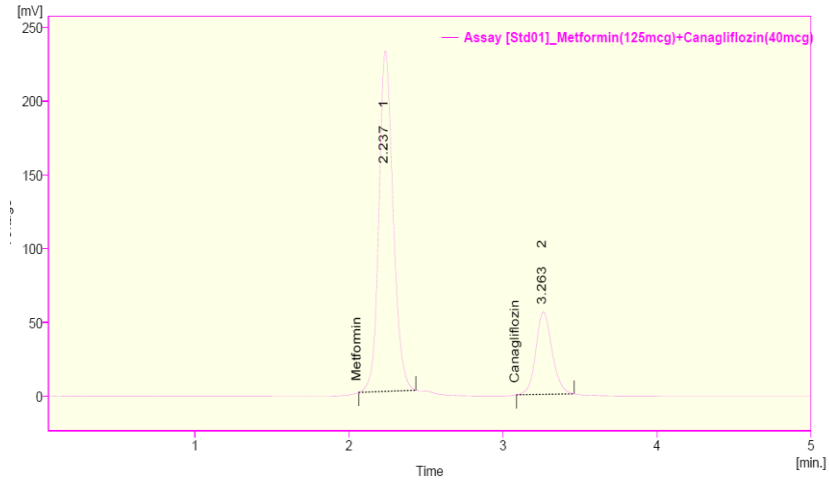


Fig: Chromatogram of Assay standard preparation-1

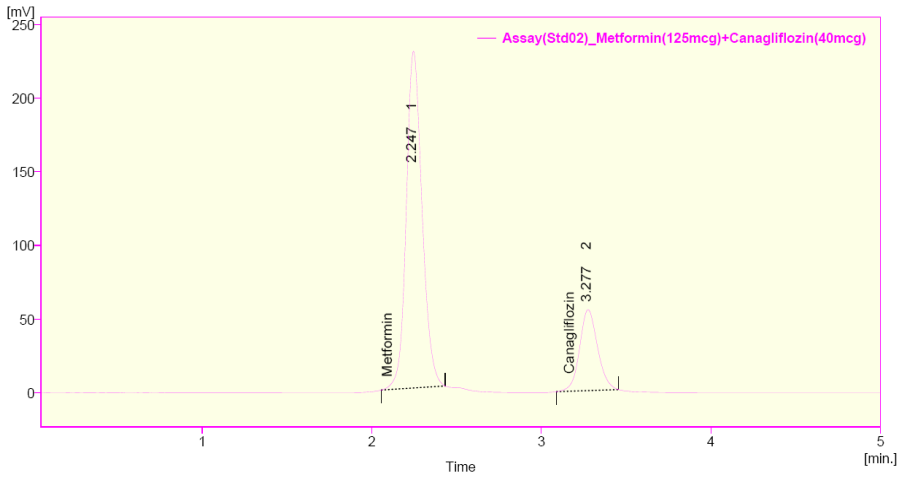


Fig: Chromatogram of Assay standard preparation-2

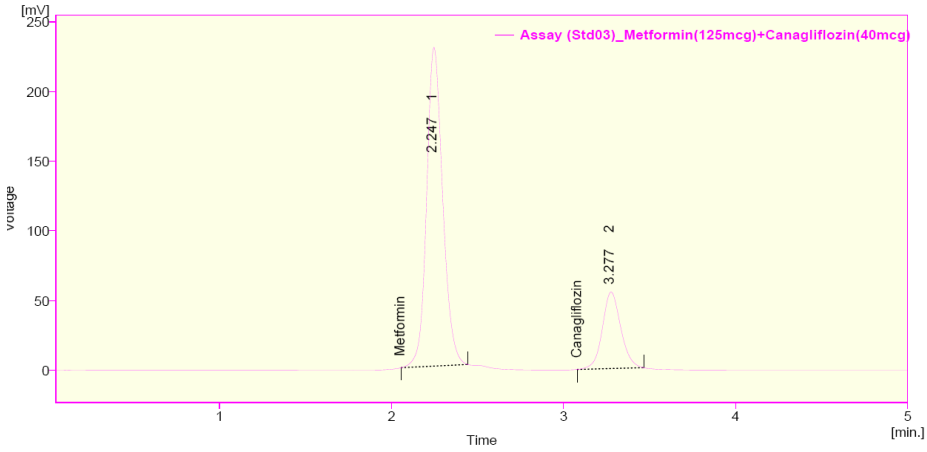


Fig: Chromatogram of Assay standard preparation-3

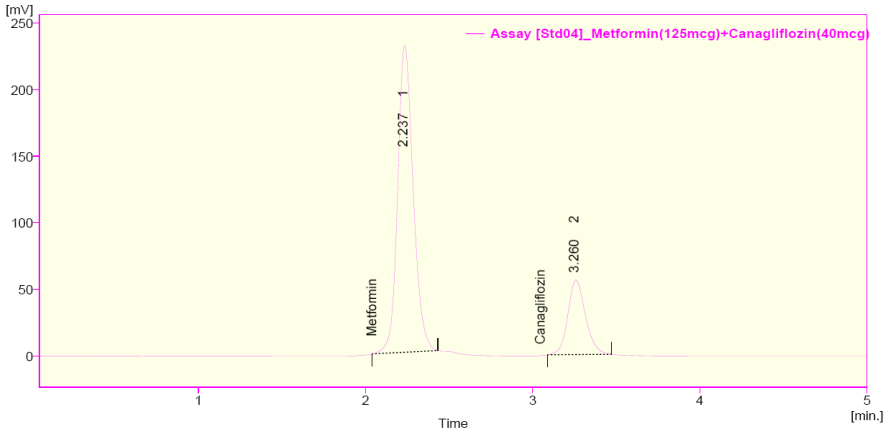


Fig: Chromatogram of Assay standard preparation-4

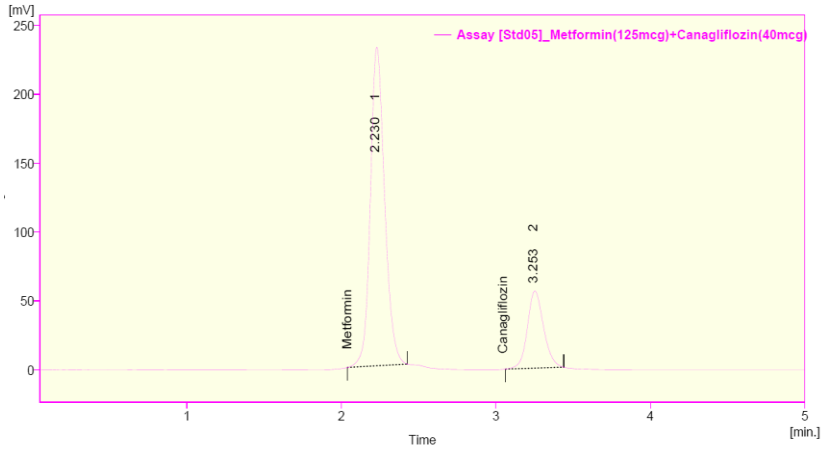


Fig: Chromatogram of Assay standard preparation-5

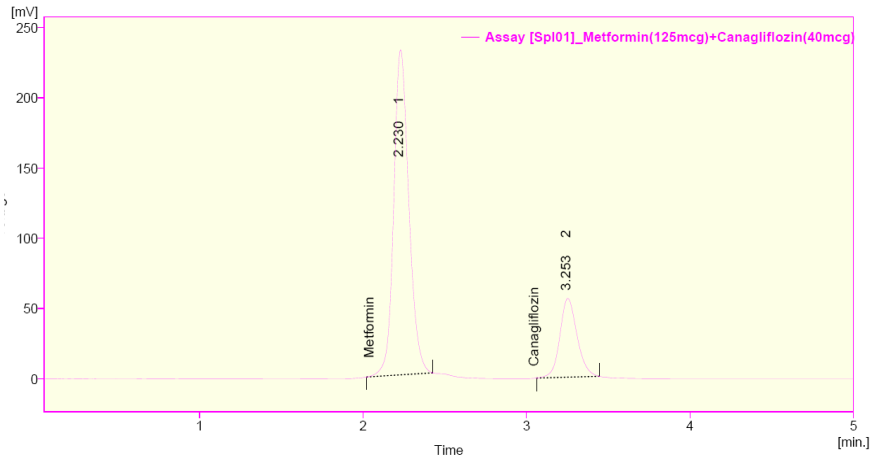


Fig: Chromatogram of Assay sample preparation-1

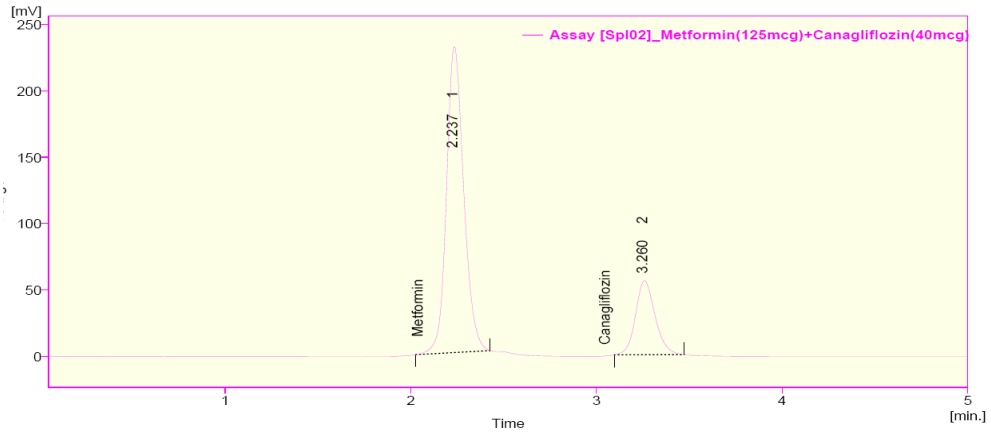


Fig: Chromatogram of Assay sample preparation-2

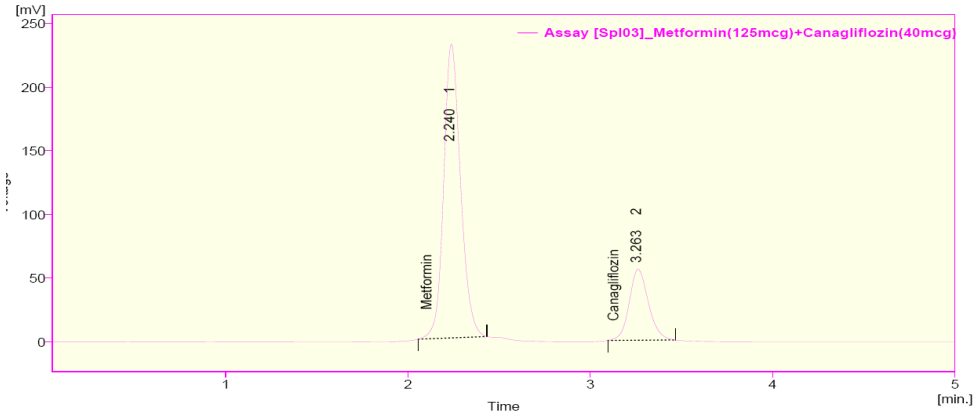


Fig: Chromatogram of Assay sample preparation-3

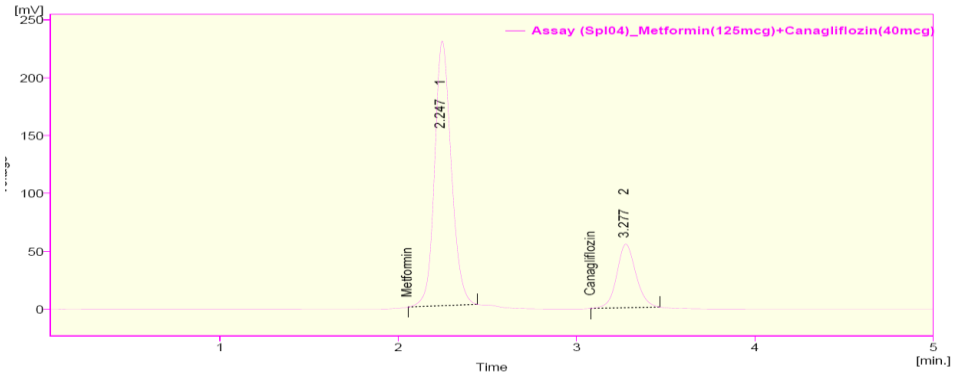


Fig: Chromatogram of Assay sample preparation-4

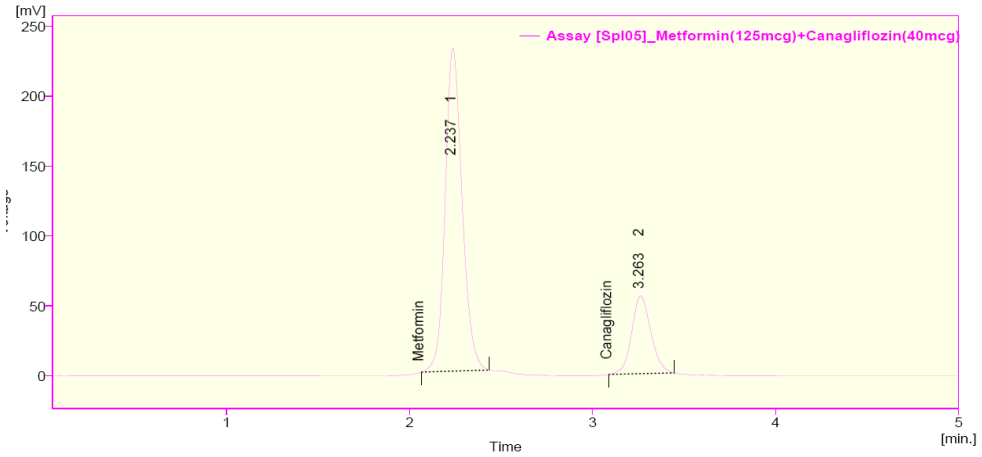


Fig: Chromatogram of Assay sample preparation-5

METFORMIN			**CANAGLIFLOZIN**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1487.1	1487.447	410.632	409.505
Injection-2	1483.265	1488.48	404.609	409.228
Injection-3	1488.429	1483.804	409.31	408.099
Injection-4	1489.131	1488.429	411.211	409.31
Injection-5	1483.538	1487.1	407.001	405.125
Average Area	1486.293	1487.052	408.5526	408.2534
Assay(%purity)	100.051094		99.9267659	

Observation

The amount of CANAGLIFLOZIN and METFORMIN present in the taken dosage form was found to be 100.05% and 99.92% respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 10mg of METFORMIN and 10 mg of CANAGLIFLOZIN in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of METFORMIN and CANAGLIFLOZIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains CANAGLIFLOZIN– 50mg METFORMIN -500 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of CANAGLIFLOZIN and METFORMIN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of CANAGLIFLOZINand 20 mg of METFORMIN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of CANAGLIFLOZINand METFORMIN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

