"DEVELOPMENT & VALIDATION OF LC-MS/MS METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND CANAGLIFLOZIN IN TABLET & CHARACTERIZATION OF DEGRADANT BY LC-MS/MS"

Ms. Maitriben Vasudevbhai Patel
Ms. Seju Patel
Ms. Bhumi Patel
Dr. Chaitanya Bhatt
A-ONE PHARMACY COLLEGE

Abstract:

We are performing the force degradation study on molecules of 'METFORMIN' and 'CANAGLIFLOZIN' by using LC-MS/MS.

Metformin is a medicine used to treat type 2 diabetes mellitus. It helps control blood sugar levels and thus prevent serious complications of diabetes. Canagliflozin is used by itself or in combination with other medicines to treat type 2 diabetes mellitus. It helps control the high blood sugar levels seen in diabetes. This reduces the chances of serious complications of diabetes and also helps prevent heart disease. Canagliflozin anhydrous is a sodium glucose cotransporter 2 inhibitor.

As per literature review, there is no Force degradation reported for the estimation of Metformin and Canagliflozin in combined pharmaceutical dosage form by LC-MS/MS. Various publication are available regarding the UV, HPLC simultaneous estimation and UV & HPLC method development of Metformin and Canagliflozin either alone or in combination with other drug in pharmaceutical dosage form.

Keywords: LC-MS/MS, Metformin, Canagliflozin, Force degradation.

1. INTRODUCTION

1.1. INTRODUCTION TO DIABETES MELLITUS [01-02]

1.1.1. Diabetes Mellitus [01]

Diabetes mellitus, commonly known as just diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Symptoms often include frequent urination, increased thirst and increased appetite. If left untreated, diabetes can cause many health complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced.

1.1.2 Classification and Causes of Diabetes Mellitus[02]

- **Type 1 Diabetes Mellitus**
- **Type 2 Diabetes Mellitus**

1.2. INTRODUCTION TO ANTIDIABETIC DRUGS [03-04]

1.2.1. Antidiabetic Drugs

Drugs used in diabetes treat diabetes mellitus by altering the glucose level in the blood. With the exceptions of insulin, most GLP receptor agonists and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors.

Diabetes mellitus type 1 is a disease caused by the lack of insulin. Insulin must be used in type 1, which must be injected.

Diabetes mellitus type 2 is a disease of insulin resistance by cells. Type 2 diabetes mellitus is the most common type of diabetes. Treatments include agents that (1) increase the amount of insulin secreted by the pancreas, (2) increase the sensitivity of target organs to insulin, (3) decrease the rate at which glucose is absorbed from the gastrointestinal tract, and (4) increase loss of glucose through urination.

Several groups of drugs, mostly given by mouth, are effective in type 2, often in combination. The therapeutic combination in type 2 may include insulin, not necessarily because oral agents have failed completely, but in search of a desired combination of effects. The great advantage of injected insulin in type 2 is that a well-educated patient can adjust the dose, or even take additional doses, when blood glucose levels measured by the patient, usually with a simple meter, as needed by the measured amount of sugar in the blood.

1.2.2. Classification of Antidiabetic Drugs

a. Insulin

b. Sensitizers

c. Secretagogues

d. Alpha-glucosidase inhibitor

e. Gliflozin

2. AIM & OBJECTIVES

Aim:

Literature review reveals that numbers of individual analytical methods available for estimation of Metformin and Canagliflozin in their individual dosage forms and combined dosage form. But LC-MS/MS method has not been reported for simultaneous estimation of Metformin and Canagliflozin in combined pharmaceutical dosage form. So it is thought to develop LC-MS/MS method for the simultaneous estimation of Metformin and Canagliflozin in Combined Dosage Form.

So Aim of present work is to develop simple, accurate, precise, rapid, specific, sensitive and selective LC-MS/MS method for simultaneous estimation of Metformin and Canagliflozin in combined pharmaceutical dosage form.

Objectives:

1. To develop rapid, precise, accurate and sensitive force degradation method for characterization of Canagliflozin and Metformin using LC-MS/MS.

2. Developing a LC-MS/MS method that is able to separate and quantify Canagliflozin and Metformin from their combined pharmaceutical formulation.

