Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

METHOD DEVLOPMENT AND VALIDATION OF METFORMIN AND EMPAGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORMS IN RP-HPLC

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the determination of Metformin and Empagliflozin in bulk and Pharmaceutical dosage form. The separation was effected on aC18 column (150 mm x 4.6 mm;5µ)using a mobile phase mixture 50 volumes of methanol and 50 volumes of phosphate buffer in a ratio of40:60 v/v with a flow rate of 1ml/min. The detection was made at 255 nm. Calibration curve was linear over the concentration range of 60-140 µ g/ml of Metformin and 3-7µg/ml of Empagliflozin. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and Pharmaceutical dosage form.

KEYWORDS

Buffer, Acetonitrile, Metformin and Empagliflozin and RP-HPLC.

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INTRODUCTION

Empagliflozin

Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes. SGLT2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycaemia and also assists weight loss and blood pressure reduction.

Categories

- Drugs Used in Diabetes
- Alimentary Tract and Metabolism
- Blood Glucose Lowering Drugs, Excl. Insulin's.

Weight: 450.91

Chemical Formula: $C_{23}H_{27}ClO_7$

IUPAC Name: (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol

Metformin

Metformin is a biguanide anti-hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral anti-hyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea. Dose titration and/or use of smaller divided doses may decrease side effects. Metformin should be avoided in those with severely compromised renal function (creatinine clearance < 30 ml/min), acute/decompensated heart failure, severe liver disease and for 48 hours after the use of iodinated contrast dyes due to the risk of lactic acidosis. Lower doses should be used in the elderly and those with decreased renal function. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels. In 2012, a combination tablet of linagliptin plus metformin hydrochloride was marketed under the name Jentadueto for use in patients when treatment with both linagliptin and metformin is appropriate.

EXPERIMENTAL

Chemicals and solvents

Mobile Phase

A mixture of 50 volumes of methanol and 50 volumes of pH. Method development and validation of metformin and empagliflozin in pharmaceutical dosage forms in RP-HPLC.

Phosphate buffer were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of Mixed Phosphate Buffer

1.625 gm of potassium di hydrogen phosphate (KH2PO4) and 0.3 gm of Dipotassium hydrogen phosphate was weighed and dissolved in 100ml of water and volume was made up to 550ml with water. Adjust the pH to 4.0 using or tho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

RESULTS AND DISCUSSION

Solubility Studies

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

Metformin

Soluble in methanol and in water, very slightly soluble in phosphate buffer.

Empagliflozin

Freely soluble in water, soluble in acetonitrile,spraingly soluble in methanol.

Determination Of Working Wavelength (λmax)

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 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.

Preparation of standard stock solution of EMPAGLIFLOZIN

10mg of EMPAGLIFLOZIN 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.

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 are shown in the Figure No.1,2 and 3 and the absorption curve shows characteristic absorption maxima at 240 nm for METFORMIN, 229 nm for EMPAGLIFLOZIN and 255nm for the combination.

Figure No.3 UV-VIS spectrum of METFORMINand EMPAGLIFLOZIN and the isosbestic point was 255nm.

Observation

The Isobestic point was found to be 255nm for METFORMINand EMPAGLIFLOZIN in combination and was shown in Figure No.3.

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both EMPAGLIFLOZIN and METFORMIN.
- The efficiency was more than 2000 EMPAGLIFLOZIN and METFORMIN.
- Resolution between two peaks >1.5.
- The details are given in the Table No.8.3.8 and Figure No.8.3.8, hence this method was for optimized.
- Optimized chromatographic conditions

Linearity and range

Preparation of standard stock solution

Standard stock solutions of METFORMIN and EMPAGLIFLOZIN (microgram/ml) were prepared by dissolving 100 mg of METFORMIN and 5 mg of EMPAGLIFLOZIN dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Further dilutions were given in the Table No 8.3.1

Acceptance criteria

The relationship between the concentration of METFORMIN and EMPAGLIFLOZIN and area of METFORMIN and EMPAGLIFLOZIN 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 EMPAGLIFLOZIN is 0.994 and 0.995. The relationship between the concentration of METFORMIN and EMPAGLIFLOZIN and area of METFORMIN and EMPAGLIFLOZIN is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

ASSAY

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 100 mg of METFORMIN and 5 mg of EMPAGLIFLOZIN 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 1000 µg/ml of METFORMIN and 50 µg/ml of EMPAGLIFLOZIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample 10 tablets (each tablet contains EMPAGLIFLOZIN-5mg METFORMIN-100mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of EMPAGLIFLOZIN and METFORMIN (150 µg/ml) were prepared by dissolving weight equivalent to 5 mg of EMPAGLIFLOZIN and 100 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 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 50µg/ml of EMPAGLIFLOZIN and 1000 µg/ml of

METFORMIN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Observation

The amount of METFORMIN and EMPAGLIFLOZIN present in the taken dosage form was found to be 99.89% and 100.9% respectively.

Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Phosphate	AR Grade
Acetonitrile	HPLC Grade
Disodium hydrogen phosphate	AR Grade

Drugs used

Metformin and Empagliflozin drugs	Gift Samples obtained from Chandra labs, Hyd.
Metformin and Empagliflozin(500mg/25mg)	Obtained from local pharmacy

Mobile phase	Methanol: KH_2PO_4
pH	4.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^oC)
Sample temperature	Room temperature(20-25^oC)
Wavelength	255
Injection volume	20 µl
Run time	5 min
Retention time	About 2.463 min for METFORMIN and 4.210 min for EMPAGLIFLOZIN.

Table No.1: Linearity Preparations

S.No	Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
					METFORMIN	EMPAGLIFLOZIN
1	Preparation 1	0.6	0.3	10	60	3
2	Preparation 2	0.8	0.4	10	80	4
3	Preparation 3	1.0	0.5	10	100	5
4	Preparation 4	1.2	0.6	10	120	6
5	Preparation 5	1.4	0.7	10	140	7

Table No.2: linearity of METFORMIN

S.No	Conc.(µg/ml)	Area
1	60	2409.102
2	80	3241.021
3	100	3991.542
4	120	4809.091
5	140	5593.013

Table No.3: linearity of EMPAGLIFLOZIN

S.No	Conc.(µg/ml)	Area
1	3	231.895
2	4	312.713
3	5	389.139
4	6	466.353
5	7	540.788

Table No.4: Assay Results

S.No	METFORMIN			EMPAGLIFLOZIN	
1		Standard Area	Sample Area	Standard Area	Sample Area
2	Injection-1	3941.191	3933.444	378.411	372.761
3	Injection-2	3925.782	3930.759	367.951	371.408
4	Injection-3	3941.042	3936.783	375.523	370.373
5	Injection-4	3925.782	3920.484	550.591	541.451
6	Injection-5	3941.191	3945.931	384.450	378.411
7	Average Area	3937.618	3933.479	411.385	406.880
8	Standard deviation	0.824		0.752	
9	%RSD	2.0		0.9	
10	Assay(%purity)	99.89		100.9	

