Analytical Method Development and Validation for Simultaneous Estimation of Saxagliptin, Dapagliflozin and Metformin Hydrochloride by HPLC in Synthetic Mixture

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Abstract- A Simple, linear, precise, robust and accurate method of reverse phase high performance liquid chromatography for simultaneous estimation of Saxagliptin, Dapagliflozin and Metformin HCl in their Synthetic mixture has been developed. The separation was achieved by LC- 20 AT C_{18} (250mm x 4.6 mm x 2.6 µm) column and buffer pH 4.0: Acetonitrile (50:50) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 258 nm. Retention time of Saxagliptin, Dapagliflozin and Metformin HCl were found to be 3.6 min, 4.2 min, 8.4 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Metformin HCl 250-750 µg/ml, for Saxagliptin 1.25-3.75 µg/ml and for Dapagliflozin 1.25-3.75 µg/ml. Developed method was found to be Simple, linear, precise, robust and accurate for simultaneous estimation of Saxagliptin, Dapagliflozin and Metformin HCl in their synthetic mixture.

I. INTRODUCTION[1-14]

Diabetes mellitus type 2 previously known as noninsulin-dependent mellitus is such a disorder where these blood glucose levels are above their normal levels due to lack of partial or complete response to insulin by the human body.

Saxagliptin is an orally active hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. It works by affecting the action of natural hormones in the body called incretins. Dapagliflozin is in a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. Metformin is a first-line therapy for type 2 diabetes mellitus (T2DM, formerly 'non-insulin-dependent diabetes mellitus'), and is one of the most commonly prescribed drugs worldwide. A drug combination of these drugs has recently been approved by FDA by the name of 'Qternmet XR' marketed by Astrazeneca.

High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography, is a separation technique which involves solid stationary phase and a liquid

mobile phase. Today, HPLC is most widely used analytical separation method. The method is popular because it is non-destructive and may be applied to thermally liable compounds (unlike GC); it is also a very sensitive technique since it incorporates a wide choice of detection methods. With the use of post column derivatization methods it improves selectivity and detection limits, HPLC can easily be extended to trace determination of compounds that do not usually provide adequate detector response. So this was utilized to develop a method for these three drugs in their Synthetic mixture.

Validation proves that a procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.
The various validation parameters are

1. Accuracy.
2. Precision (repeatability and intermediate precision).
3. Selectivity and/or Specificity.
4. Limit of detection (LOD) and Limit of quantitation (LOQ).
5. Linearity and range.
6. Robustness and Ruggedness.

Literature review reveals that numbers of analytical methods are available for estimation of Saxagliptin, Dapagliflozin and Metformin HCl in their bulk and pharmaceutical formulation individually and in combined Dosage form.No method has been reported for simultaneous estimation of Saxagliptin, Dapagliflozin and Metformin HCl in their Synthetic mixture by HPLC. No reported or official method available for their estimation as the drug combination has been recently approved by USFDA in May 2019. Therefore, it was thought worthwhile to develop HPLC Method for the Simultaneous Estimation of Saxagliptin, Dapagliflozin and Metformin HCl in Synthetic mixture

II. MATERIALS AND METHODS

A. Apparatus and Instrument

Sr. No.	Name of Equipment	Manufacturer
1	UV Spectrophotometer	LabMan
2	HPLC	Shimadzu
3	Analytical balance	Electronic Balance (Shimadzu AUX220)
4	pH Meter	LabMan
5	Melting Point Apparatus	LabMan
6	Ultrasonicator	Dolphin
7	Hot air oven, MITEC-784	Patel Scientific
8	FT-IR	Bruker, Alpha-II

B. Reagents and Materials.

Methanol, Water and Acetonitrile of HPLC grade.
Potassium di-hydrogen ortho phosphate and Ortho-phosphorice acid of AR grade.

C. Preliminary Analysis of Drug.[9-12]

1) Description

The sample of Saxagliptin, Dapaglifozin, Metformin HCl was observed for its color and texture.

2) Melting point

The sample of Saxagliptin, Dapaglifozin, Metformin HCl was taken in capillary and place into the melting point apparatus. Observed the melting point and compared with the reference.

Drug	Melting Point [Observed]	Melting Point [Standard]
Dapagliflozin	54-56 °C	55-58 °C
Metformin HCl	222-226 °C	223-226 °C
Saxagliptin	202-206 °C	203-206 °C

3) Identification Test

Potassium Bromide IR disc was prepared using 1mg of Saxagliptin, Dapaglifozin, Metformin HCl on Hydraulic Pellet Press. This disc was scanned in the region of 4000–400cm^{-1} in FTIR and obtained IR spectrum was compared with the reference spectrum of Metformin HCl.