3. To conduct forced degradation study under various stressed conditions like hydrolysis (acid-alkali), oxidation, thermal and photolysis.

4. To develop LC-MS/MS method for simultaneous estimation of Metformin and Canagliflozin in pharmaceutical dosage form.

5. Applying the newly developed, validated analytical method for the estimation of Metformin and Canagliflozin in formulations.

3. MATERIAL & METHOD

- **List of Instruments:**

Instruments Name	Manufacturer
LC	Shimadzu LC-20 AT
MS/MS	ABScix API 200
Analytical balance	Shimadzu ATX-224
Sonicator	Frontline 1870
pH meter	Analab Scientific Private Ltd
Hot Air Oven	Kesar Control System
Photo stability Chamber	Kesar Control System

- **List of Reagents:**

Chemicals	Grade	Manufacturer
Acetonitrile	LC-MS	J.T. Baker
Water	LC-MS	Aquarch
Methanol	LC-MS	J.T. Baker
Metformin	Intas Pharmaceutical	
Canagliflozin	Labron Healthcare Pvt Ltd	

- **LC-MS/MS Parameter Optimization:**

Instrument	Liquid chromatography Mass spectrometer (API-2000) equipped with auto sample, auto injector, column oven, ion source ESI electron spray ionizer with Q1 and collision energy.		
Ion Source setting		***Scan setting***	
Ion source	ESI	Polarity	Positive ion
Curtain Gas	20psi	Scan type	MRM
Ion Spray Voltage	5000	Scan time	1-10 min
Temperature	400°C	Declustering Potential	50
Ion Source Gas(GS1)	50psi	Focusing Potential	400
Ion Source Gas(GS2)	60psi	Entrance Potential	10
Scan type	Metformin	MRM:(Q1)130.200 Da and (Q3) 59.400 Da	
	Canagliflozin	MRM:(Q1)467.100 Da and (Q3) 427.200 Da	
	Met DP1	MRM:(Q1)131.400 Da and (Q3) 87.100 Da	
	Met DP2	MRM:(Q1)116.300 Da and (Q3) 59.300 Da	
	Met DP3	MRM:(Q1)102.600 Da and (Q3) 86.100 Da	
	Cana DP1	MRM:(Q1)482.500 Da and (Q3) 281.400 Da	
	Cana DP 2	MRM:(Q1)499.100 Da and (Q3) 366.600 Da	
	Cana DP3	MRM:(Q1)499.100 Da and (Q3) 194.800 Da	

- **Chromatographic condition:**

Column	:	Agilent, Zorbax, C18, (150mm x 4.6mm), 5µm			
Flow rate	:	1.0 mL/min	Injection volume	:	20 µL
Column oven temperature	:	35 °C	Run time	:	8 min
Column oven compartment	:	Ambient	Mode	:	Isocratic
Metformin R.T	:	About 3.0 min			
Canagliflozin R.T	:	About 5.9 min			
Met DP1 R.T	:	About 1.6 min			
Met DP2 R.T	:	About 3.6 min			
Met DP3 R.T	:	About 1.8 min			
Cana DP1 R.T	:	About 4.1 min			
Cana DP2 R.T	:	About 4.8 min			
Cana DP3 R.T	:	About 6.8 min			

- **Mobile Phase preparation:**
  Prepare 0.1% Formic Acid in water and Methanol in the ratio of 30:70.

  Diluent: Water: Methanol (50:50)

- **Standard Stock Solution of Metformin:** Weigh and transfer about 10.0mg of Metformin into a 10ml volumetric flask and make up volume with diluent (1000mcg/ml).

- **Standard Stock Solution of Canagliflozin:** Weigh and transfer about 50.0mg of Canagliflozin into a 100ml volumetric flask and make up volume with diluent. (500mcg/ml).

- **Working Standard Solution(Combine std prepn):** Take 0.1ml from Metformin stock solution and 0.1ml from Canagliflozin stock solution into 100ml volumetric flask and make up the volume with diluent.
  (MET-1.0mcg/ml and CANA-0.5mcg/ml)

Note: Inject above working standard preparation for mobile phase selection.