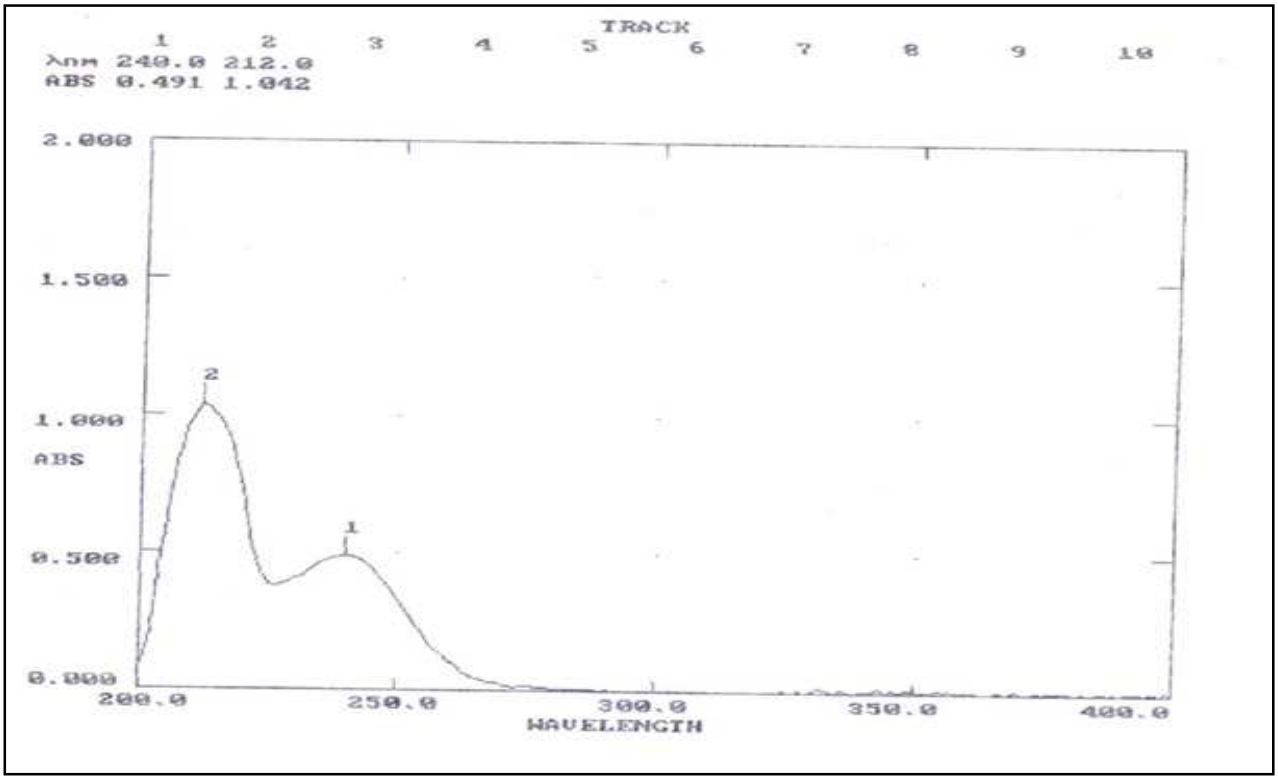


Figure No.1: UV-VIS spectrum of METFORMIN

Observation: λ_{max} was found to be 240nm for METFORMIN shown in the Figure 1.1

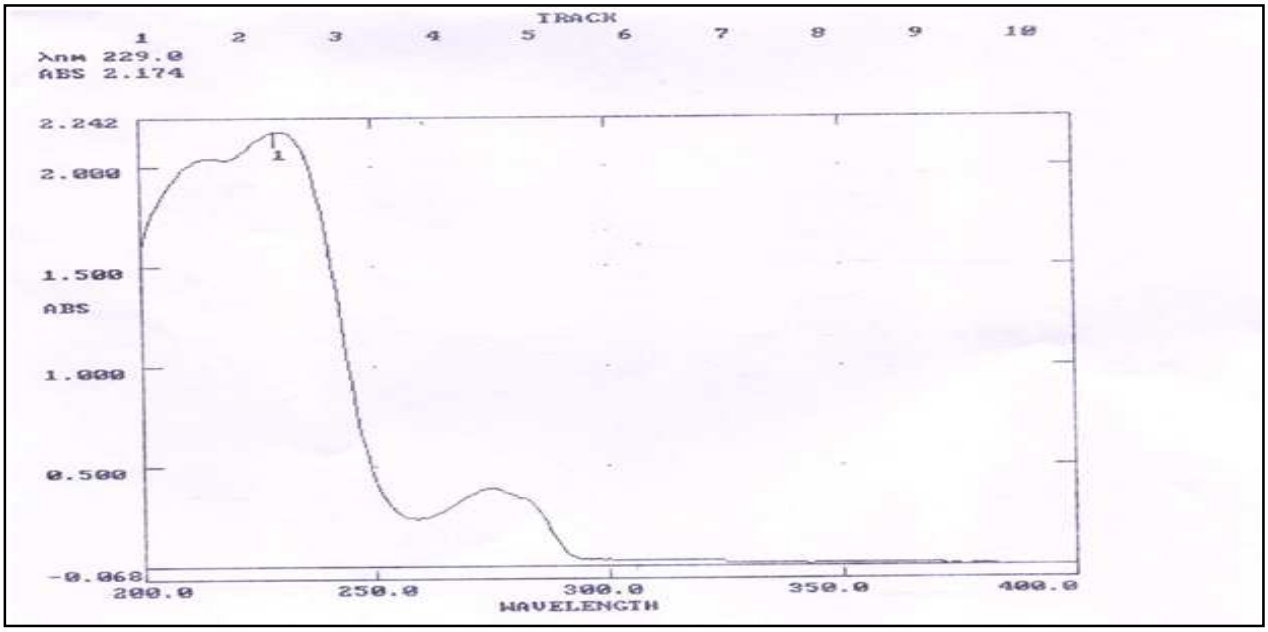
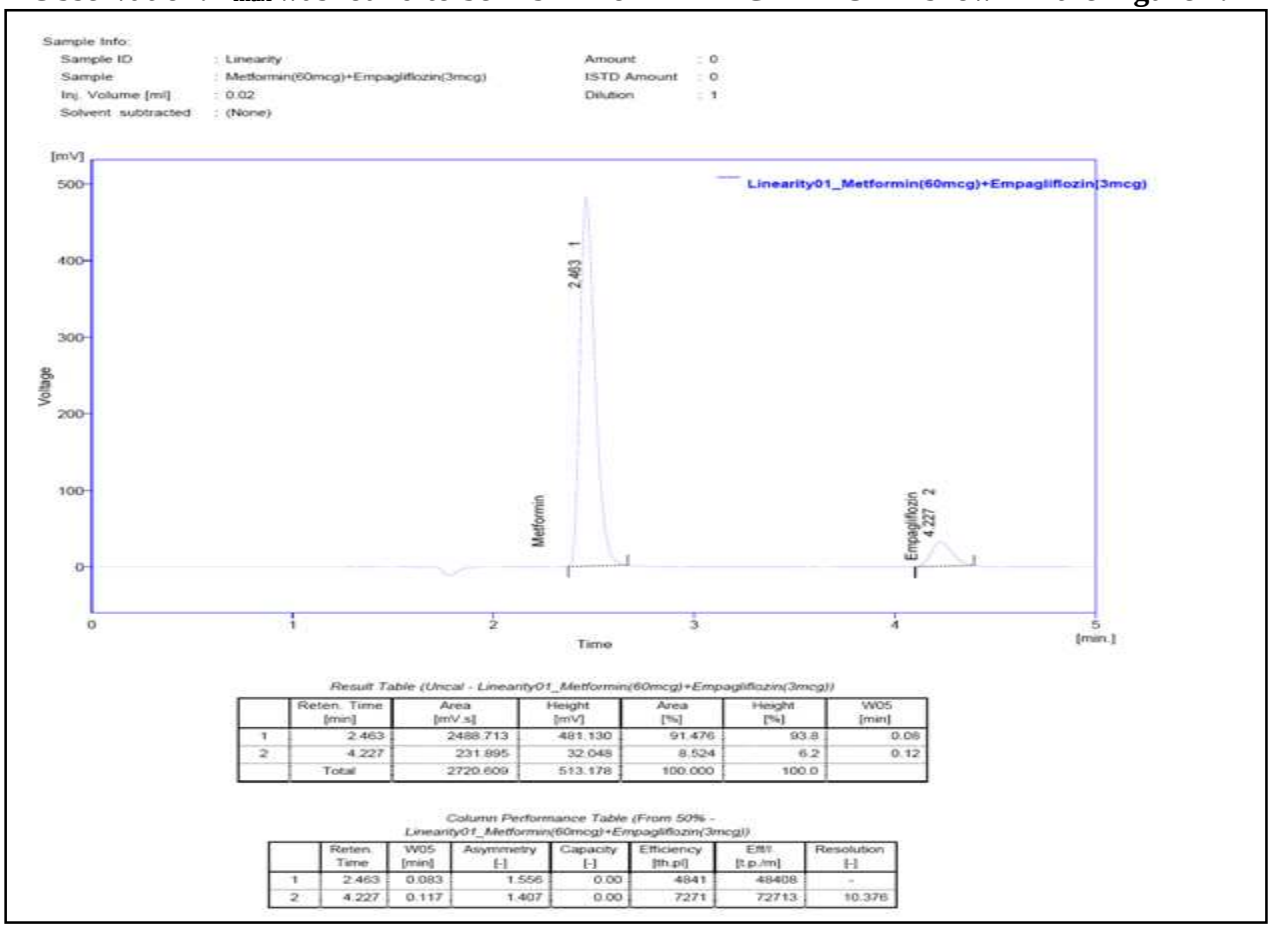


