A NOVEL LC-MS METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN IN PURE AND IN DOSAGE FORM.

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Article History: Received: 10/07/2022 Revised: 16/08/2022 Accepted: 12/09/2022

ABSTRACT:

Aim:

The aim of the present method is to develop and validate a specific, sensitive, precise, and accurate liquid chromatography-mass spectrometry (LC-MS) method for the simultaneous estimation of Saxagliptin and Dapagliflozin .

Methodology:

The effective separation was achieved by using Symmetry C18 (50 × 4.6 mm, 3.5 µm) column and a mobile phase composition (75:25 v/v) A: Acetonitrile : B:2mM Amonium Acetate with 0.2% Formic acid using 0.12 ml/min flow rate and 10 µl of injection volume using Methanol: Water in the ration of 50:50 as diluent. The seperation was monitored on atomic pressure chemical ionization mode mass spectrometer with positive polarity mode. The method is validated as per ICH guidelines.

Results

The retention time of Saxagliptin and Dapagliflozin were found to be at 0.96 min and 1.23 min respectively. The limit of detection (LOD) and limit of quantification (LOQ) were observed at 3 ppb and 10 ppb concentration respectively for the both the drugs. the linear range was found in the concentration ranges from 25 ppb to 150 ppb with regression coefficient of 0.9978 and accuracy in the range of 97.50–102.10%. The percentage relative standard deviation (% RSD) for six replicates said to be injections were less than 10%.

Conclusion

The proposed method was validated successfully as per ICH guidelines. Hence, this is employed for the simultaneous determination Saxagliptin and Dapagliflozin

Key Words: *Saxagliptin, Dapagliflozin,chemical ionisation,LC/MS, ICH*

Introduction:

Saxagliptin [2, 7] (SAXA) acting as Anti Diabetic agent (Dipeptidyl peptidase-4 inhibitor) which boosts post prandial insulin release, decrease Glucagon secretion and lower mean time as well as fasting blood glucose in type 2 diabetes. [1] This agent is used in combination with other oral hypoglycemic agents. Dapagliflozin [2,5] (DAPA) acting as Sodium-Glucose cotransporter-2(SGLT2) inhibitors. This agent is used in combination with diet and exercise to improve glycemic control in adult with type -2 Diabetes. SGLT 2 is major transporter of glucose whose inhibition induces glucosuria and lower blood sugar in type 2 diabetes mellitus. [2] Several analytical methods are available which can determine SITA and DAPA individually or in combination with another drug. From detailed review of literature, it was found that no analytical method is available for determination of DAPA and SITA from simulated mixture or formulation, [3,4,6] In general, various analytical methods like high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy, and liquid chromatography-mass spectrometry (LC-MS) are used for identification, characterization, and quantification of the drug substances and drug product in combined form.[8-13] Based on literature review, few analytical methods like high-performance liquid chromatography methods and spectrophotometric methods were available for separation of Saxagliptin and Dapagliflozin But as of now, no LC-MS method has been reported for the simultaneous determination of Saxagliptin and Dapagliflozin. Hence, we aimed to develop a LC-MS method for the simultaneous determination Saxagliptin and Dapagliflozin

Materials and Methodology: [14-18]

Saxagliptin and Dapagliflozin reference standard (claim 99.32%) were procured from Horizon Pharma, Hyderabad, as a gift sample.

All UPLC grade solvents were obtained from the Merck India Limited, Mumbai, India. All the solvents and solutions used were filtered through millipore (0.25 μm) filters.

Chemicals Details:

Ammonium Acetate	Fischer chemical	Optima
Formic acid	Merck	Emplura
Acetonitrile	J T Baker	HPLC
Methanol	J T Baker	HPLC
Milli-Q Water	Millipore	Milli-Q

Instrument Details

UPLC ID.: Waters Acquity UPLC

MASS ID.: Waters Quattro Premier XE

Optimized LC-MS method conditions:

The Waters LC-MS with auto sampling system was used to perform the method. Separation of the phenyl vinyl sulfone was successfully achieved by the Symmetry C18 (50 × 4.6 mm, 3.5 µm) column and a mobile phase composition of 0.1%v/v ammonia buffer to methanol (5:95 v/v), using 0.45 ml/min flow rate and 20 µl of injection volume, with methanol used as diluent. The temperature maintained in the auto sampler and column was 5 °C and ambient respectively. MS parameters were mentioned in the table