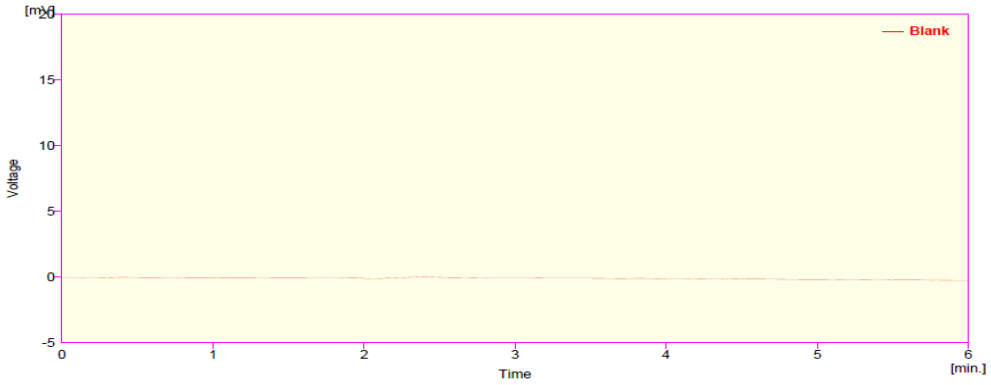


Fig: Blank chromatogram for specificity by using mobile phase

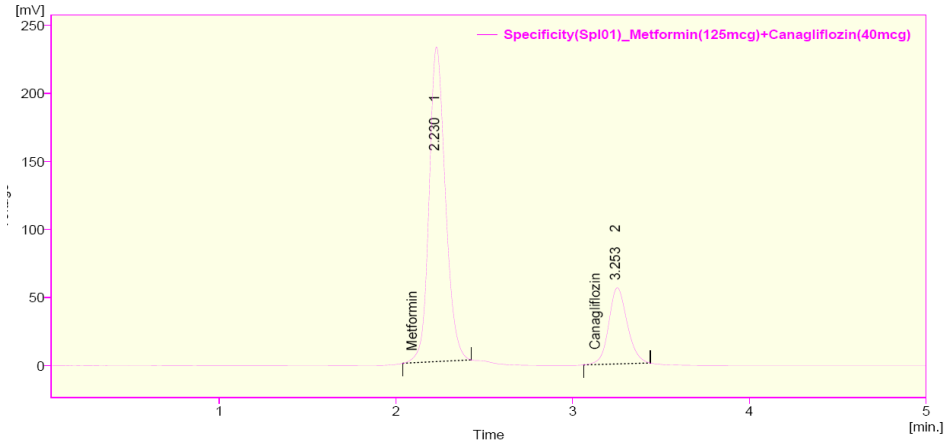


Fig: Chromatogram for specificity of CANAGLIFLOZIN and METFORMIN sample

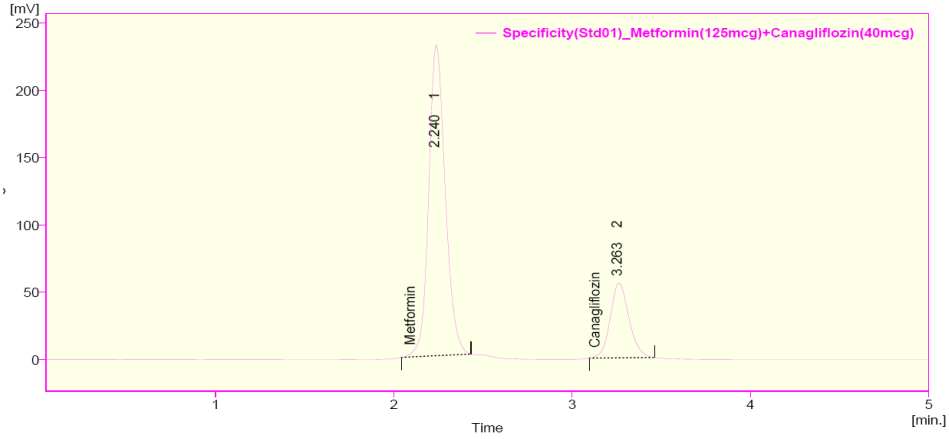


Fig: Chromatogram for Specificity of CANAGLIFLOZIN and METFORMIN standard

Observation

It is observed from the above data; diluents or excipients peaks are not interfering with the CANAGLIFLOZIN and METFORMIN peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of METFORMIN and CANAGLIFLOZIN (microgram/ml) were prepared by dissolving 10 mg of METFORMIN and CANAGLIFLOZIN dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Further dilutions were given in the table

Table: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				METFORMIN	CANAGLIFLOZIN
Preparation 1	0.75	0.24	10	75	24
Preparation 2	1	0.32	10	100	32
Preparation 3	1.25	0.4	10	125	40
Preparation 4	1.50	0.48	10	150	48
Preparation 5	1.75	0.56	10	175	56

