Int. J. Adv. Pharm. Biotech., 2018; 4(1): 10-22
doi.org/10.38111/ijapb.20180401002

IJAPB

International Journal of Advances in Pharmacy and Biotechnology
Vol.4, Issue-1, 2018, 10-22
ISSN: 2454-8375
Research Article
Open Access

A NOVEL SIMULTANEOUS STABILITY-INDICATING UPLC ASSAY METHOD FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN IN ORAL SOLID DOSAGE FORM

Vijay Kumar Rekulapally[1*], Sripal Reddy Palavai[2], Raghunadha Reddy Seelam[3]

[1]Dr. Reddy's Laboratories Ltd. IPDO, Bachupally, Hyderabad, Telengana, 500090.

[2]Aurobindo Pharma USA,INC, Raleigh , North Carolina, USA - 27606 .

[3]Department of Pharmaceutical Sciences, University of Maryland, School of Pharmacy, Baltimore, MD, United States

Corresponding author E- mail: vijaykmpharm@gmail.com

Received: 28 January 2018 Revised: 12 February 2018 Accepted: 24 February 2018

ABSTRACT:

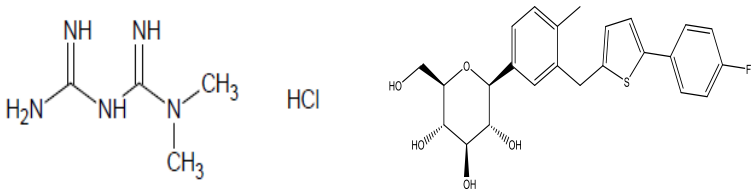
A Novel, Simultaneous stability indicating RP-HPLC method was developed for combined estimation of Metformin Hydrochloride (CANA) and Canagliflozin (MET) in fixed dose pharmaceutical formulations. The developed chromatographic method for the separations of CANA and MET and its degradation products by the use of stationary phase BEH C18 column, 50×2.1 mm, 1.7 µm column with mobile phase consisting of sodium dihydrogen phosphate buffer (30 mM) and10mM of 1-Octane Sulphonic acid in 1000ml of water in mobile phase A, mobile phase B mixture of Methanol: Acetonitrile 50:50 v/v ratio at a flow rate of 0.3 mL/min, with reproducible gradient program. Chromatographic separations was made of analytical peaks and degradation peaks occurring within 10 min. Detection of the analytes - Metformin at 230 nm& Canagliflozin at 290 nm using PDA detector. Method validation was performed as per ICH guidelines. Linearity of the method was established from 2–170 µg/ml for Metformin hydrochloride and 12–180µg/ml for Canagliflozin ($R^2$0.999). The method can be considered as stability-indicating and can be used for simultaneous determination of CANA and MET in fixed dose pharmaceutical formulations and drug product stability studies.

Keywords: *UPLC; Metformin; Canagliflozin; Stability indicating, Stress induced degradation products; ICH guidelines*

1. INTRODUCTION

Metformin hydrochloride is chemically designated as 1,1-Dimethylbiguanide hydrochloride (Fig. 1A). It is used in the treatment of type 2 diabetes[1]. Canagliflozin (Fig. 1B) chemically designated as (2S, 3R, 4R, 5S, 6R)-2-{3-[5-[4-Fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol.

It is used to treat type 2 diabetes[2-6]. Both CANA and MET been analyzed by various techniques either alone or in combination with other drugs. Analytical methods existed for Metformin hydrochloride included determination by HPLC[7] and UPLC[8]. Analytical methods existing for Canagliflozin included determination by high performance liquid chromatography[9-10], and CANA and MET combination analyzed by using HPLC[11-12]. The combination of CANA and MET has not been discussed any official pharmacopoeia like USP, BP or EP, etc. There not been any literature found for drug substance and drug product stability indicating UPLC method (with or without forced degradation studies) for simultaneous determination of both drugs. Hence tried and succeeded to develop and validate [13-16] a simultaneous simple, precise, and sensitive, stability indicating UPLC method for the combined determination of CANA and MET in drug product.



(A) **(B)**

Fig. 1 Chemical structure of Metformin HCl (A), Canagliflozin (B)

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

Canagliflozin hydrochloride, Metformin hydrochloride standard were supplied by Dr. Reddy's Laboratories (Hyderabad, India). The UPLC-grade Acetonitrile analytical grade, NaH_2PO_4 monohydrate and Ortho-phosphoric acid were sourced from Merck (Darmstadt, Germany). Water was sourced from Millipore MilliQ.

2.2. Equipments

Analysis was performed with a Waters (Milford, MA) Acuity UPLC system equipped with a quaternary solvent manager, sample manager, column-heating compartment and photodiode array detector. This system was controlled by Waters Empower3 software.

An Acquity UPLC BEH C18 column, 50×2.1 mm, 1.7 µm (Waters) was employed for chromatographic separation. Thermo Scientific multifuge centrifuge machine, heating oven (MACK Pharmatech, Hyderabad, India), photo stability chamber, water baths and Milli Volt controller (Julabo, Seelbach, Germany) were used for hydrolysis studies.