Analytical Method:

A summary of the chromatographic and mass spectrometric conditions are as follows:

HPLC	:	Waters Acquity UPLC
MASS	:	Waters Quattro Premier XE
Ion source	:	Electrospray ionization (ESI)
Detection ions (m/z)		
Polarity	:	Positive ion mode
Saxagliptin	:	316.20* amu (parent), 180.10* amu (product)
Dapagliflozin	:	426.10* amu (parent), 135.10* amu (product)
Column	:	Agilent XDB Zorbax C18 2.1 × 50 mm, 3.5 µm.
Column oven temperature	:	30 ºC
Autosampler temperature	:	10 ºC
Mobile phase	:	Mobile Phase : Isocratic flow (75:25 v/v) A: Acetonitrile : B:2mM Amonium Acetate with 0.2% Formic acid in water
Flow rate	:	0.120 mL/min
Volume of injection	:	10 µL
Run time	:	3.0 minutes
Diluent	:	Methanol: Water (50: 50)

□□This parameter may change by 0.5 units.		
MRM Conditions**		
Positive ion mode:		
Capillary (KV)	:	3.00
Extractor (V)	:	3
Source Temperature	:	120 ºC
Desolvation Temperature	:	400 ºC
Cone Gas Flow(L/Hr)	:	100
LM1 Resolution	:	15
HM1 Resolution	:	15
Multiplier (V)	:	550

Test Item Name	Cone (Voltage)**	Collision Energy (CE)**
Saxagliptin	30	25
Dapagliflozin	30	25

** These parameters may vary from one instrument to another instrument due to their Mass calibration parameters

Preparation of Reagents/Buffers/Mobile Phase/Diluent:

Preparation of 2mM Ammonium Acetate with 0.2% formic acid in water (v/v)

To approximately 500 mL of water, added about154 mg of Ammonium Acetate and then added 2.000 ml of formic acid mix well and make up to 1000 mL and sonicate. Label and store the solution at ambient temperature.

Preparation of Acetonitrile (v/v)

To 1000 mL bottle add approximately 1000 mL of Acetonitrile was taken, label and store the solution at ambient temperature. Record the details in 'Reagent / Solution Preparation Form'.

Preparation of 50% Methanol in Water (Diluent-1)

To 50 mL of Methanol, add 50 mL of water and sonicate. Label and store the solution at ambient temperature.

Preparation of 50% Methanol in Water (Rinsing Solution)

To 500 mL of Methanol, add 500 mL of water and sonicate. Label and store the solution at ambient temperature.

Saxagliptin and Dapagliflozin stock solution (100.000 µg/mL):

Weigh about 1.000 mg of Saxagliptin and Dapagliflozin separately and dissolve in 10mL of methanol: HPLC Water (50:50) to obtain a known concentration of100.000µg/mL. Label the stock solution and store in refrigerator (2ºC to8ºC).

Preparation of working standard dilution:

Take around 50µL stock solution of Saxagliptin and Dapagliflozin and dilute to 50 mL with diluent to obtain approximately concentration of 100.000 ng/mL. Label the ISTD dilution and store in refrigerator (2ºC to 8ºC).

Method validation

System suitability test

The system suitability of the method was performed by injecting blank solution once and 100% level standard solution of Saxagliptin and Dapagliflozin for six times into LC-MS system. The system suitability was confirmed by assessing the % RSD.

Linearity

The linearity of a method represents the direct proportional relationship between concentration and test result. It was conducted for the Saxagliptin and Dapagliflozin in the range of LOQ level (3 ppm) to about 150% of limit (10 ppm) by injecting each level of concentration two times and plotted a curve between average peak area versus concentration in ppm to find out the regression coefficient value.

Accuracy

The accuracy of the method has been done by performing the recovery studies, at LOQ level, 50%, 100%, and 150% level. A known amount of Saxagliptin and Dapagliflozin spiked separately to pre-analyzed samples of the mentioned levels. Each spiked level was injected for three times into the LC-MS system and calculated the percent recovery of each level.

Method precision

The method precision was performed by spiking the sample with phenyl vinyl sulfone at 100% of the specified limit with respect to the sample concentration. Six homogenous replicates were injected and calculated the content of phenyl vinyl sulfone to determine the % RSD.