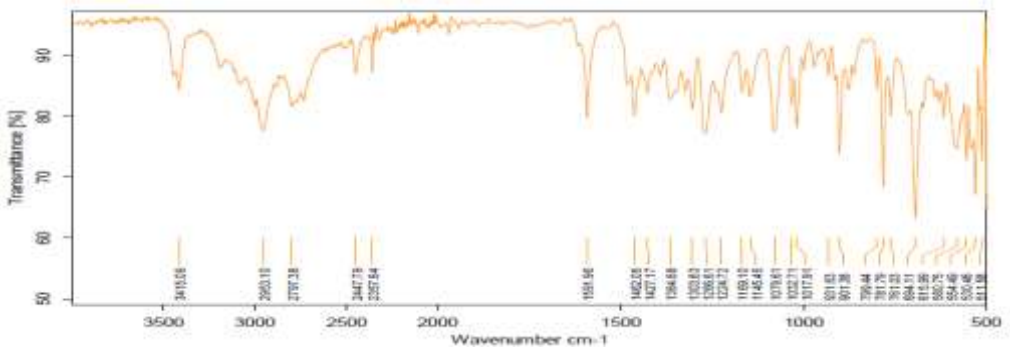


Figure 1 IR spectra of Sample Metformin HCl

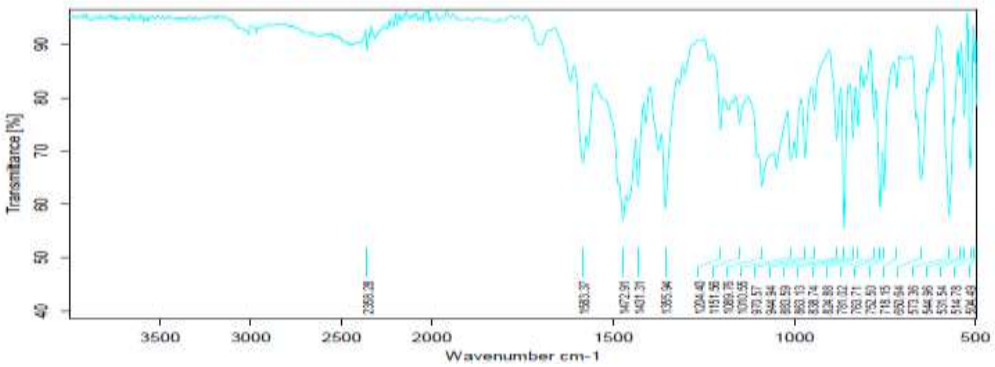


Figure 2 IR spectra of Sample Dapagliflozin

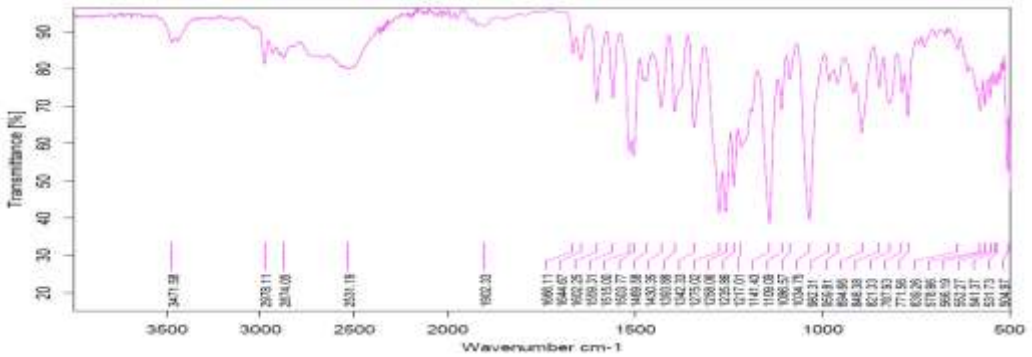


Figure 3 IR spectra of Sample Saxagliptin

4) Solubility

The samples of Saxagliptin, Dapaglifozin and Metformin HCl were taken in test tubes and observed for solubility in various solvents like water, methanol, 0.1 N HCl and 0.1 N NaOH.