2.3. Chromatographic conditions

The column used was UPLC BEH C18 column, 50×2.1 mm, 1.7 µm as stationary phase. The mobile phase A buffer was prepared by dissolving 4.15g of Sodium dihydrogen phosphate and 1.0g of 1-Octane sulphonic acid in 1000ml of water, filtered through 0.22µ filters and mobile phase B was constituted as a mixture of Methanol: Acetonitrile 50:50 v/v ratio. A mixture of Milli Q water and Acetonitrile in 50:50 v/v was used as a diluent to prepare solutions. The gradient program Time (minutes)/% mobile phase A was set as 0/100, 8/0, 8.1/100, 10/100 respectively. Before use, the mobile phase was mixed thoroughly and degassed. The mobile phase was pumped at 0.3 ml/min. The eluted compound CANA and its impurities monitored at 230 nm, MET and its impurities were monitored at 290 nm. The column used at 25°C temperature. The injection volume for samples and standards set as 0.4 µL.

2.4. Preparation of standard stock solution of CANA

Accurately 60 mg of CANA working standard was weighed into a 50 ml volumetric flask, dissolved in diluent and made up to volume with diluent and mixed well.

2.5. Preparation of standard stock solution of MET

Accurately 50 mg of MET working standard was weighed into a 50 ml volumetric flask, dissolved in and diluted to volume with diluent and mixed.

2.5.1. Preparation of standard olution of CANA& MET

Accurately 5ml each of CANA and MET standard stock solutions were pippetted into a 100 ml volumetric flask, dissolved in diluent and made up to volume with diluent and mixed well.

2.6. Preparation of sample solution

Sample stocks were made up to 3000 µg/ml of CANA and 20000 µg/ml of MET by using diluent, and they were further diluted to achieve final concentrations of 12 µg/ml of CANA and 80 µg of MET with diluent. The solution was filtered through 0.45 µ nylon filter.

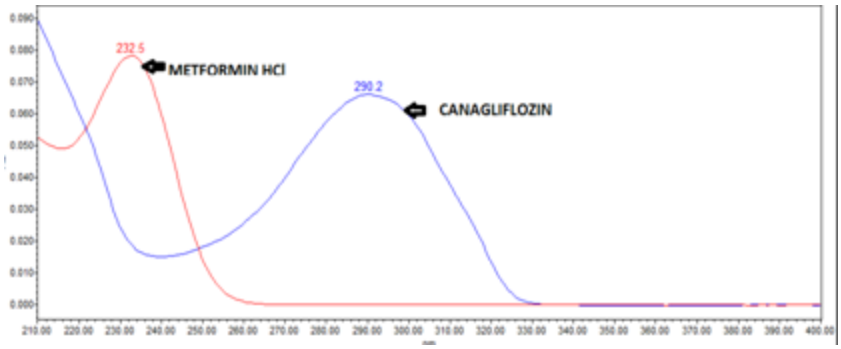


Fig. 2 Finalization of Chromatographic conditions

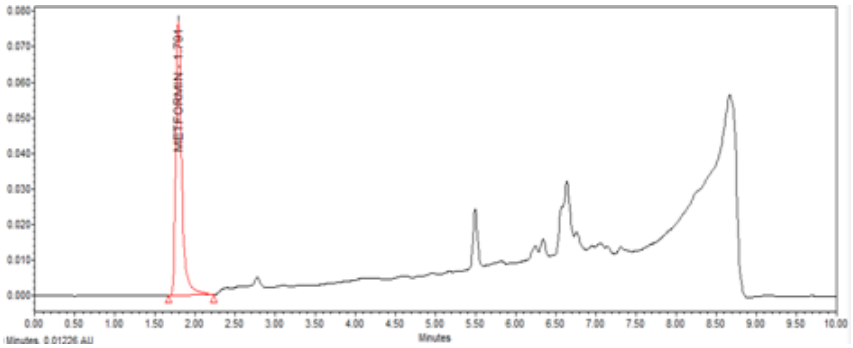


Fig. 3 Finalized standard chromatogram of MET at 230 nm

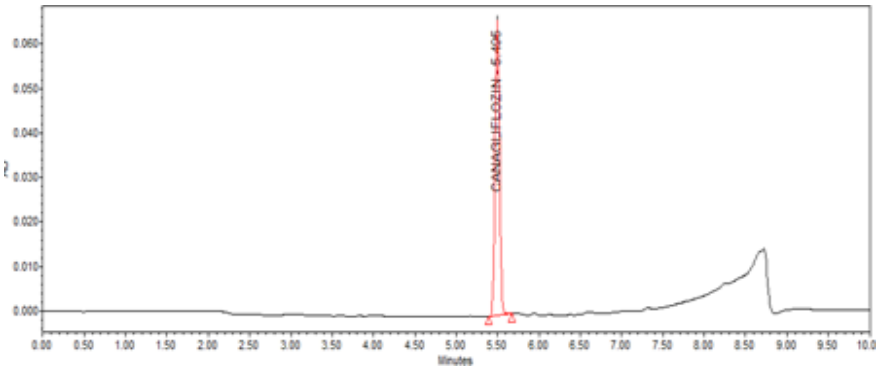


Fig. 4 Final standard chromatogram of CANA at 290 nm

2.7. Precision

Performed six sample preparations on the same day and inter-day, the %RSD of the peak area were then calculated to represent precision.