- **Chromatographic Trials**

Mobile Phase	Ratio (v/v)	Retention Time	Remarks
Metformin and Canagliflozinin Water: Methanol	20:80	6.24 (Metformin) 3.01 (Canagliflozin)	Both the peak is observed but peak shape is not good.
Metformin and Canagliflozinin Water: Methanol	25:75	6.75 (Metformin) 3.75 (Canagliflozin)	Both the peak is observed but still peak shape is not good and retention time is increased.
Metformin and Canagliflozinin Water: Methanol	30:70	6.84 (Canagliflozin)	only canagliflozin peak is observed and retention time is increased.
Metformin and Canagliflozinin Water: Methanol: 0.1% formic acid	20:80	2.06 (Metformin) 2.70 (Canagliflozin)	Both the peak is observed but retention time of both the peak is almost same.
Metformin and Canagliflozinin Water: Methanol: 0.1% formic acid	30:70	3.04 (Metformin) 5.91 (Canagliflozin)	Both the peak is observed and peak shape is good and retention time is different of both the peaks.

- **Forced Degradation solution preparation to find out Degradant Compound from Standard (By infusing sample into Mass Spectrometer):**

Metformin Degradation Procedure

1) Acid Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N HCL and put the volumetric for 3 hours on water bath at 80^0C. After 3 hour make up the volume with diluent. (1.0mcg/ml).

2) Base Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N NaOH and put the volumetric for 4hours on water bath at 80^0C.After 4 hour make up the volume with diluent. (1.0mcg/ml).

3) Oxidation Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 30.0% Hydrogen Peroxide and put the volumetric for 4 hours on water bath at 80^0C.After 4 hour make up the volume with diluent. (1.0mcg/ml).

4) Thermal Degradation:
Put about 100.0mg of Metformin standard into petridish and place the petridish into hot air oven at 105^0C for 3 days. After 3 days' weigh and transfer about 10.0mg of above powder into a 10ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (1.0 mcg/ml)

5) Photo Degradation:
Put about 100.0mg of Metformin standard into petridish and place the petridish into a photo stability chamber for 5 days.After 5 days' weigh and transfer about 10.0mg of above powder into a 10ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (1.0 mcg/ml).

Canagliflozin Degradation Procedure

1) Acid Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N HCL and put the volumetric for 4 hours on water bath at 80^0C. After 4 hour make up the volume with diluent. (0.5mcg/ml).

2) Base Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N NaOH and put the volumetric for 8 hours on water bath at 80^0C.After 8 hour make up the volume with diluent. (0.5mcg/ml).

3) Oxidation Degradation:
Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 30.0% Hydrogen Peroxide and put the volumetric for 5hours water bath at 80^0C.After 5 hours make up the volume with diluent.(0.5mcg/ml).

4) Thermal Degradation:
Put about 100.0mg of Canagliflozin standard into petridish and place the petridish into hot air oven at 105^0C for 3 days. After 3 days weigh and transfer about 50.0mg of above powder into a 100ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (0.5 mcg/ml)

5) Photo Degradation:
Put about 100.0mg of Canagliflozin standard into petridish and place the petridish into a photo stability chamber for 5 days. After 5 days weigh and transfer about 50.0mg of above powder into a 100ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (0.5 mcg/ml)

Note: The forced degradation preparation solution of Metformin and Canagliflozin is infused into the mass spectrometer to identify the degradation product of Metformin and Canagliflozin.

- **Forced Degradation Solution preparation for LC-MS/MS**

Sample Stock Solution for forced degradation:
Weight equivalent about 5mg of CANA/100mg of MET into a 100ml volumetric flask and add 60 ml of diluent and Shake for 15 minutes in sonicator. Make up volume with diluent up to the mark. Filter this solution with whatman filter paper no-1. Take 0.1ml of this solution into 100ml volumetric flask and make up to the mark with diluent.
(MET-1.0mcg/ml and CANA-0.5mcg/ml).

Metformin Degradation Procedure

1) Acid Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N HCL and put the volumetric for 3 hours on water bath at 80^0C. After 3 hour make up the volume with diluent. (1.0mcg/ml).

2) Base Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N NaOH and put the volumetric for 4 hours on water bath at 80^0C. After 4 hour make up the volume with diluent. (1.0mcg/ml).

3) Oxidation Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 30.0% Hydrogen Peroxide and put the volumetric for 4 hours on water bath at 80^0C.After 4 hour make up the volume with diluent. (1.0mcg/ml).