Intermediate precision

The intermediate precision was performed by spiking the sample with phenyl vinyl sulfone at 100% of the specified limit with respect to the sample concentration in six preparations. The intermediate precision study was carried out on different days with different analysts. We calculated the content of Saxagliptin and Dapagliflozin in spiked preparations and determined the % RSD.

Sensitivity (LOD and LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were determined by using signal to noise ratio. The LOD solution was prepared in such a way to obtain the S/N ratio is about 3:1 to 5:1. Based on the concentration of LOD, the LOQ solution was prepared (3 times to LOD concentration) to obtain the signal to noise ratio of about 10:1 to 15:1.

Results and Discussion:

Initially, the solubility of the Saxagliptin and Dapagliflozin was checked in various solvents. It was found that Saxagliptin and Dapagliflozin **were** soluble in methanol and water.

Method optimization

The retention time of Saxagliptin and Dapagliflozin were found to be at 0.96 min and 1.23 min respectively. The limit of detection (LOD) and limit of quantification (LOQ) were observed at 3 ppb and 10 ppb concentration respectively for the both the drugs. the linear range was found in the concentration ranges from 25 ppb to 150 ppb with regression coefficient of 0.9978 and accuracy in the range of 97.50–102.10%. The percentage relative standard deviation (% RSD) for six replicates said to be injections were less than 10%.

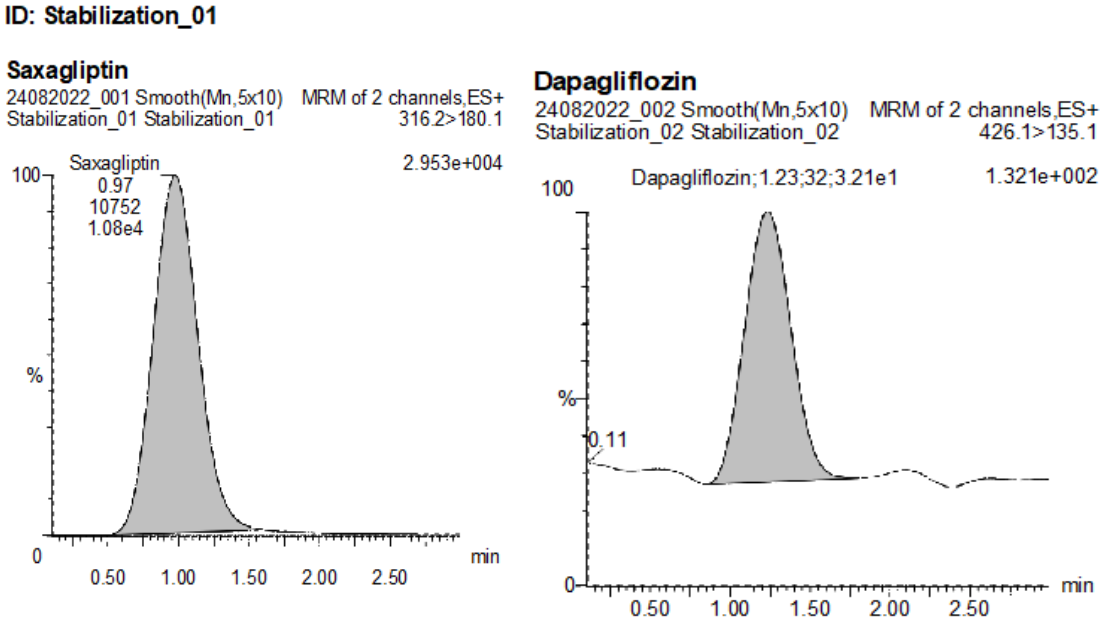

Figure No:1 Method Stabilisation

The developed method was validated as per Q2 specifications of the ICH guidelines.

Linearity

The linearity of the present method was done for Saxagliptin and Dapagliflozin in the range of LOQ level to about 150% of limit. The regression coefficient (R^2) value was calculated from the calibration curve, which was constructed by plotting between obtained peak area and concentrations.

R^2 value of the calibration curve was 0.9980. The results are shown in Fig.