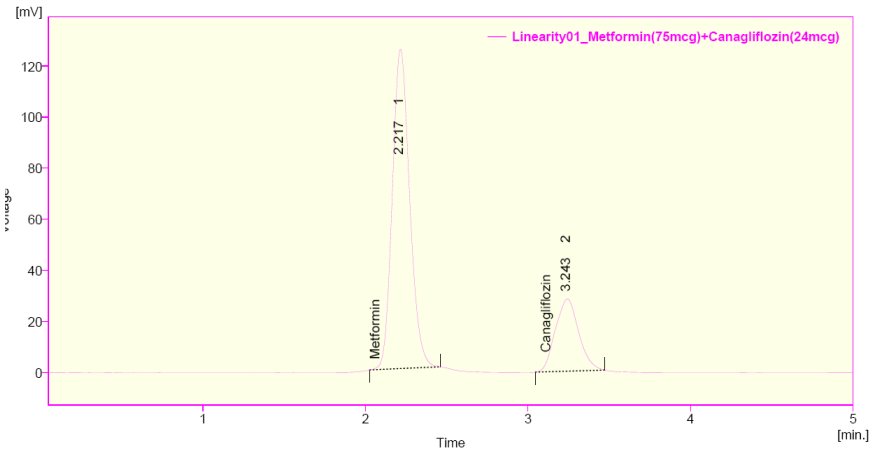


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN preparation-1

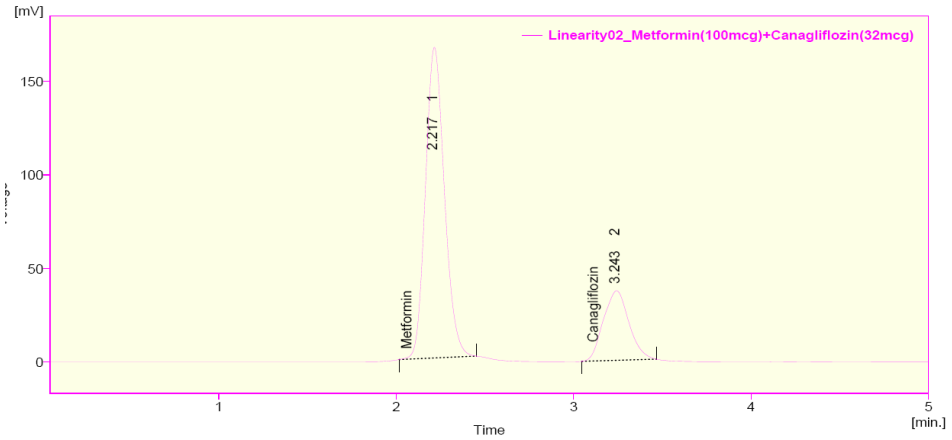


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN preparation-2

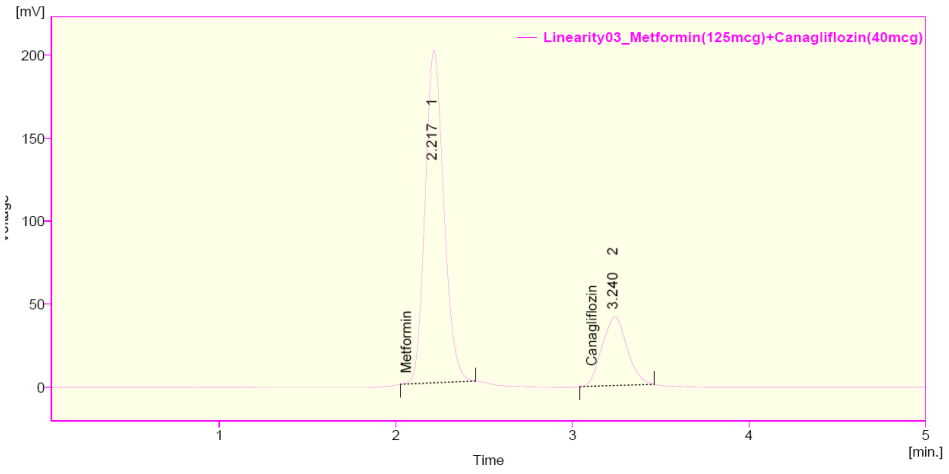


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN preparation-3

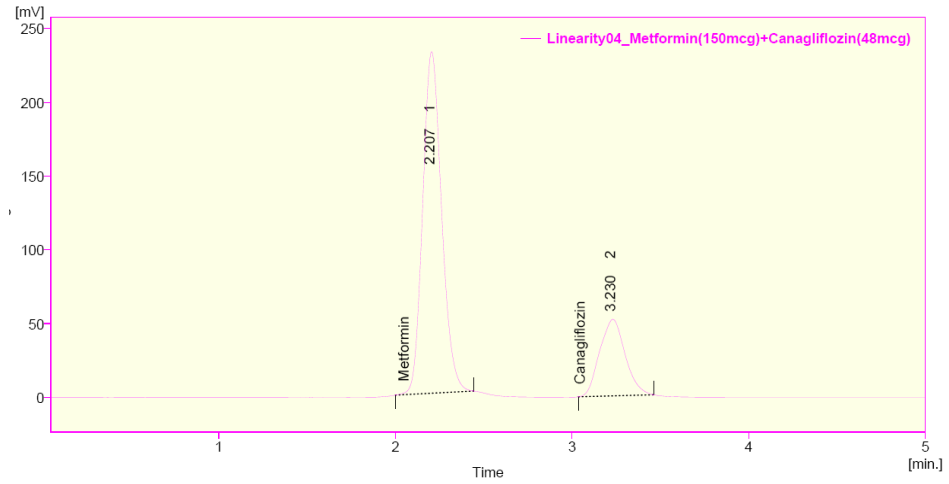


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN preparation-4

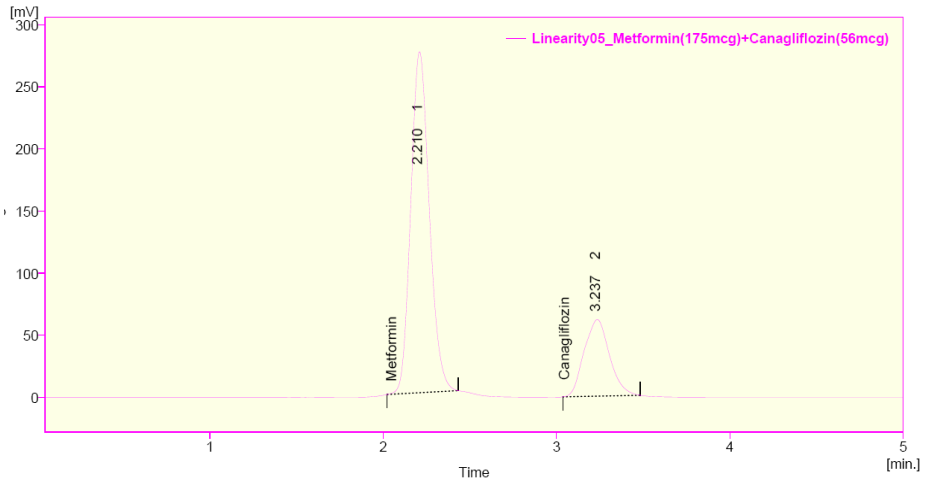


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN for preparation-5

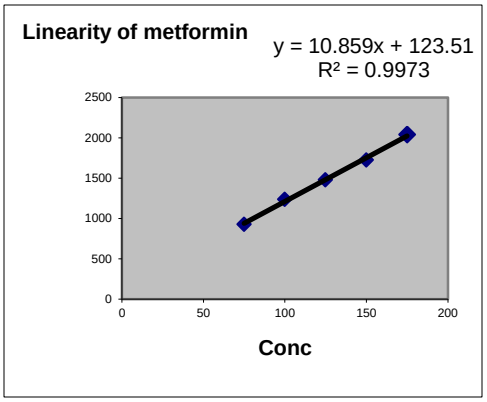
Linearity of METFORMIN

S.No.	Conc.(µg/ml)	Area
1	75	927.341
2	100	1235.752
3	125	1477.14
4	150	1722.958
5	175	2041.082

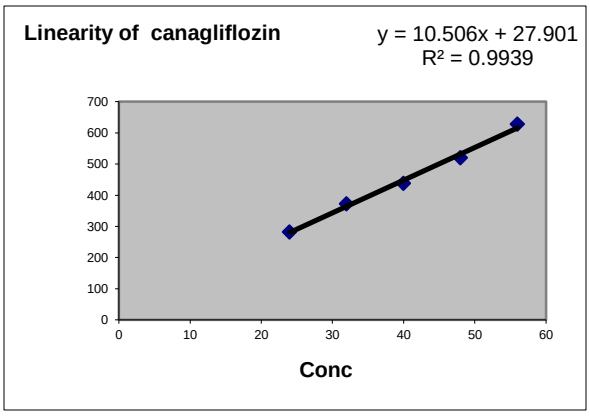
Linearity of CANAGLIFLOZIN

S.No.	Conc.(µg/ml)	Area
1	24	281.784
2	32	372.204
3	40	438.317
4	48	520.564
5	56	627.846

Linearity graph of METFORMIN



Linearity graph of CANAGLIFLOZIN



Acceptance criteria

The relationship between the concentration of METFORMIN and CANAGLIFLOZIN and area of METFORMIN and CANAGLIFLOZIN should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of METFORMIN and CANAGLIFLOZIN is 0.999 and 0.996. The relationship between the concentration of METFORMIN and CANAGLIFLOZIN and area of METFORMIN and CANAGLIFLOZIN is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%

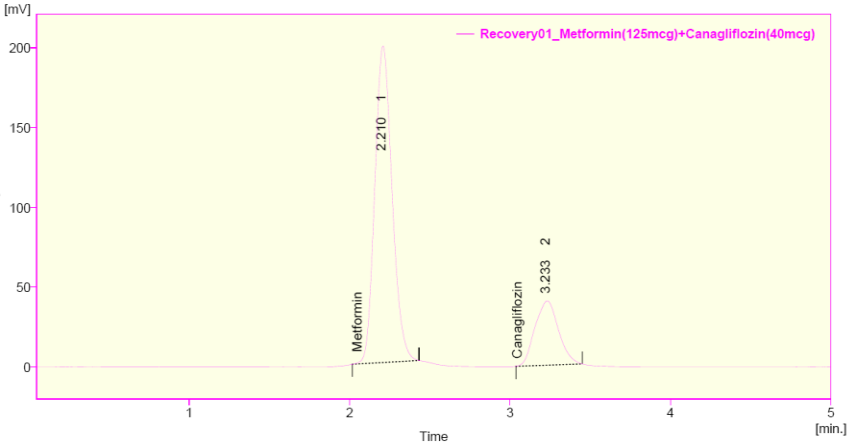


Fig: Chromatogram of 50% recovery (injection 1)

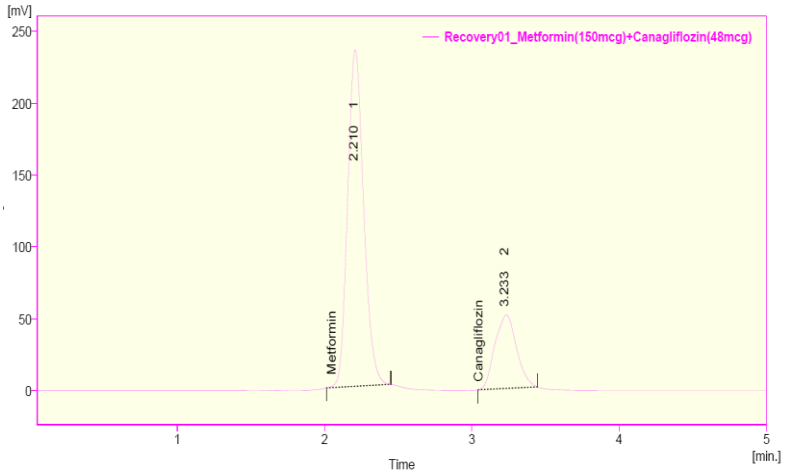


Fig: Chromatogram of 100% recovery (injection 2)

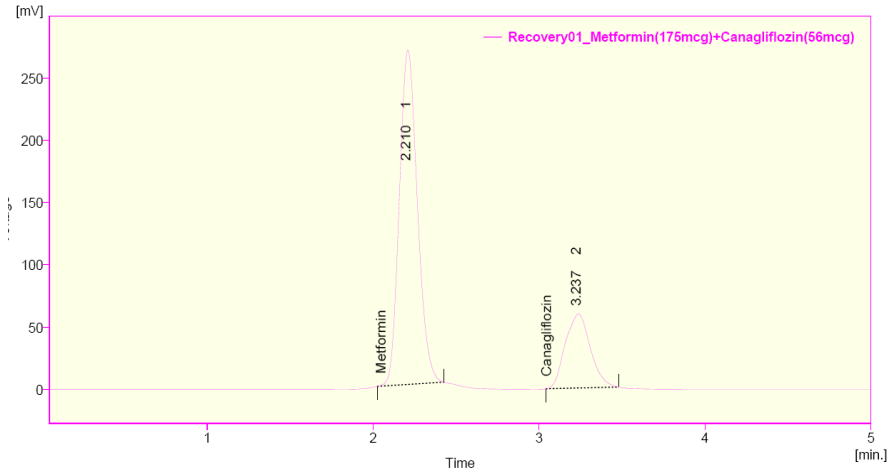


Fig: Chromatogram of 150% recovery (injection 3)