4) Thermal Degradation:
Standard Preparation: Put about 100.0mg of Metformin standard into petridish and place the petridish into hot air oven at 105^0C for 3 days. After 3 days' weigh and transfer about 10.0mg of above powder into a 10ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (1.0 mcg/ml)

5) Photo Degradation:
Standard Preparation: Put about 100.0mg of Metformin standard into petridish and place the petridish into a photo stability chamber for 5 days. After 5 days' weigh and transfer about 10.0mg of above powder into a 10ml

volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (1.0 mcg/ml).

Canagliflozin Degradation Procedure

1) Acid Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N HCL and put the volumetric for 4 hours on water bath at 80^0C. After 4 hour make up the volume with diluent. (0.5mcg/ml).

2) Base Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 1N NaOH and put the volumetric for 8 hours on water bath at 80^0C. After 8 hour make up the volume with diluent. (0.5mcg/ml).

3) Oxidation Degradation:
Standard Preparation: Transfer 0.1ml of standard stock solution into a 100ml volumetric flask, add 1.0ml 30.0% Hydrogen Peroxide and put the volumetric for 5 hours water bath at 80^0C. After 5 hours make up the volume with diluent. (0.5mcg/ml).

4) Thermal Degradation:
Standard Preparation: Put about 100.0mg of Canagliflozin standard into petridish and place the petridish into hot air oven at 105^0C for 3 days. After 3 days weigh and transfer about 50.0mg of above powder into a 100ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (0.5 mcg/ml)

5) Photo Degradation:
Standard Preparation: Put about 100.0mg of Canagliflozin standard into petridish and place the petridish into a photo stability chamber for 5 days. After 5 days weigh and transfer about 50.0mg of above powder into a 100ml volumetric flask and make up volume with diluent. Transfer 0.1ml solution into a 100ml volumetric flask and make up volume with diluent. (0.5 mcg/ml)

- **Method Validation:**
- **Linearity and Range:**

1. **Range for Metformin**: 50% to 150% of the standard preparation.

2. **Range for Canagliflozin**: 50% to 150% of the standard preparation

3. **Standard stock solution of Metformin**: 10mg→10ml with diluent (1000mcg/ml)

4. **Standard stock solution of Canagliflozin**: 50mg→ 100ml with diluent (500mcg/ml)

Standard solutions preparation for Linearity

Linearity Level	Std stock soln of Metformin	Std stock soln of Canagliflozin	Make up with diluent (ml)	Concof Canagliflozin	Conc of Metformin
50%	0.50ml +	0.50ml→	1000ml	0.25 mcg/ml	0.5 mcg/ml
75%	0.75ml +	0.75ml→	1000ml	0.375 mcg/ml	0.75 mcg/ml
100%	1.00ml +	1.00ml→	1000ml	0.50 mcg/ml	1.0 mcg/ml
125%	1.25ml +	1.25ml→	1000ml	0.625 mcg/ml	1.25 mcg/ml
150%	1.50ml +	1.50ml→	1000ml	0.75 mcg/ml	1.50 mcg/ml

- **Precision**

1. **Intraday precision**

Intraday precision was performed on same day for 3 concentration of the given range (n=3)
For Metformin standard preparation:
Lower concentration- 50% (0.5 mcg/ml)
Middle concentration- 100%(1.0 mcg/ml)
Higher concentration- 150%(1.50 mcg/ml)

For Canagliflozin standard preparation:
Lower concentration- 50% (0.250 mcg/ml)
Middle concentration- 100% (0.50 mcg/ml)
Higher concentration- 150% (0.750 mcg/ml)

Limit: %RSD for area at each level should not be more than 2.0%

2. **Interday precision**

Interday precision performed on three consecutive days for 3 concentration of the given range(n=3)
For Metformin standard preparation:
Lower concentration- 50% (0.5 mcg/ml)
Middle concentration- 100%(1.0 mcg/ml)

Higher concentration- 150% (1.50 mcg/ml)

For Evogliptin Tartrate standard preparation:
Lower concentration- 50% (0.250 mcg/ml)
Middle concentration- 100% (0.50 mcg/ml)
Higher concentration- 150% (0.750 mcg/ml)