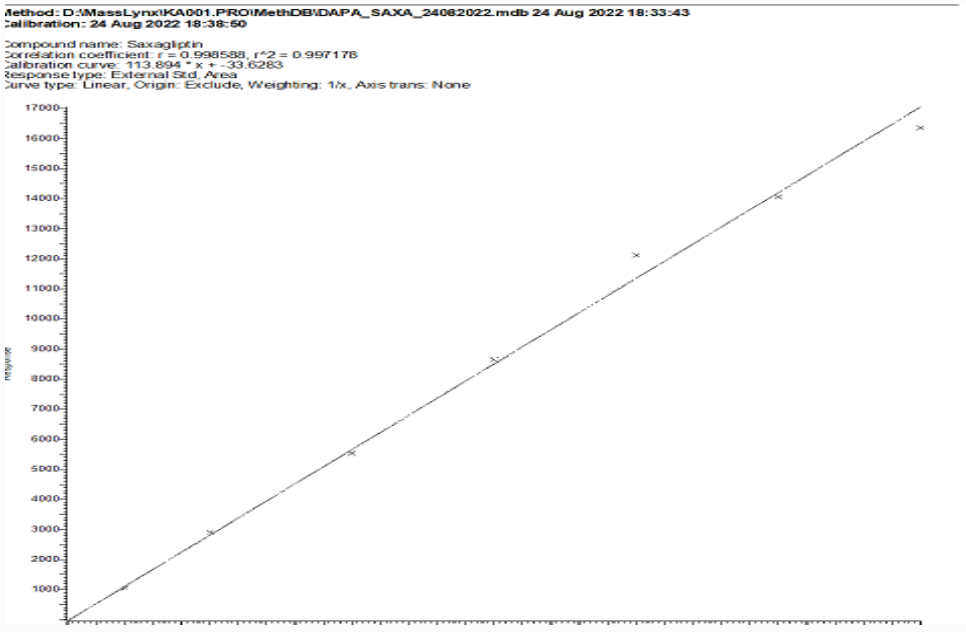

Figure No 2: Linearity of Saxagliptin

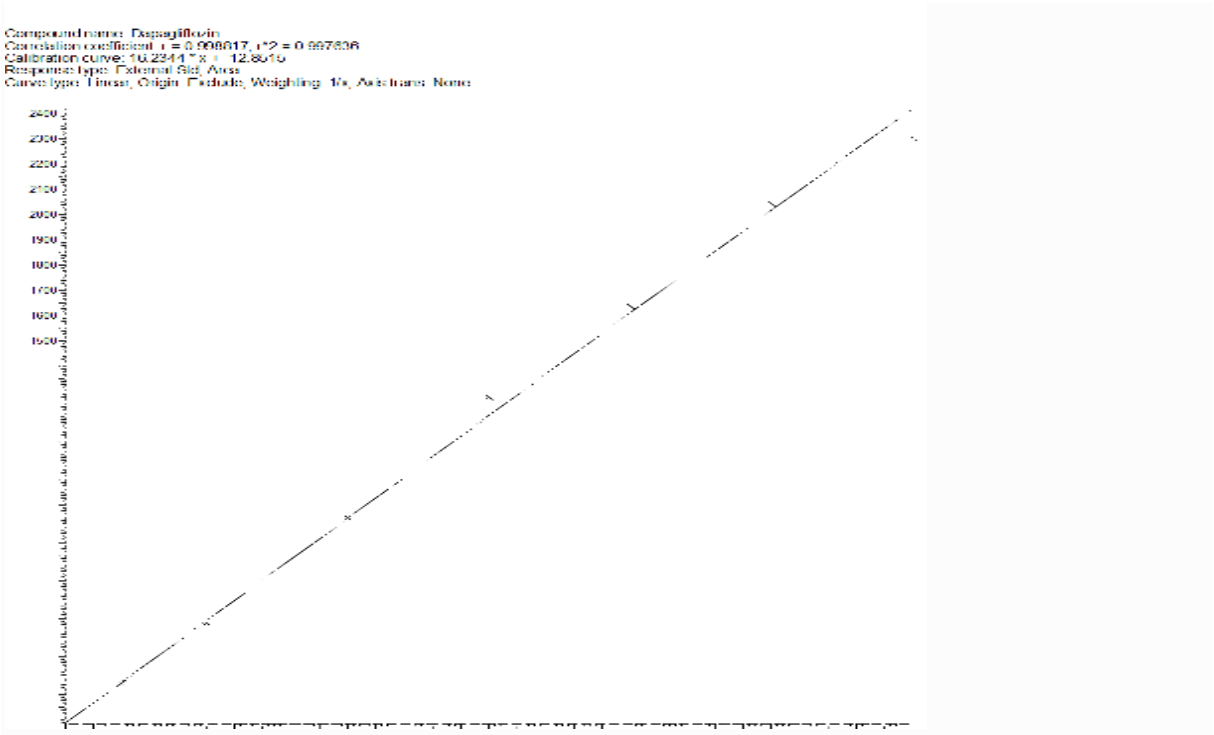

Figure No 3: Linearity of Dapagliflozin

Accuracy

The percentage recovery of Saxagliptin and Dapagliflozin from the different levels of spiked sample solutions was in the range of 97.50–102.10% (Table) which indicates that accuracy of the proposed method was very accurate and the