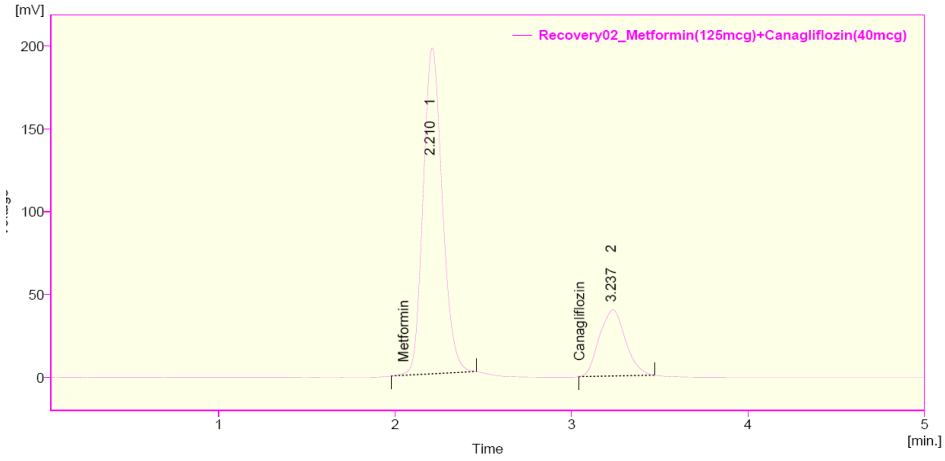


Fig: Chromatogram of 50% recovery (injection 1)

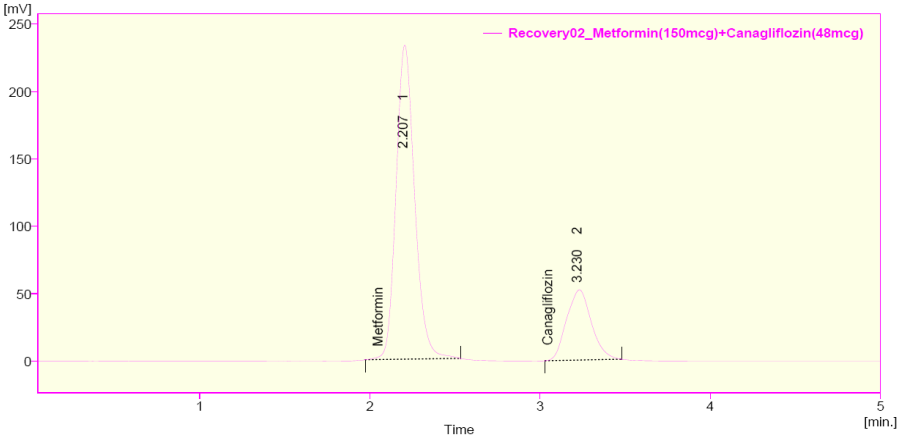


Fig: Chromatogram of 100% recovery (injection 2)

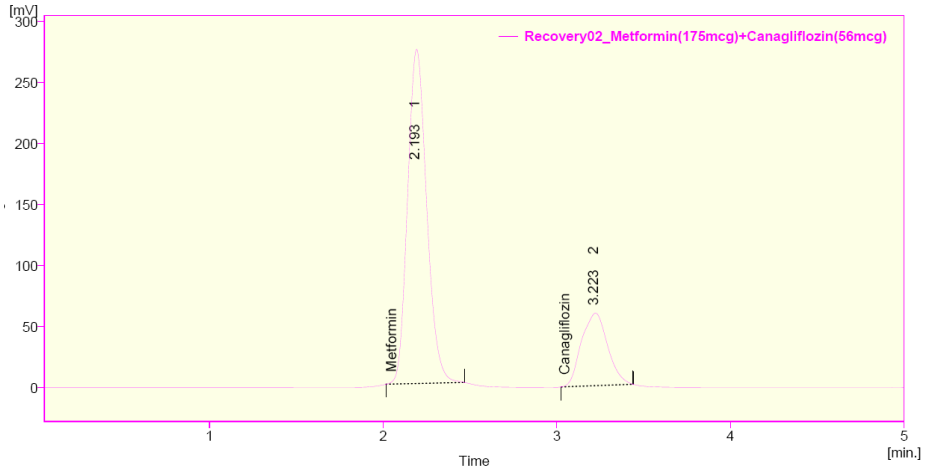


Fig: Chromatogram of 150% recovery (injection 3)

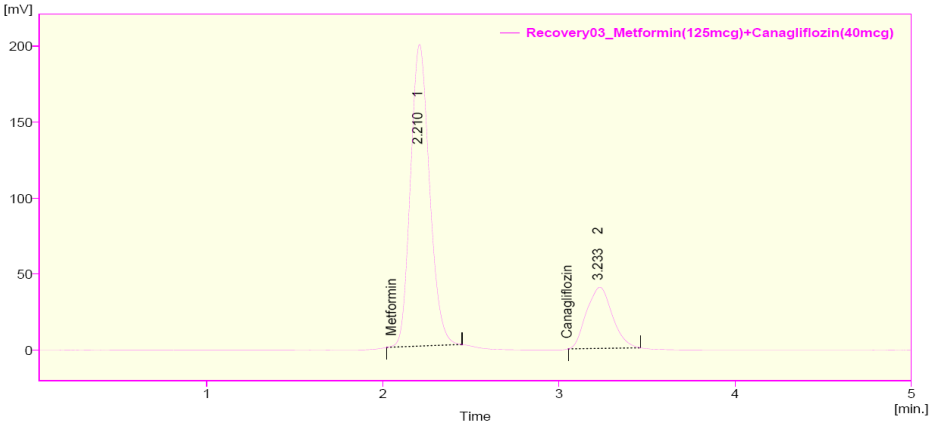


Fig: Chromatogram of 50% recovery (injection 1)

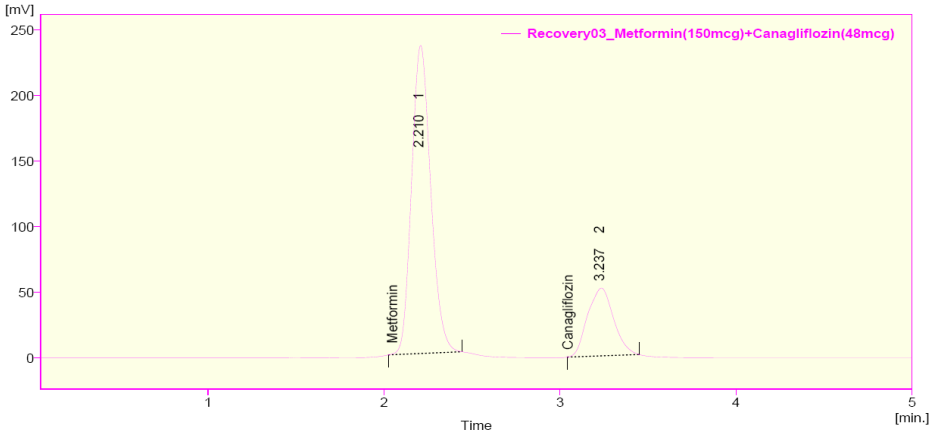


Fig: Chromatogram of 100% recovery (injection 2)

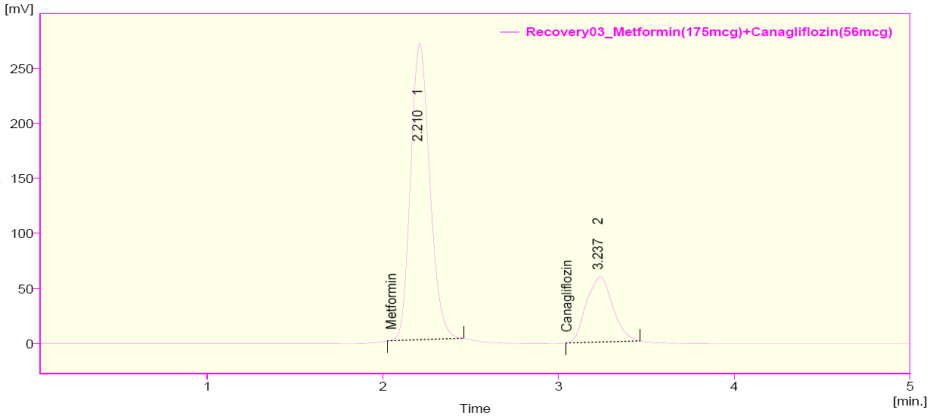


Fig: Chromatogram of 150% recovery (injection 3)

Recovery level	Accuracy METFORMIN			Average % Recovery
	Amount taken(mcg/ml)	**Area**	**%Recovery**	
100	**75**	**410.811**		
	75	**411.371**	**197.6411668**	**102.83**
	75	**411.224**	**197.9105828**	
			197.8398611	**99.36**
120	**125**	**522.084**		
	125	**527.341**	**125.5873028**	
	125	**529.651**	**126.8518741**	**99.103**
			127.4075446	
140	**175**	**621.351**		
	175	**621.351**	**89.67958741**	
	175	**615.264**	**89.67958741**	
			88.80105072	

Acceptance criteria: The % recovery of CANAGLIFLOZIN and METFORMIN should lie between 98% and 110%.