Figure No.2: UV-VIS spectrum of EMPAGLIFLOZIN

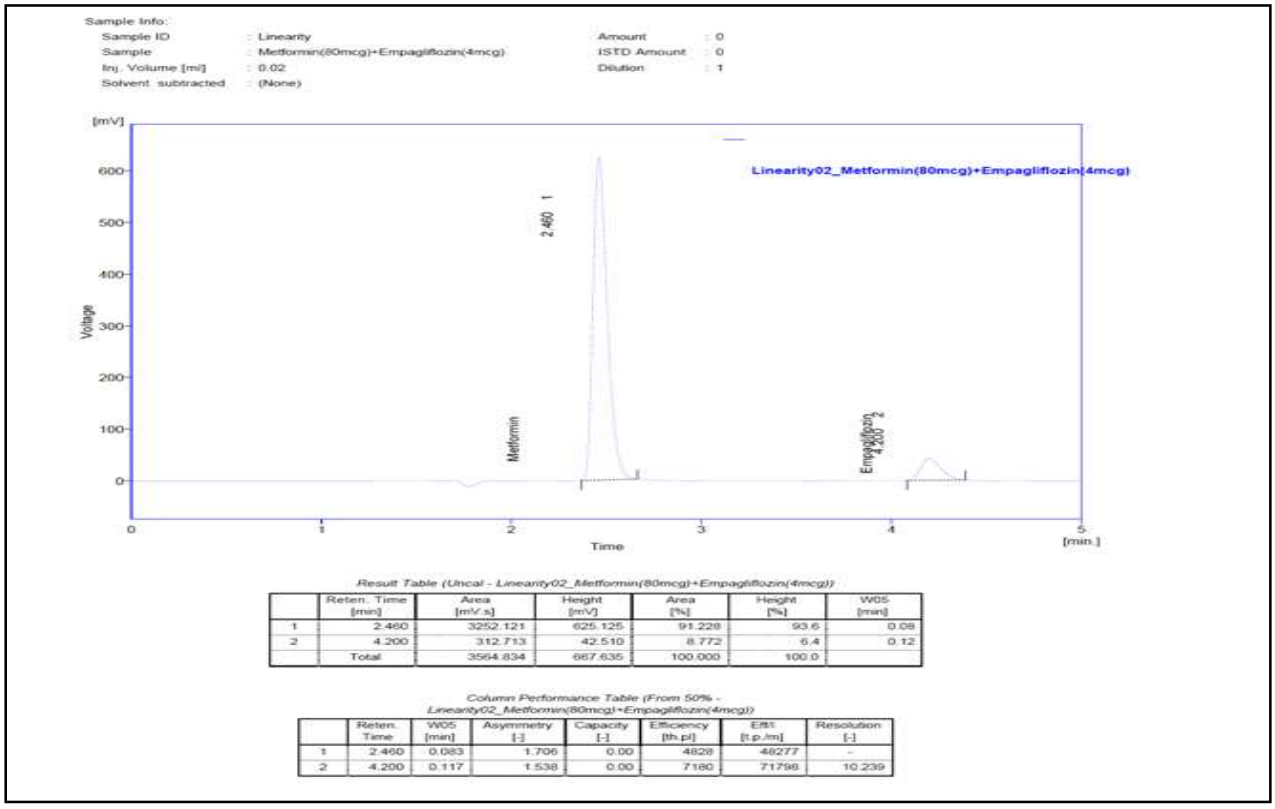
Observation: λ_{max} was found to be 229nm for EMPAGLIFLOZINshown in the Figure 1.2