results were lied within the acceptance limits (± 25%) of the ICH guidelines.

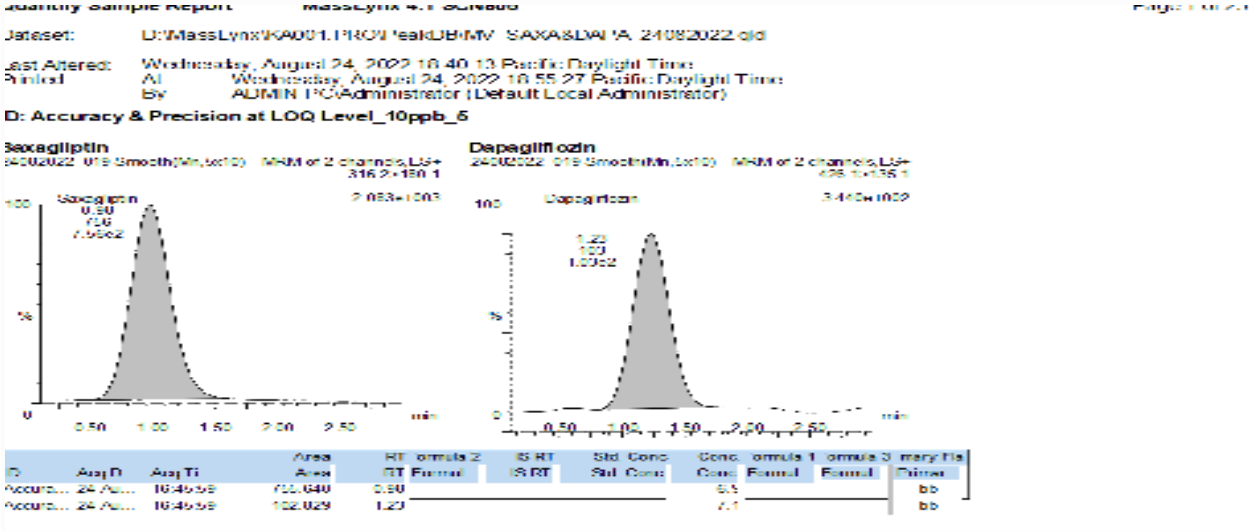

Figure No 4 : Accuracy

Precision

The % RSD values for method precision and intermediate precision of the Saxagliptin and Dapagliflozin were found to 1.88 and 1.91 for the 100% level concentration . The lower values (≤ 10) of both precisions represents that the method has good precision.