Table: Recovery results for CANAGLIFLOZIN

Recovery level	Accuracy CANAGLIFLOZIN			Average % Recovery
	Amount taken(mcg/ml)	**Area**	**%Recovery**	
100	**24**	**410.811**		
	24	**411.371**	**197.6411668**	**102.83**
	24	**411.224**	**197.9105828**	
			197.8398611	**99.36**
120	**40**	**522.084**	**125.5873028**	
	40	**527.341**	**126.8518741**	

	40	**529.651**		**99.103**
			127.4075446	
140	**56**	**621.351**		
	56	**621.351**	**89.67958741**	
	56	**615.264**		
			89.67958741	
			88.80105072	

Observation

The percentage mean recovery of METFORMIN and CANAGLIFLOZIN is 99.19 % and 99.89 % respectively.

Precision

Method precision

Method precision

Prepared sample preparations of METFORMIN AND CANAGLIFLOZIN as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of METFORMIN AND CANAGLIFLOZIN should be not more than 2.0%.

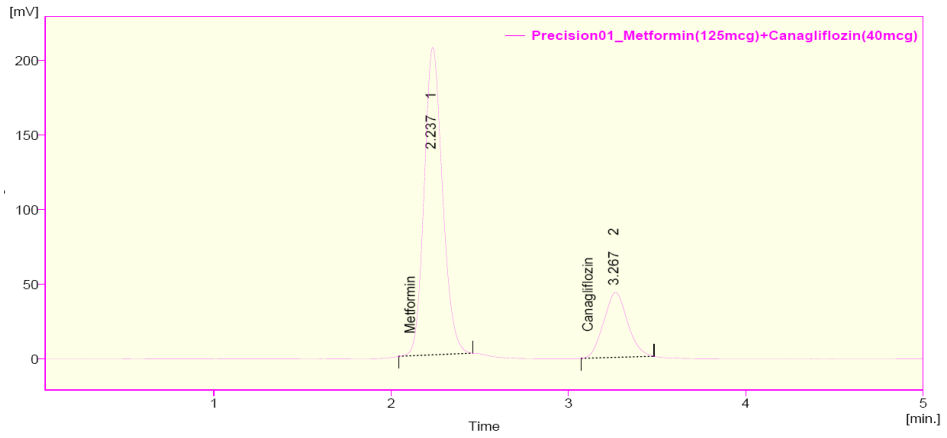


Fig: Chromatogram of precision injection 1

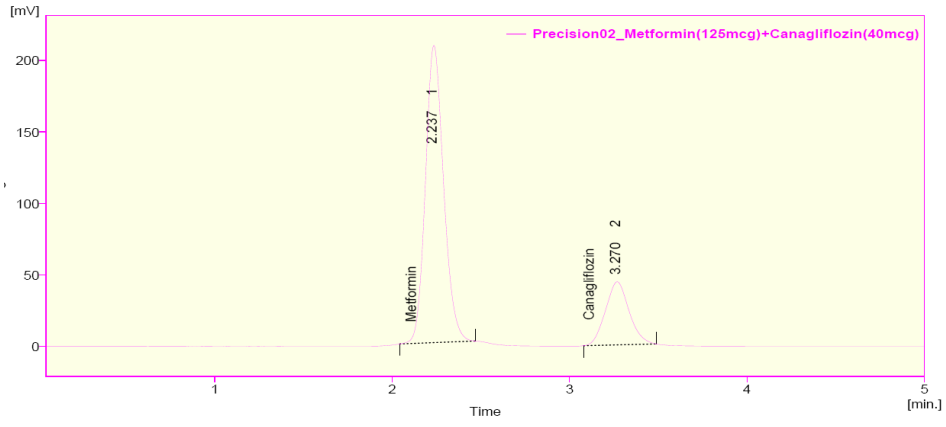


Fig: Chromatogram of precision injection 2

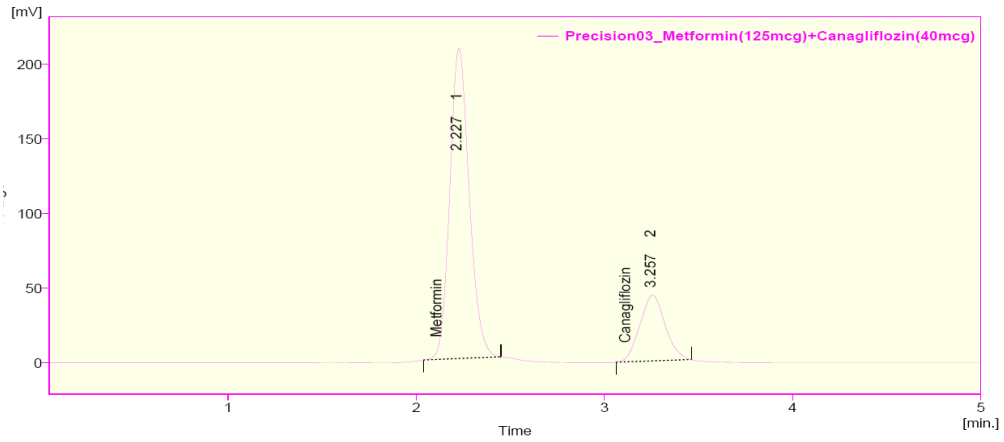


Fig: Chromatogram of precision injection 3

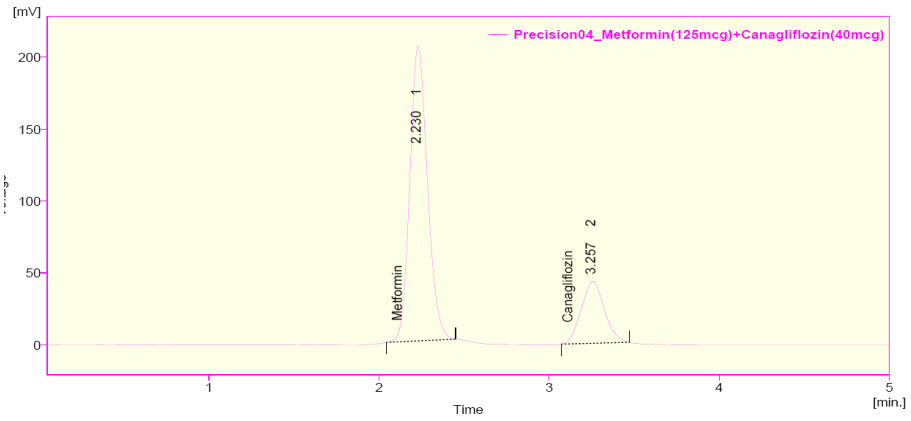


Fig: Chromatogram of precision injection 4

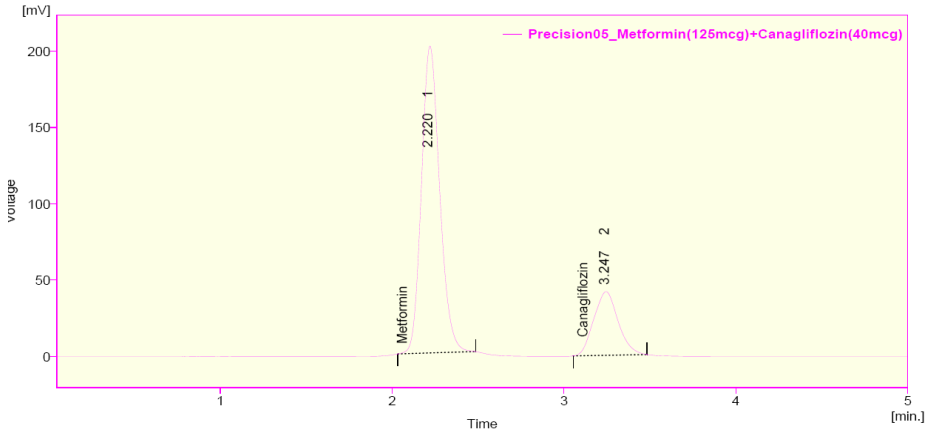


Fig: Chromatogram of precision injection 5

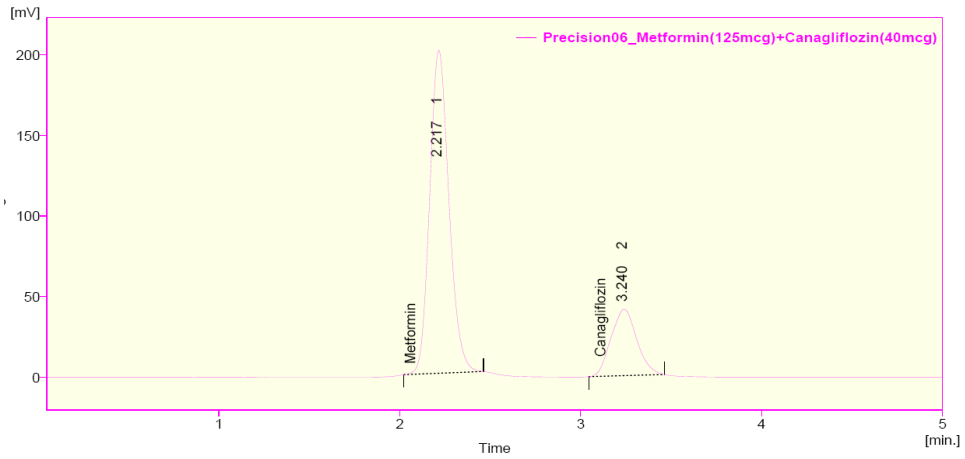