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.463	2488.713	481.130	91.476	93.8	0.08
2	4.227	231.895	32.048	8.524	6.2	0.12
	Total	2720.609	513.178	100.000	100.0	

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/ [t.p./m]	Resolution [-]
1	2.463	0.083	1.556	0.00	4841	48408	-
2	4.227	0.117	1.407	0.00	7271	72713	10.376

Figure No.3: Chromatogram of METFORMIN and EMPAGLIFLOZIN preparation-1



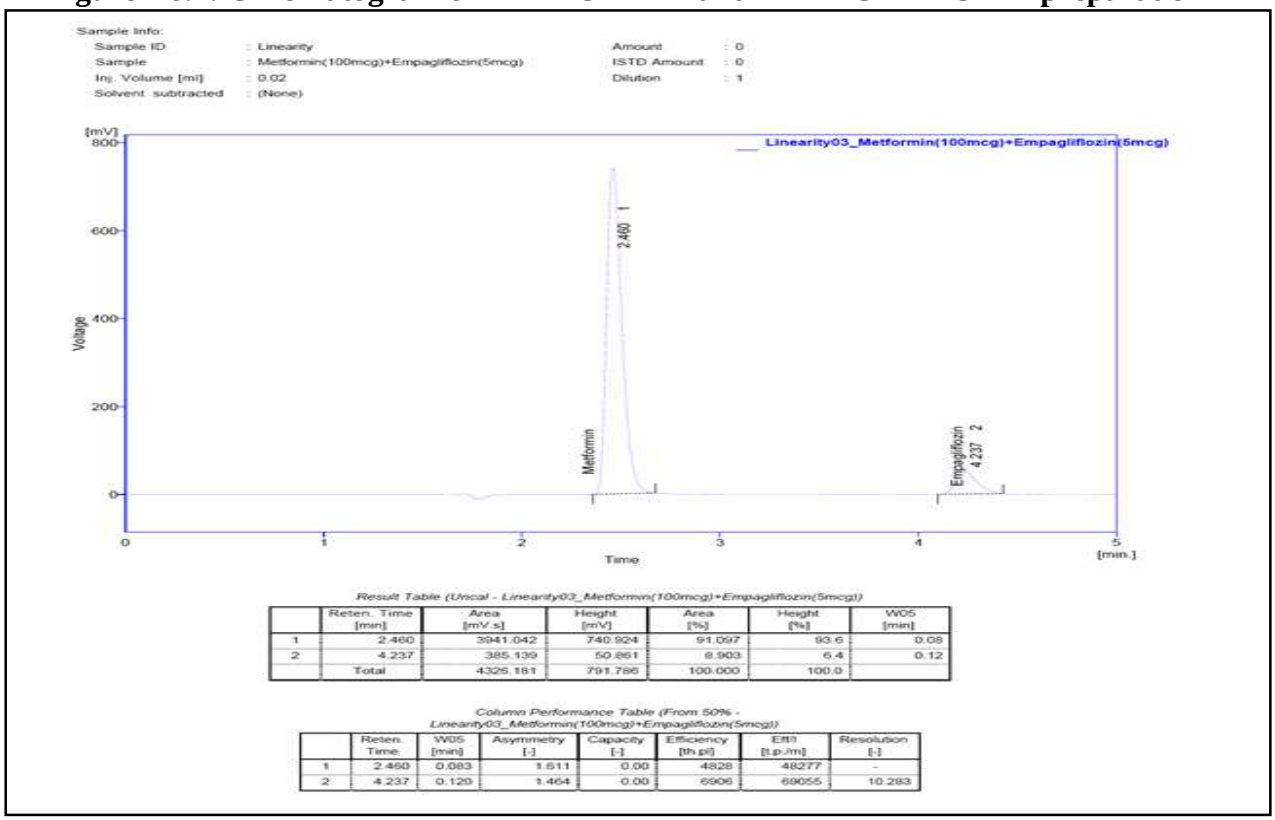
Result Table (Uncal - Linearity02_Metformin(80mcg)+Empagliflozin(4mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.460	3252.121	625.125	91.228	93.6	0.08
2	4.200	312.713	42.510	8.772	6.4	0.12
	Total	3564.834	667.635	100.000	100.0	