Solvent	Metformin HCl	Dapagliflozin	Saxagliptin
Distilled Water	Freely soluble	Insoluble	Slightly soluble
0.1 M HCl	Soluble	Soluble	Insoluble
0.1 M NaOH	Insoluble	Slightly Soluble	Insoluble
Methanol	Freely soluble	Soluble	Soluble
Acetonitrile	Freely soluble	Soluble	Soluble

D. Preparation of standard solutions

I. Preparation of Metformin HCl Stock solution (5000µg/mL)

Accurately weigh 500mg of Metformin HCl and Transferred to 100ml volumetric flask and volume was made up 100 ml with methanol.

II. Preparation of Dapagliflozin Stock solution (25µg/mL)

Accurately weigh 25mg of Dapagliflozin and Transferred to 100ml volumetric flask and volume was made up 100ml with methanol. Taken 10ml from this solution and transfer to 100ml volumetric flask and volume was made up 100ml with methanol.

III. Preparation of Saxagliptin Stock solution (25µg/mL)

Accurately weigh 25mg of Saxagliptin and Transferred to 100ml volumetric flask and volume was mad up 100ml with methanol. Taken 10ml from this solution and transfer to 100ml volumetric flask and volume was made up 100ml with methanol.

IV. Preparation of standard working standard solution (Saxagliptin 2.5µg/mL, Dapagliflozin 2.5µg/mL and Metformin 500µg/mL)

1 mL from Saxagliptin stock solution,1 mL from Metformin stock solution and 1ml from Dapagliflozin stock solutions were transferred to 10 mL volumetric flask and volume was made up to the mark by Mobile phase which was used in particular trials.

V. Preparation of formulation solution

Sample Stock Solution (Saxagliptin 25 µg/mL, Dapagliflozin 25 µg/mL and Metformin 5000 µg/mL)

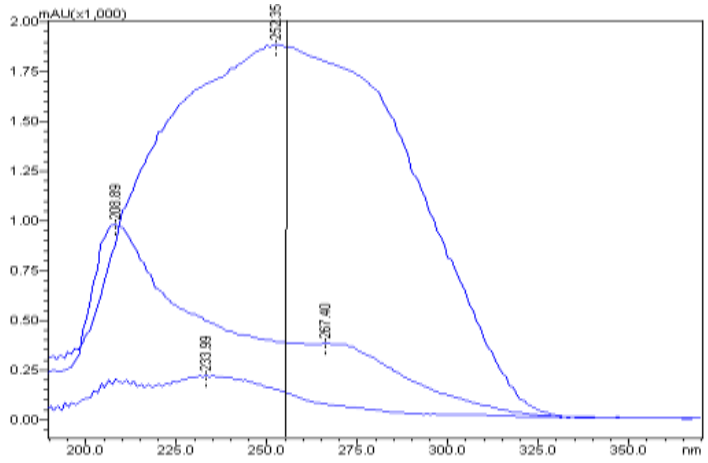
Accurately weighed synthetic mixture powder equivalent to 500mg of Metformin, 2.5mg Dapagliflozin and 2.5mg of Saxagliptin was transferred to a 100 ml volumetric flask, 60 ml Mobile phase was added and Shake for 15 min and volume was made upto the mark with mobile phase. The solution was filtered through whatman filter paper no. 42.

Working Sample Preparation (Saxagliptin 2.5µg/mL, Dapagliflozin 2.5µg/mL and Metformin 500µg/mL):

1 mL from sample stock solution was transferred to 10 ml volumetric flask and volume was made up to the mark with Mobile phase.

E. Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. The drug solutions were scanned in UV region of 200-400 nm and overlay spectrums were recorded.



F. Selection of Mobile Phase

Trials contain various mobile phase which are considered of Methanol, Water, Acetonitrile and Buffer in different proportions and different volumes at different flow rate were tried. On the basis of various trails the mixture of Buffer at pH 4.0: Acetonitrile (50:50), at 1.0 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