Fig: Chromatogram of precision injection 6

Table 9.5.7: Results for Method precision of METFORMIN and CANAGLIFLOZIN

METFORMIN			**CANAGLIFLOZIN**		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	3.145	978370.000	1	6.211	340457
2	3.165	962064.000	2	6.224	341907
3	3.151	967422.000	3	6.212	339323.000
4	3.148	955774.000	4	6.194	339473.000
5	3.126	951906.000	5	6.168	339074
6	3.116	962532.000	6	6.170	340503.000
avg	3.1418	963011.333	avg	6.197	340122.833
stdev	0.0178	9297.067	stdev	0.023	1058.443
%RSD	**0.57**	**0.97**	**%RSD**	**0.38**	**0.31**

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

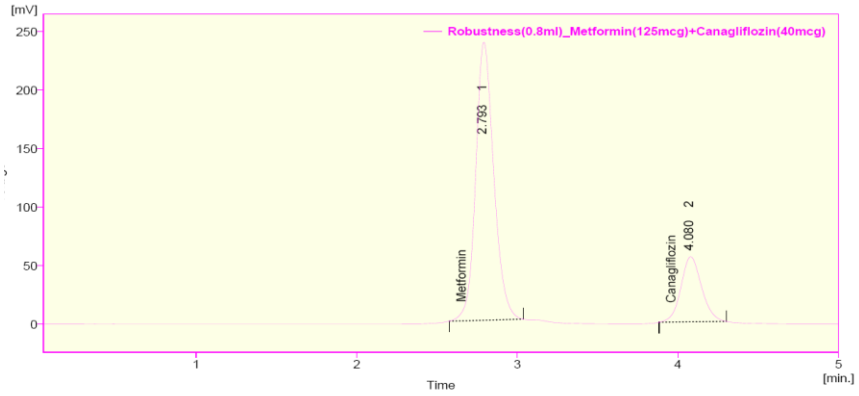


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN Robustness (0.8 ml/min)

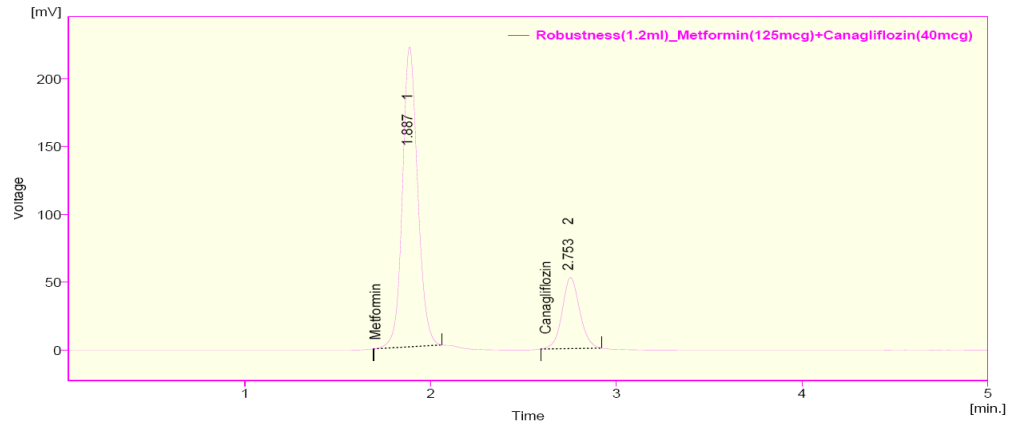


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN for Robustness (1.2 ml/min)

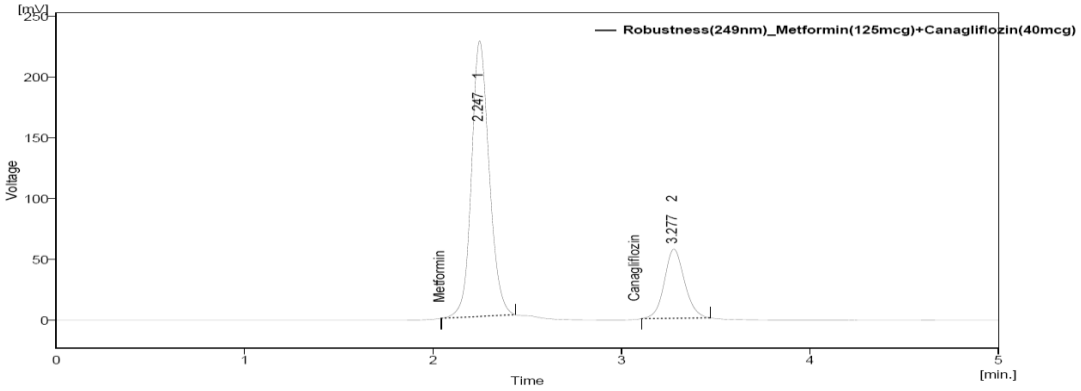


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN for Robustness (249nm)

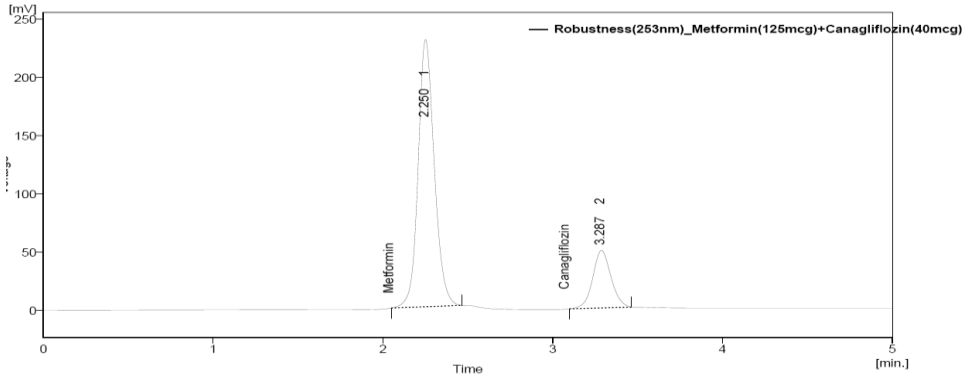


Fig: Chromatogram of CANAGLIFLOZIN and METFORMIN for Robustness (253nm)

Result of Robustness study

Parameter	METFORMIN		CANAGLIFLOZIN	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	2.562	1.679	5.059	1.263
1.2 ml/min	2.148	1.678	4.235	1.264