Column Performance Table (From 50% - Linearity02_Metformin(80mcg)+Empagliflozin(4mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.460	0.083	1.706	0.00	4828	48277	-
2	4.200	0.117	1.538	0.00	7180	71798	10.239

Figure No.4: Chromatogram of METFORMIN and EMPAGLIFLOZIN preparation-2



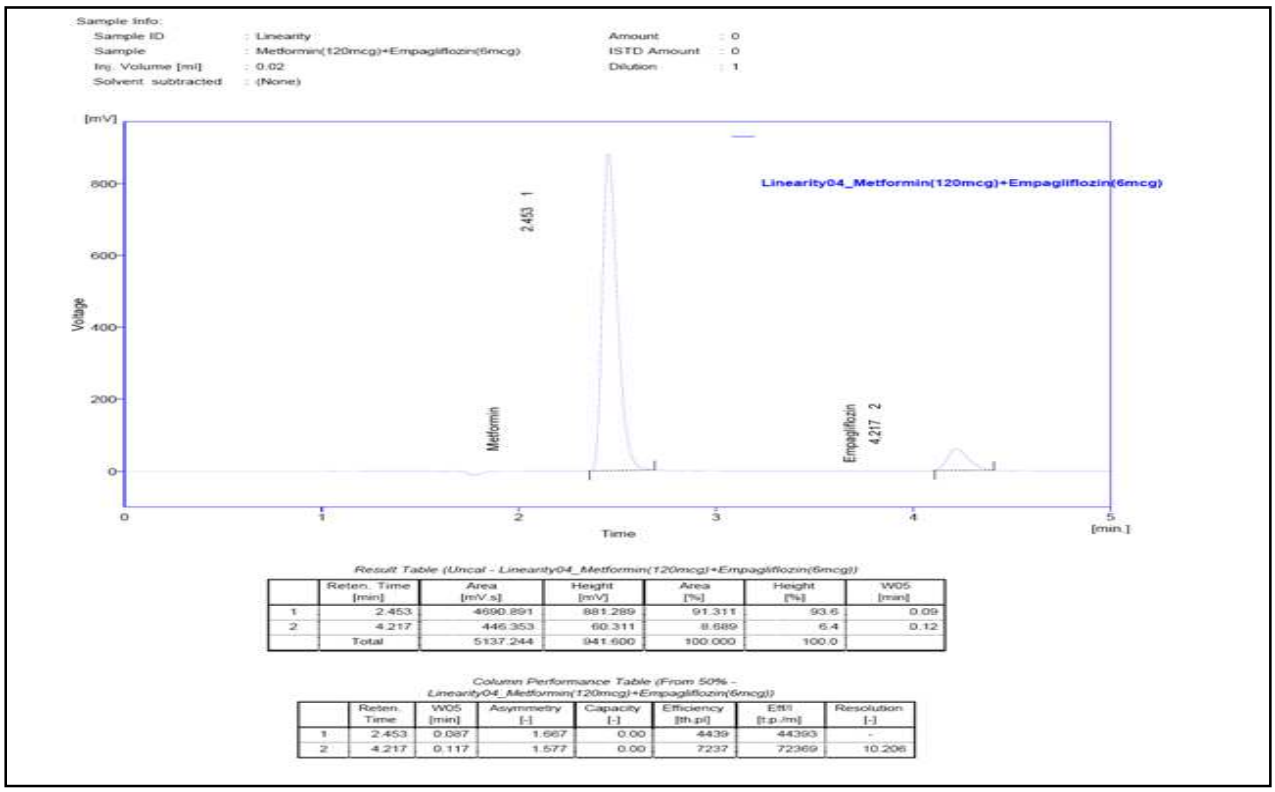
Result Table (Uncal - Linearity03_Metformin(100mcg)+Empagliflozin(5mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.460	3941.042	740.924	91.097	93.6	0.08
2	4.237	385.139	50.861	8.903	6.4	0.12
	Total	4326.181	791.786	100.000	100.0	

Column Performance Table (From 50% - Linearity03_Metformin(100mcg)+Empagliflozin(5mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.460	0.083	1.611	0.00	4828	48277	-
2	4.237	0.120	1.464	0.00	6906	69055	10.283