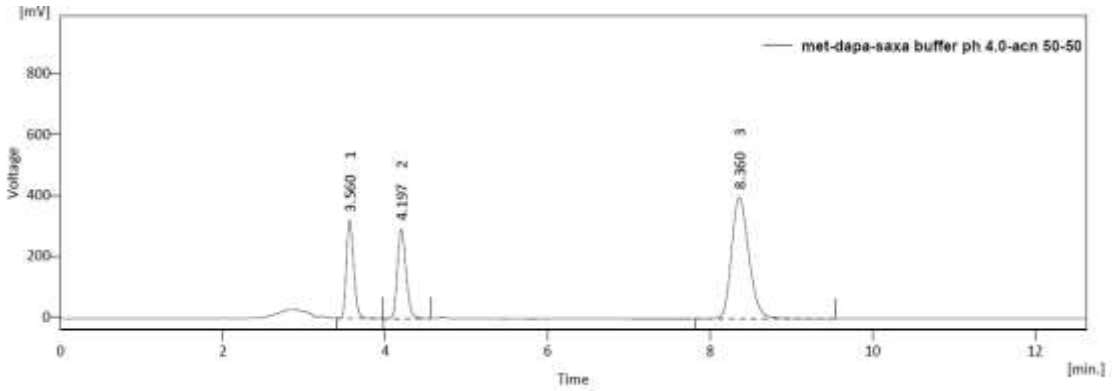


Figure 4: HPLC Chromatogram of Saxagliptin 2.5µg/mL, Dapagliflozin 2.5µg/mL and Metformin 500µg/mL in Buffer, pH 4.0: Acetonitrile (50:50)

G. System suitability test

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Parameters	Saxagliptin	Dapagliflozin	Metformin HCl
Retention Time	3.560	4.197	8.360
Theoretical Plates	7021	6776	7319
Asymmetry	1.391	1.321	1.352
Resolution	-	3.41	14.00

H. Optimized Chromatographic conditions

Column: C_{18} (250 mm × 4.6 mm, 5 µm)
Mobile Phase: Buffer (pH 4.0): Acetonitrile (50:50)
Flow Rate: 1.0 ml/min
Detection Wavelength: 258 nm
Run time: 20 min
Injection volume: 20.0 µl

III. METHOD VALIDATION[7]

A. Specificity

To Perform the Specificity Individual and Combined Solution of Metformin HCl 500µg/mL, Saxagliptin 2.5µg/mL and Dapagliflozin 2.5µg/mL were injected and interference was checked with the Chromatogram of Blank

B. Linearity

Correlation co-efficient for calibration curve of Metformin HCl, Saxagliptin and Dapagliflozin was found to be 0.999, 0.997 and 0.997 respectively.

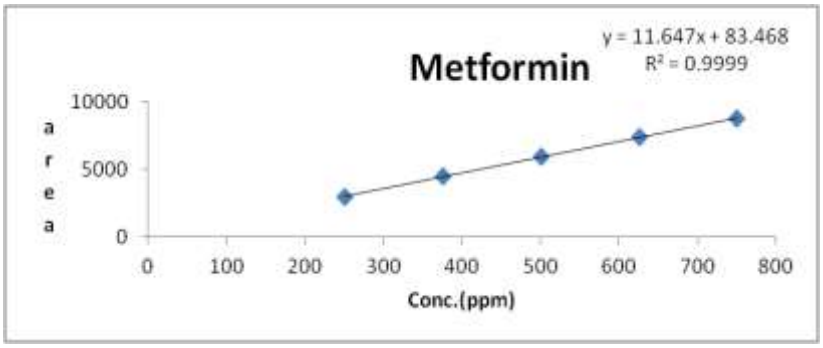


Figure 5 Callibration curve for Metformin HCl.