Limit: %RSD for area at each level should not be more than 2.0%

3. **Repeatability**

Performed for 1 concentration (n=6) for at 100% level

For Metformin standard preparation:
Middle concentration- 100% (1.0 mcg/ml)

For Canagliflozin standard preparation:
Middle concentration- 100% (0.5 mcg/ml)

Limit: %RSD for area at each level should not be more than 2.0%

- **Recovery**

1. **Standard stock solution of Metformin**: 10.0mg→10ml with diluent (1000mcg/ml)
2. **Standard stock solution of Canagliflozin**: 50.0mg→100ml with diluent (500mcg/ml)

Recovery solution preparation:

Recovery Level	Solution 1	Std stock soln of Metformin	Std stock soln of Canagliflozin	Diluted with diluent
80%	0.5ml +	0.4ml +	0.4ml -→	100ml
100%	0.5ml +	0.5ml +	0.5ml -→	100ml
120%	0.5ml +	0.6ml +	0.6ml -→	100ml

Limit:

- % Recovery should be between 98.00% to 102.00% at each level.
- % RSD for % Recovery should be less than 2.0%

- **Robustness:**

Inject working standard preparation for different flow rateand different mobile phase composition:
Flow rate: +0.2ml/mint and -0.2ml/mint
Solvent % in mobile phase: +2% solvent and -2% solvent in mobile phase.

- **Assay of Marketed Formulation:**

- **Standard Stock Solution of Metformin:** Weigh and transfer about 10.0mg of Metformin into a 10ml volumetric flask and make up volume with diluent (1000mcg/ml).

- **Standard Stock Solution of Canagliflozin:** Weigh and transfer about 50.0mg of Canagliflozin into a 100ml volumetric flask and make up volume with diluent. (500mcg/ml).

- **Working Standard Solution(Combine stdprepn):** Take 0.1ml from Metformin stock solution and 0.1ml from Canagliflozin stock solution into 100ml volumetric flask and make up the volume with diluent. (MET-1.0mcg/ml and CANA-0.5mcg/ml)

  Assay preparation (Marketed formulation)
  Label claim:
  CANA-50.0mg and MET-1000.0mg
  Sample Stock Solution for assay:
  Weight equivalent about 5mg of CANA/100mg of MET into a 100ml volumetric flask and add 60 ml of diluent and Shake for 15 minutes in sonicator. Make up volume with diluent upto the mark. Filter this solution with whatman filter paper no-1.

  Sample Working Solution for assay:
  Take 0.1ml of this solution into 100ml volumetric flask and make up to the mark with diluent.(MET-1.0mcg/ml and CANA-0.5mcg/ml).

Note: Inject working standard preparation and working sample preparation for assay analysis.

4. CONCLUSION

Table 4.1: Summary of Validation Parameters of LC-MS/MS Method for Metformin and Canagliflozin

Sr No.	Parameter		Metformin	Canagliflozin
1	Specificity		Specific	specific
2	Linearity & Range		0.5-1.5	0.25-0.75
3	Regression equation		y = 80.48x – 19.12	y = 6880x – 438.3
4	Correlation co-efficient (r^2)		0.995	0.997
5	Precision (% RSD)	Repeatability	1.762	1.539
		Interday	1.015 -1.435	0.813-1.215
		Intraday	0.934-1.680	1.153-1.330
6	Accuracy (% recovery)		99.964-100.188	99.913-100.741
7	Limit of Detection(LOD)		59.302µg/ml	2.639
8	Limit of Quantification(LOQ)		2.639 µg/ml	7.998 µg/ml
9	Robustness (% RSD)		< 2.0 in each parameters	< 2.0 in each parameters
10	% Assay		99.677 ± 1.386	100.377± 0.848

5. REFERENCES

1. De B; Sen S and Chakraborty R. Diabetes Mellitus Analysis and Advancement; 1st Edition; CBS Publishers & Distributors, India, 2008, pp 105-124.
2. Diabetes Mellitus; Central Council for Research in Homoepathy; 1st Edition; Central Chinmaya Mission Trust, India, 2010, pp 96-118.
3. Banerjee S.Oral Anti-diabetics - Current Concepts; 3rd Edition; Scientific Publishing, New Delhi, India, 2018, pp 221-247.
4. Tripathi KD. Pharmacological Classification of Drugs; 6th Edition, Jaypee Brothers Medical Publishers, India, 2021, pp 116-131.