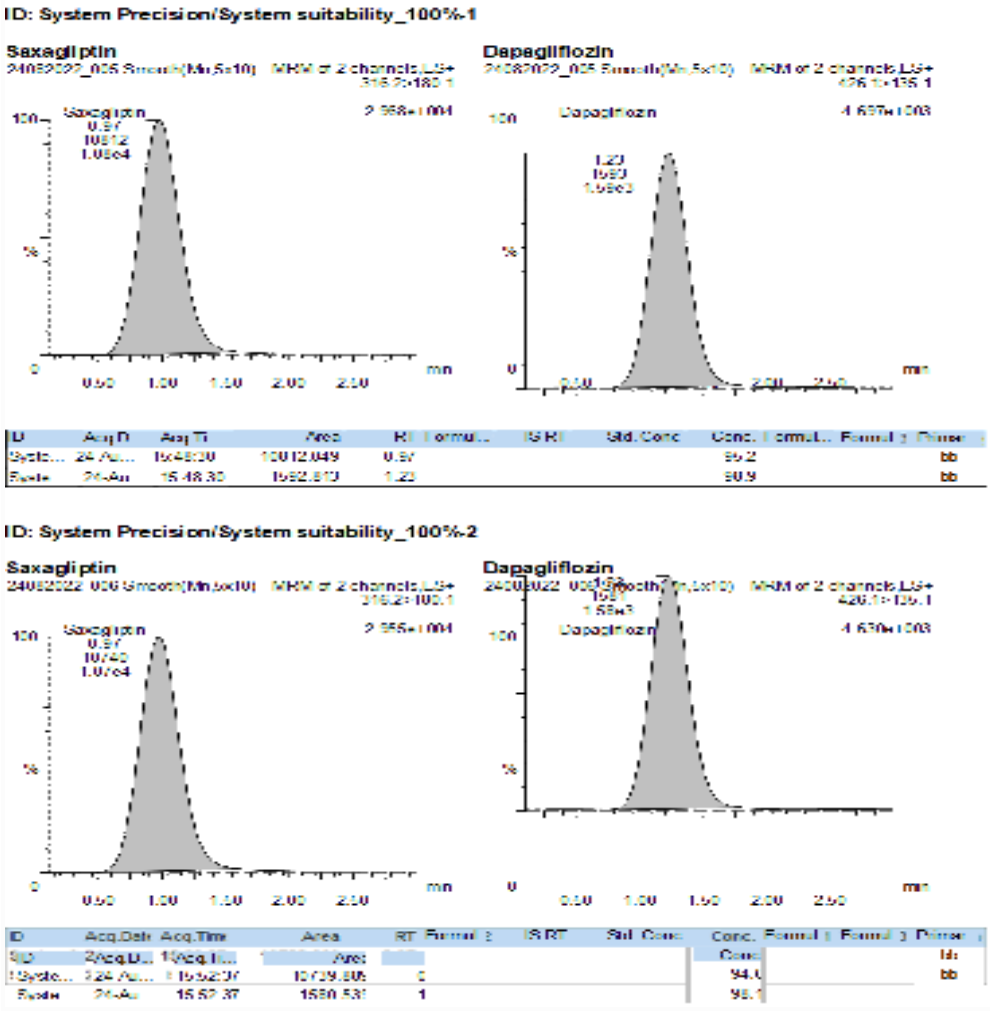

Figure No 5: Precision

Sensitivity

The LOD and LOQ of the phenyl vinyl sulfone were found to be 3 ppm and 10 ppm, respectively, which indicates that the method has good sensitivity. Table shows the LOD and LOQ results of the Saxagliptin and Dapagliflozin.

ID	Acq.D...	Acq.Ti...	Area	RT
LOD_3...	24-Au...	16:17:14	188.316	0.98
LOD_3...	24-Au...	16:17:14	26.074	1.23

ID	Acq.D...	Acq.Ti...	Area	RT	Formul...	IS RT	Std. Conc	Conc.	Formul...	Formul...	Primar...
LOQ_...	24-Au...	16:21:20	758.139	0.97				7.0			bb
LOQ_...	24-Au...	16:21:20	113.301	1.23				7.8			bb

As per extensive literature review, as of now, no LC/MS method has been reported for the estimation of Saxagliptin and Dapagliflozin. Among the various analytical techniques, the LC-MS method is a proficient and insightful technique to separate, identify, and quantify the impurities of the drug substance and drug product . Few analytical methods were reported for the estimation Saxagliptin and Dapagliflozin .The developed LC-MS method was highly effective to separate, identify, and quantify the polyvinyl sulfone. The current method has good sensitivity with a detection level of 3 ppm of Saxagliptin and Dapagliflozin with high specificity. Symmetry C18 (50 × 4.6 mm, 3.5 µm) column and a mobile phase composition (75:25 v/v) A: Acetonitrile : B:2mM Amonium Acetate with 0.2% Formic acid using 0.12 ml/min flow rate and 10 µl of injection volume using Methanol:Water in the ration of 50:50 as diluent.a simple solvent system represents the cost effectiveness of the method. A Novel Rapid analysis for the simultaneous estimation Saxagliptin and Dapagliflozin

Conclusion:

A specific and sensitive LC-MS method was developed for the simultaneous estimation Saxagliptin and Dapagliflozin pure and pharmaceutical dosage form. The proposed method has very good sensitivity, accuracy, and specificity. Hence, this method has intended use in the process chemistry department and quality control department.