Figure No.5: Chromatogram of METFORMIN and EMPAGLIFLOZIN preparation-3



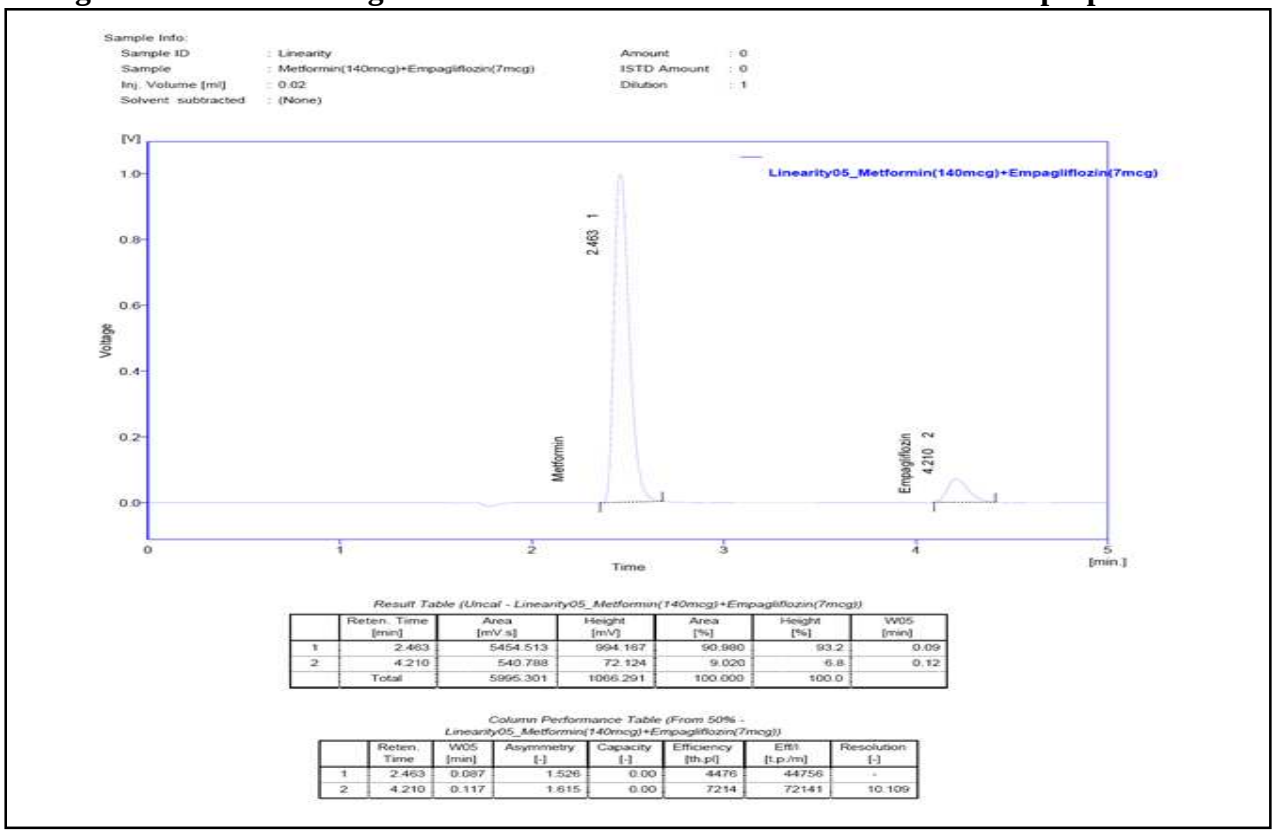
Result Table (Uncal - Linearity04_Metformin(120mcg)+Empagliflozin(6mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.453	4690.891	881.289	91.311	93.6	0.09
2	4.217	446.353	60.311	8.689	6.4	0.12
	Total	5137.244	941.600	100.000	100.0	

Column Performance Table (From 50% - Linearity04_Metformin(120mcg)+Empagliflozin(6mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.453	0.087	1.667	0.00	4439	44393	-
2	4.217	0.117	1.577	0.00	7237	72369	10.206

Figure No.6: Chromatogram of METFORMIN and EMPAGLIFLOZIN preparation-4



Result Table (Uncal - Linearity05_Metformin(140mcg)+Empagliflozin(7mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.463	5454.513	994.167	90.980	93.2	0.09
2	4.210	540.788	72.124	9.020	6.8	0.12
	Total	5995.301	1066.291	100.000	100.0	

Column Performance Table (From 50% - Linearity05_Metformin(140mcg)+Empagliflozin(7mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.463	0.087	1.526	0.00	4476	44756	-
2	4.210	0.117	1.615	0.00	7214	72141	10.109