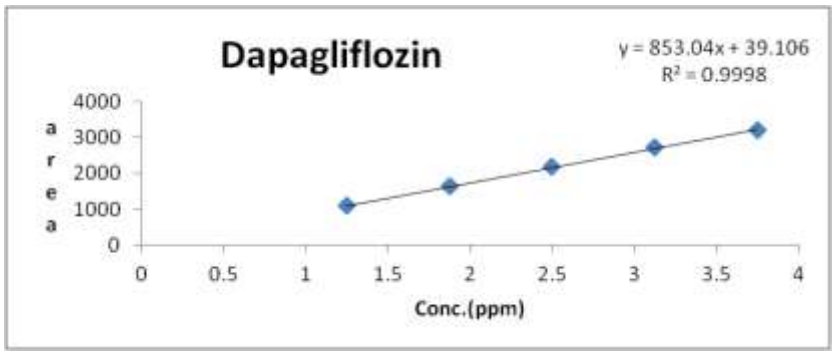


Figure 6 Callibration curve for Dapagliflozin

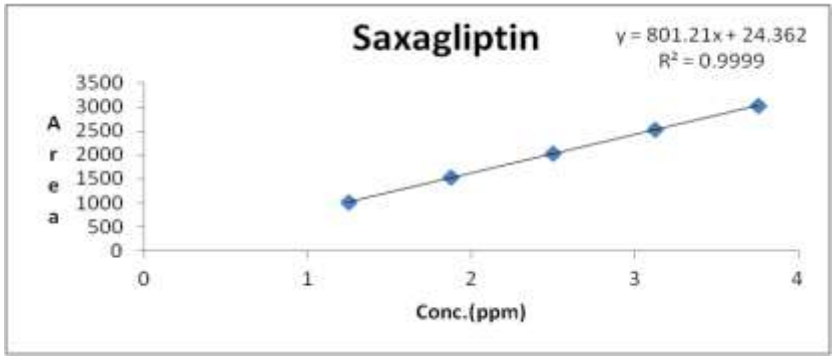


Figure 7 Callibration curve for Saxagliptin

C. Precision

a. Repeatability

The % RSD for Metformin HCl, Dapagliflozin and Saxagliptin was found to be 1.13,1.32 and 1.47 respectively.

b. Intra day Precision

The Intraday Precision for Metformin HCl at concentration of 250,500 and 750 µg/ml was found to be 1.25, 1.45 and 1.14 %RSD resp.

The Intraday Precision for Dapagliflozin at concentration of 1.25,2.5 and 3.75 µg/ml was found to be 0.85, 1.60 and 1.41 %RSD resp.

The Intraday Precision for Saxagliptin at concentration of 1.25,2.5 and 3.75 µg/ml was found to be 1.68, 1.08 and 1.59 %RSD resp.

c. Inter day Precision

The Interday Precision for Metformin HCl at concentration of 250,500 and 750 µg/ml was found to be 1.23, 0.93 and 0.90 %RSD resp.

The Interday Precision for Dapagliflozin at concentration of 1.25, 2.5 and 3.75 µg/ml was found to be 1.18, 1.21 and 1.55 %RSD resp

The Interday Precision for Saxagliptin at concentration of 1.25, 2.5 and 3.75 µg/ml was found to be 1.41, 1.00 and 0.87 %RSD resp

D. Accuracy

Recovery data for Metformin HCl

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	250	200	203.30	101.65	100.32 ± 1.19
2		250	200	198.73	99.37	
3		250	200	199.88	99.94	
4	100 %	250	250	254.20	101.68	100.57 ± 1.03
5		250	250	250.95	100.38	
6		250	250	249.14	99.65	
7	120 %	250	300	303.49	101.16	99.69 ± 1.57
8		250	300	299.60	99.87	
9		250	300	294.12	98.04	

Recovery data for Dapagliflozin

Sr. No.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1.25	1	1.02	101.58	99.91 ± 1.47
2		1.25	1	0.99	99.33	
3		1.25	1	0.99	98.82	
4	100 %	1.25	1.25	1.27	101.66	100.59 ± 0.98
5		1.25	1.25	1.25	100.35	
6		1.25	1.25	1.25	99.75	
7	120 %	1.25	1.5	1.52	101.11	100.16 ± 1.17
8		1.25	1.5	1.51	100.50	
9		1.25	1.5	1.48	98.85	

Recovery data for Saxagliptin

Sr. No.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1.25	1	1.02	101.61	100.58 ± 1.15
2		1.25	1	0.99	99.34	
3		1.25	1	1.01	100.81	
4	100 %	1.25	1.25	1.27	101.66	100.79 ± 0.76
5		1.25	1.25	1.25	100.35	
6		1.25	1.25	1.25	100.35	
7	120 %	1.25	1.5	1.52	101.12	100.40 ± 0.78
8		1.25	1.5	1.51	100.51	
9		1.25	1.5	1.49	99.58	

IV CONCLUSION

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Metformin HCl, Saxagliptin and Dapagliflozin in their Synthetic mixture. The separation was achieved by LC- 10 AT C_{18} (250mm x 4.6 mm x 2.6 µm) column and Buffer, pH 4.0: Acetonitrile (50:50) as mobile phase, at a flow rate of 1 ml/min and detection wavelength at 258 nm with retention time (RT) 3.56 min, 4.19 min, 8.36 min for Saxagliptin, Dpagliflozin and Metformin HCl respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Metformin HCl 250-750 µg/ml, for Dapagliflozin 1.25-3.75 µg/ml and for Saxagliptin 1.25-3.75 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Metformin HCl ,Saxagliptin and Dapagliflozin in their Synthetic mixture.

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