References:

1.Williams DM, Stephens JW. Combination Therapy with Saxagliptin and Dapagliflozin for the Treatment of Type 2 diabetes. *Expert Opin Pharmacother.* 2015;16:2373–2379.

2. FDA Approves Once-Daily Qtern (dapagliflozin and saxagliptin) Tablets for Adults with Type-2 Diabetes [Internet]. Drugs.com.

3. Manasa S, Dhanalakshmi K, Nagarjunareddy G, Sreenivasa S. Development and Validation of a RP-HPLC Method for the Estimation of Dapagliflozin in API. *Int J Pharm Sci.* 2014;5:5394–5397.

4. Shyamala M, Nidhi B, Kavitha M, Pooja Sharma JV. Validated RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Dapagliflozin in Tablet Dosage Form. *AJBPR.* 2015;2:109–113.

5. Dapagliflozin National Center for Biotechnology Information. PubChem Compound Database. U.S. National Library of Medicine; May 23, 2017

6. Dave DJ. Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus. J Pharmacol Pharmacother. 2011;2:230–235

7. Saxagliptin [Internet]. National Center for Biotechnology Information. PubChem Compound Database; U.S. National Library of Medicine; accessed May 23, 2017.

8. Gumieniczek A, Berecka A. Analytical tools for determination of new oral antidiabetic drugs, glitazones, gliptins, gliflozins and glinides, in bulk materials, pharmaceuticals and biological samples. Open Chem. 2016;14:215–242.

9. El-Bagary RI, Elkady EF, Ayoub BM. Spectrophotometric Methods Based on Charge Transfer Complexation Reactions for the Determination of Saxagliptin in Bulk and Pharmaceutical Preparation. Int J Biomed Sci. 2012;8:204–208.

10. Aubry AF, Gu H, Magnier R, Morgan L, Xu X, Tirmenstein M, Wang B, Deng Y, Cai J, Couerbe P, Arnold M. Validated LC-MS/MS Methods for the Determination of Dapagliflozin, a Sodium-Glucose Co-Transporter 2 Inhibitor in Normal and ZDF Rat Plasma. *Bioanalysis.* 2010;2:2001–2009.

11. Batta N, Pilli N, Derangula V, Vurimindi H, Damaramadugu R, Yejella R. A Rapid and Sensitive LC-MS/MS Assay for the Determination of Saxagliptin and its Active Metabolite 5-Hydroxy Saxagliptin in Human Plasma and its application to a pharmacokinetic study. *Drug Res.* 2014;65:133–140.

12. Pawanjeet JC, Balaji M, Srinivasarao V, Ramakrishna K, Apparao KM. Devolpment and validation of simple stability indicating RP-HPLC method for analysis of saxagliptin and its forced degradation impurities in bulk drug and pharmaceutical dosage form. *Int J Res Dev Pharm L Sci.* 2014;3:993–1003.

13. Scheeren LE, Marcolino AIP, Adams AIH, Rolim CMB. Stability indicating RP-LC-PDA method for the quantitative analysis of saxagliptin in pharmaceutical dosage form. *Braz J Pharm Sci.* 2015;51:461–466

14.Awuchi CG, Twinomuhwezi H, Awuchi CG (2022) Hyphenated techniques. In: Analytical techniques in biosciences. Elsevier, pp 125–145.

15.Wilson ID, Brinkman UAT (2003) Hyphenation and hyphenation. J Chromatogr A 1000:325–356.

16. Kumar A, Vyas J, Mishra SB, Patel AB, Patel NK, Shah SR, Sheth DB (2022) A brief review on liquid chromatography-mass spectrometry/LCMS and its application. AJPA 12:203–210

17. Srivastava B, Haribabu Y (2021) Method development and validation of simultaneous estimation of emtricitabine and tenofovir alafenamide in bulk and tablet dosage form using LC-MS/MS. RJPT 25:2493–2496.

18.Farré M, Petrovic M, Barceló D (2007) Recently developed GC/MS and LC/MS methods for determining NSAIDs in water samples. Anal Bioanal Chem 387:1203–1214.

19. A Validated LC-MS/MS method for determination of dapagliflozin in tablet formulation, IOSR Journal Of Pharmacy. 2019;9(8)Series. II:01-06.