5. Marvin C. McMaster. LC-MS/MS: A Practical User Guide. 3rd Edition; John Wiley & Sons, Inc Publication, 2005, pp 311-341.
6. Dass C. Fundamentals Of Contemporary Mass Spectrometry; 1st Edition; John Wiley, US, 2007, pp 321-343.
7. Wiley VCH. The HPLC-MS Handbook For Practitioners; 4th Edition; John Wiley & Sons Publication, 2017, pp 110-135.
8. Lee SM. LC/MS Application in Drug Development. 2nd Edition;John Wiley & Sons, 2003, pp 101-127.
9. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
10. FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration", Rockville, US Department of Health and Human Services, 2000.
11. "Drug profile for Metformin", http://www.drugbank.ca/drugs/DB00331
12. "Drug profile for Canagliflozin", http://www.drugbank.ca/drugs/DB08907
13. Indian Pharmacopoeia. Volume II, Ghaziabad: Indian Pharmacopoeia Commisssion, 2018, pp 2544.
14. British Pharmacopoeia. Volume I, British Pharmacopoeia Commission, 2013, pp 0931.
15. The United State Pharmacopoeia 30, National Formulary 25, United State Pharmacopoeial Convention, Inc. 2010, pp 4283.
16. Madhukkar et.al., "Simple and Sensitive Analytical Method Development and Validation of Metformin Hydrochloride by RP-HPLC", *Int. J. Pharm. & Pharm. Sci.* **2011**, 3(3), 117-120.
17. P. Sowjanya et.al., "RP-HPLC Method Development of Metformin in Pharmaceutical DosageForm", *Res. & Rev. J. Pharm. Ana.***2015**, 4(4), 09-20.
18. S. M. Honmane et.al., "Development and Validation of UV-Spectrophotometric Method for Estimation of Metformin in Bulk and Tablet Dosage Form", *Ind. J. Pharm. Edu. & Res.* **2017**, 51(4), 764-770.
19. C.R. Dass et.al., "Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide", *J. Food. & Drug. Ana.* **2019**, 315-322.

20. V. Swetha et.al., "Method Development and Validation for Simultaneous Estimation of Metformin Hydrochloride and Gliclazide by RP-HPLC in Bulk and Tablet Dosage Form", *Asi. J. Pharm. Ana. & Med. Chem.* **2017**, 5(1), 13-22.
21. K. K. Patel et.al., "Development and Validation of Stability Indicating HPTLC Method for Estimation of Glimepiride and Metformin Hydrochloride", *Int. J. Pharm. Sci. & Res*, **2015**, 6(3), 1222-1229.
22. S. Elda et.al., "New Analytical Method Development and Validation for SimultaneousEstimation ofMetformin and Glibenclamide in Bulk and Tablet Dosage Form using RP-HPLC", *Rasayan. J. Chem.* **2014**, 7(1), 55-63.
23. N. R. Doredla et.al., "Method Development and Validation of RP-HPLC Method for Simultaneous Analysis of Three Component Tablet Formulation containing Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide", *Int. J. Pharm. Tech Res.*, **2012**, 4(3), 948-956.
24. M. V. Priya et.al., "RP-HPLC Method for Simultaneous Estimation of Metformin HCL, Ramipril and Glimepiride in Bulk and Their Combination Tablet Dosage Form", *IOSR J. Pharm. & Bio. Sci.* **2016**, 11(3), 16-23.
25. D. Ramesh et.al., "Stability Indicating RP-HPLC Method for the Simultaneous Determination of Atorvastatin Calcium, Metformin Hydrochloride, and Glimepiride in Bulk and Combined Tablet Dosage Form", *Int. Sch. Res. Not.* **2014**, 01-08.
26. A.Rajasekaran et.al., "Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in pharmaceutical formulation", *Wor. J. Pharm. Sci.* **2014**, 2(4), 317-327.
27. N. Sirigiri et.al., "Stability Indicating Method Development and Validation for Simultaneous Estimation of Linagliptin and Metformin HCl in Tablets by HPLC", *Der. Pharm. Chem*. **2017,** 9(21), 100-106.
28. R. Surisetti et.al., "Analytical method development and validation for the estimation of Saxagliptin and Metformin by RP HPLC method", *Int. J. Adv. Res. & Dev.* **2018**, 3(10), 23-28.