Figure No.7: Chromatogram of METFORMIN and EMPAGLIFLOZIN for preparation-5

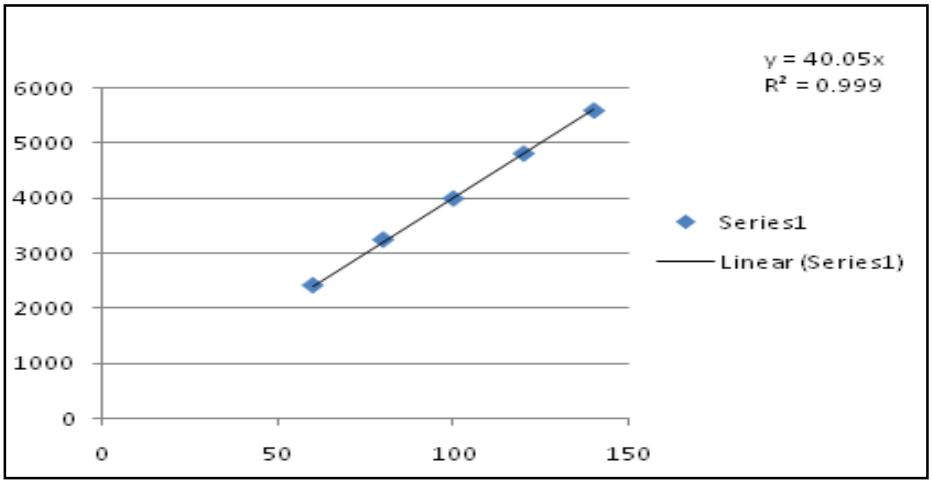


Figure No.8: Linearity graph of METFORMIN

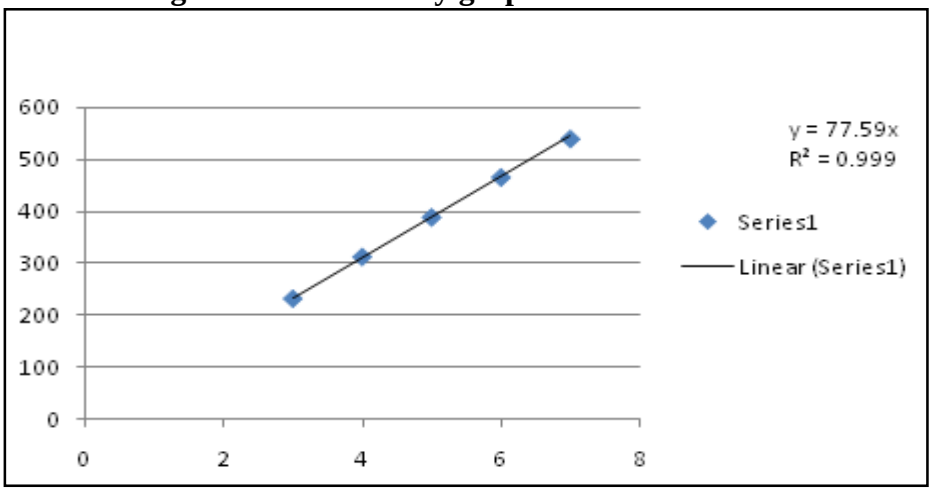


Figure No.9: Linearity graph of EMPAGLIFLOZIN

CONCLUSION

The results was concluded that, a newly developed method for the simultaneous estimation Metformin and empagliflozin drugs was found to be simple, precise, accurate and high resolution and shorter retention time makes this method is more acceptable, cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

ACKNOWLEDGEMENT

The authors are sincerely thanks to Safa College of Pharmacy, Kurnool, Andhra Pradesh, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Sharma B K. HPLC, *Instrumental methods of chemical analysis*, *Goel publishers*, 24th edition, 2005, 286-300.
2. Gurudeep R, Chatwal, Sharm K, Anand. HPLC, *Instrumental methods of chemical analysis*, 2010, 624-639.
3. ICH, Text on Validation of Analytical Procedures, ICH - Q2A, *International Conference on Harmonization*, IFPMA, Geneva, 2-3, 1995, A-1 to A-3.
4. ICH, Validation of Analytical Procedures Methodology, ICH - Q2B, *International Conference on Harmonization*, 1996, 1-3.

5. ICH Guidelines, Q2 (R1) *Validation of Analytical Procedures Text and Methodology,* 2005, 1-6.
6. British pharmacopoeia, 1, 2011, 143-144.
7. United States pharmacopoeia 34 NF29, 2(1), 1873-1875, 1949-1951.
8. Indian pharmacopoeia, 2nd edition, 2010, 806-807, 849-850.
9. The Merck Index, an Encyclopedia of Chemical, Drugs and Biologicals, Maryadele J.O. Neil. Eds, Published by Merck Research Lab, Division of Merck and co. Inc., *Whitehouse Station, NJ,* 13th edition, 2006, 148. NJ: 2006:86.
10. Detectors - http://lipidlibrary.aocs.org/topics /detect92/file.pdf
11. Method development - http://www.pharmainfo.net/reviews/introductionanalyticalmethoddevelopmentpharmacutical-formulations.
12. Manoj K S, Pramod K S, Sambhu C M, Preet K K, Nitin K, Rupesh D. A perspective review on method development and validation by HPLC, *International Journal of Pharmaceutical Sciences,* 4(3), 2011, 1387-1413.

Please cite this article in press as: Jaffar Hussain B *et al*. Method development and validation of Metformin and Empagliflozin in pharmaceutical dosage forms in RP-HPLC, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 4(3), 2016, 91-100.