Wavelength				
249nm	2.566	1.687	5.052	1.262
253nm	2.570	1.686	5.065	1.265

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

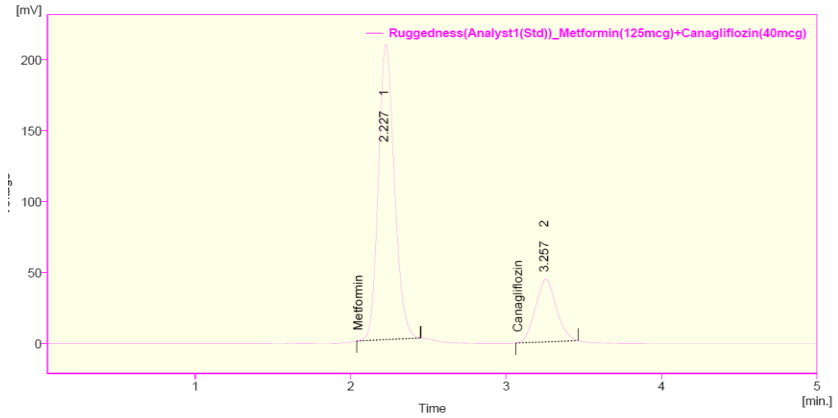


Fig: Chromatogram of Analyst 01 standard preparation

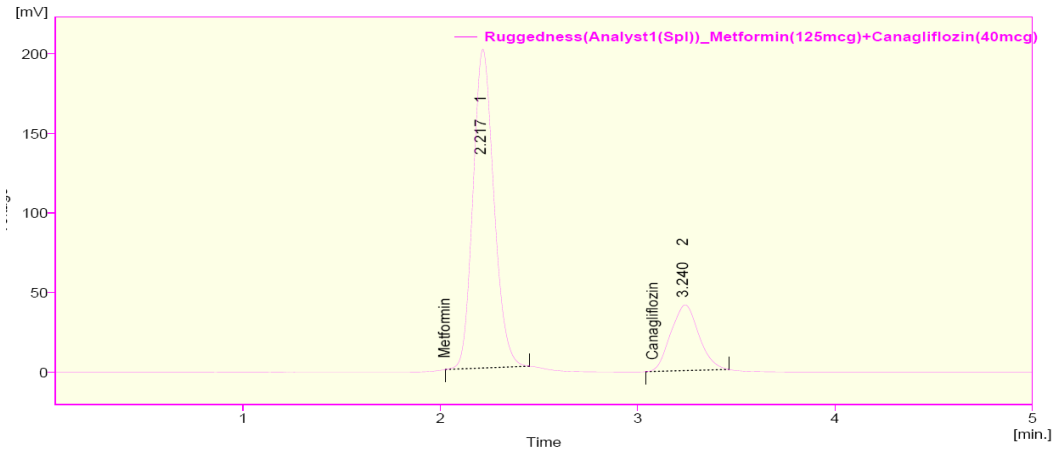


Fig: Chromatogram of Analyst 01 sample preparation

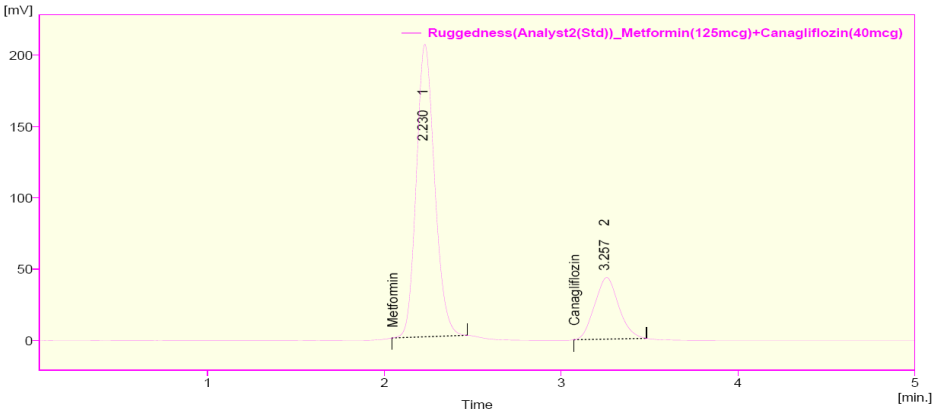


Fig: Chromatogram of Analyst 02 standard preparation

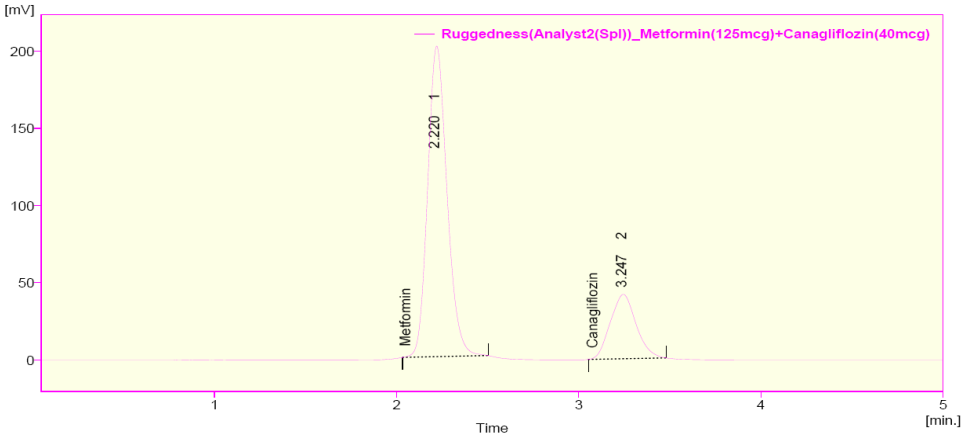


Fig: Chromatogram of Analyst 02 sample preparation

Results for Ruggedness

METFORMIN	%Assay	CANAGLIFLOZIN	%Assay
Analyst 01	100.5	**Analyst 01**	98.9
Anaylst 02	99.5	**Anaylst 02**	100.6

BIBLIOGRAPHY

[1]. Li Q, Cheng T, Wang Y, Bryant SH. "PubChem as a public resource for drug discovery.". *Drug Discov Today* **15** (23-24), 2010, 1052–7. doi:10.1016/j.drudis.2010.10.003. PMID 20970519. edit

[2]. Evan E. Bolton, Yanli Wang, Paul A. Thiessen, Stephen H. Bryant. "Chapter 12 PubChem: Integrated Platform of Small Molecules and Biological Activities". *Annual Reports in Computational Chemistry* **4**, 2008, 217–241. doi:10.1016/S1574-1400(08)00012-1.

[3]. Hettne KM, Williams AJ, van Mulligen EM, Kleinjans J, Tkachenko V, Kors JA.. "Automatic vs. manual curation of a multi-source chemical dictionary: the impact on text mining". *J Cheminform* **2** (1), 2010, 3. doi:10.1186/1758-2946-2-3. PMID 20331846. edit

[4]. Joanne Wixon, Douglas Kell. "Website Review: The Kyoto Encyclopedia of Genes and Genomes — KEGG". Yeast17 (1), 2000, 48–55. doi:10.1002/(SICI)1097-0061(200004)17:1<48::AID-YEA2>3.0.CO;2-H.

[5]. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B, Overington JP. "ChEMBL: a large-scale bioactivity database for drug discovery". Nucleic Acids Res 40 (Database issue): 2012, D1100–7. doi:10.1093/nar/gkr777. PMID 21948594. edit

[6]. Witters LA: The blooming of the French lilac. J Clin Invest. 2001 Oct;108(8):1105-7. PMID 11602616

[7]. UNGAR G, FREEDMAN L, SHAPIRO SL: Pharmacological studies of a new oral hypoglycemic drug. Proc Soc Exp Biol Med. 95(1), 1957, 190-2. PMID 13432032

[8]. Lord JM, Flight IH, Norman RJ: Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ. 25, 327(7421), 2003, 951-3. PMID 14576245

[9]. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N: Metformin in non-alcoholic steatohepatitis. Lancet. 15, 358(9285), 2001, 893-4. PMID 11567710

[10]. Nair S, Diehl AM, Wiseman M, Farr GH Jr, Perrillo RP: Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. Aliment Pharmacol Ther. 20(1), 2004, 23-8. PMID 15225167

[11]. Knox C, Law V, Jewison T, Liu P, Ly S, Frolkis A, Pon A, Banco K, Mak C, Neveu V, Djoumbou Y, Eisner R, Guo AC, Wishart DS. "DrugBank 3.0: a comprehensive resource for omics research on drugs". Nucleic Acids Res. 39 (Database issue): 2011, D1035–41. doi:10.1093/nar/gkq1126. PMC 3013709. PMID 21059682. Edit

[12]. David S. Wishart, Craig Knox, An Chi Guo, Dean Cheng, Savita Shrivastava, Dan Tzur, Bijaya Gautam, and Murtaza Hassanali. "DrugBank: a knowledgebase for drugs, drug actions and drug targets". Nucleic acids research 36 (Database issue): D901–. doi:10.1093/nar/gkm958. PMC 2238889. PMID 18048412. Edit. 2008.

[13]. Ghose, A.K., Viswanadhan V.N., and Wendoloski, J.J. "Prediction of Hydrophobic (Lipophilic) Properties of Small Organic Molecules Using Fragment Methods: An Analysis of AlogP and CLogP Methods". *J. Phys. Chem. A* **102**, 1998, 3762–3772. doi:10.1021/jp980230o.