29. M. Yunoos et.al., "Stability indicating quantitative RP-HPLC method development and validation for simultaneous determination of metformin hydrochloride and saxagliptin in bulk and combined tablet dosage form", *J. Chem. & Pharm. Res.* **2015**, 7(4), 346-355.
30. P. Chengalva et.al., "Development and Validation of RP-HPLC Method for Metformin Hydrochloride and Nateglinide in bulk and combined dosage form", *Int. J. Pharm. & Pharm. Sci.* **2016**, 8(4), 267-271.
31. K. Neelima et.al., "Analytical Method Development and Validation of Metformin, Voglibose, Glimepiride in Bulk and Combined Tablet Dosage Form by Gradient RP-HPLC", **2014**, 5(1), 27-33.
32. I. Nemallapudi et.al., "Method Development and Validation of Simultaneous Estimation of Alogliptin and Metformin Hydrochloride by RP HPLC", *Asi. J. Res. Chem. & Pharm. Sci.* **2018**, 6(4), 206-214.
33. A. M. Patel et.al., "Stability Indicating RP-HPLC Method Development and Validation for Estimation of Empagliflozin and Metformin Hydrochloride", *Wor. J. Pharm. & Pharm. Sci.* **2017,** 6(9), 872-885.
34. M. Akteruzzaman et.al., "Development and Validation of a Simple RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Rosiglitazone in Pharmaceutical Dosage Forms", *J. Pharm. Sci.* **2012**, 11(2), 157-163.
35. Darshan Bhatt, Padmini Thatavarthi, B. Rajkamal., "Analytical Method Development and Validation for the Estimation of Canagliflozin in Bulk and Formulation by RP- HPLC", *Int. J. Pharm. Sci. & Drug. Res.* **2018,** 10(3), 139-143.
36. A Suneetha and D Sharmila., "A Validated Stability Indicating RP-HPLC Method for Estimation of Canagliflozin in Dosage Form", *Res. J. Pharm. Bio. & Chem. Sci.* **2015,** 6(5), 1186-1194.
37. Hemambika Sadasivuni and Narayana Rao Gundoju., "Analytical Method Development and Validation of Canagliflozin Hemihydrate in Bulk and Pharmaceutical Dosage forms", *Mukt. Shabd. J.* **2020,** 9(6), 1690-1698.
38. Gouru Santhosh Reddy, Sonam Patel, Krishna Veni Nagappan., "Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for the

Estimation of Canagliflozin in Bulk and its Pharmaceutical Formulation", *J. Young. Pharm*. **2020**, 12(4), 321-326.

39. V. Triveni, B. Venkata Durga Ram, M. Swathi, N. Trivani, P. Durga, Dr B. Tirumaleswara Rao, Dr. Challa Sudheer., "Method Development and validation for the estimation of Canagliflozin in drug substances by RP-HPLC", *Indo. Ame. J. Pharm. Res.* **2017,** 7(3), 7973-7978.
40. Arulselvan Murugesan and Mukthinuthalapati Mathrusri Annapurna., "Validated and Quantified stability indicating stress degradation study of oral anti-diabetic agent Canagliflozin by RP-HPLC method", *Int. J. App. Pharm.* **2021,** 13(5), 358-363.
41. Ishpreet Kaur, Sharad Wakode, Harsharan Pal Singh, Satish Manachanda., "Development and Validation of a Stability-Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form", *Pharm. Meth.* **2016,** 7(1), 54-62.
42. Aswini Kumar Parida, K Srinivasa Rao, Ajay Kumar Patnaik., "RP-HPLC Method for the Estimation of Canagliflozin in Bulk and Pharmaceutical Dosage Forms", *Int. J. Pharm. Res. & Hea. Sci.* **2018,** 6(1), 2308-2313.
43. Bangaruthalli J, Prof. D.Gowri Shankar, M.N.L.Renuka , P.Akhila Divya.Vanga., "Method Development and validation of simultaneous estimation of Metformin & Canagliflozin by using RP HPLC", *Int. J. Sci. & Eng. Res.* **2018,** 9(11), 1309-1319.