2.8. Linearity

Linearity obtained over concentration ranges of 12 µg to 180 µg for CANA and 2 µg to 170 µg for MET. Each solution was prepared in triplicate.

2.9. Accuracy

Accuracy of developed method was determined by spiking API on placebo various concentrations ranging from 50% of lower strength (50 mg for CANA and 500 mg for MET) to 150% of higher strength (150 mg for

CANA and 1000 mg for MET) of target test concentration. Six preparations were lower and higher concentration for covering the data in the range and for remaining levels in triplicate preparations.

3.0. ROBUSTNESS

Robustness was performed with 10% organic variation in mobile phase composition, 0.02 ml/min flow rate. The changes in retention time, tailing factor and number of theoretical plates were studied.

3.1. Limit of detection (LOD) and limit of quantification (LOQ)

Detection and quantification limits were determined by the signal-to-noise (S/N) approach. In order to examine the LOQ and LOD solutions of dissimilar concentrations were prepared by spiking known amounts of CANA and MET spiked in diluent. The average S/N ratio from all the analyses at each concentration level was used to calculate the LOQ and LOD. The concentration which gives more than 10 readily quantified. The concentration at which gives S/N ratio 3 can be readily detected was reported as the LOD.

3.2. Specificity

A study was conducted to demonstrate the effective separation of degradents from CANA peak MET peak and by forced degradation studies of CANA and MET tablets.

3.2.1. Acid degradation studies

Acid stress was induced by subjecting to 1 M HCl at 60 °C for 24 h and keeping on a shaking water bath. After administration of the stress the solution was neutralized with 1 M NaOH, and a further sample was prepared with diluent.

3.2.2. Base degradation studies

Base stress was simulated by using 1 M NaOH at 60°C for 3 h and keeping on a shaking water bath. After induction of the stress the solution was neutralized with 1 M HCl, further sample was prepared with diluent.

3.2.3. Oxidative degradation studies

Oxidative stress was performed by using 30% H_2O_2 for 24 h in room condition. After completion of the stress, further sample prepared with diluent.

3.2.4. Thermal degradation studies

Thermal degradation was performed at 105°C for 24 h. After completion of the stress, further sample preparation was made as a control sample.

3.2.5. Photolytic degradation studies

For photolytic degradation study performed in photolytic chamber (1.2 million lux per hour and 200 watt hour/square meter) for 16 h. After completion of the stress on sample same samples used for the sample preparation.

4. RESULTS AND DISCUSSION

Liquid chromatographic analysis of Metformin and related impurities showed that they were highly polar, with low UV absorbance. CANA was moderately polar,

with potential degradation impurities. These challenges were considered for simultaneous method development.

Preliminary studies involved trying different stationary phases and testing several mobile phase compositions for the effective separation of CANA and MET. Both analytes have different retention behaviors; hence it was challenging to separate both analytes in shorter method without interfering placebo components and degradation impurities. Method development started with evaluating retention of CANA & MET and in reversed-stationary phases (C8, and C18 columns), Phosphate buffer with organic modifiers, by keeping MET retention as a target. Further chromatographic elutions were optimized by using an ion pair reagent which helped to retain MET, but the CANA peaks were not symmetrical, In order to improve the CANA peak symmetry chromatographic parameters The method was validated as per ICH guidelines (ICH 1996), Q2(R1). Evaluated validation parameters were linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

4.1 Validation

Linear curve plotted in concentration ranges of 13–181 µg/ml (13, 32, 65, 103, 129 and 181 µg/ml) for MET and 12–170 µg/ml, CANA (12, 30, 60, 96, 120 and 170 µg/ml). The linear regression equation for MET was found were evaluated using a combination of phosphate buffer and ion pair agent and commercially available stationary phases like BEH Shield C8 and HSS T3 column, BEH C18 with linear gradient programs. Finally the best separation was observed with sodium dihydrogen phosphate monohydrate and 1-octane Sulphonic acid monohydrate buffer, stationary phase BEH C18, 50 mm x 2.1 mm, 1.7 m column linear gradient program. MET λ_{max} was at 230 nm, CANA λ_{max} was at 290 nm. Measuring both compounds at a specified wavelength was not possible due to the difference in the UV absorbance characteristics.

Symmetrical and high intensity peaks of CANA and MET chromatograms were recorded in above chromatographic conditions, the retention time of Metformin and Canagliflozin were 1.77 min, 5.42 min, respectively, System suitability data are summarized in Table 1.

to be Y = 3815 X + 36.3 with correlation coefficient greater than 0.999. The linear regression equation for CANA was found to be Y = 7450 X + 5367 with correlation coefficient greater than 0.999. The limit of detection (LOD) and quantification (LOQ) were determined by making serial dilutions. LOD was found to be 0.165µg and 0.122µg for CANA and MET, respectively (signal to noise ratio of 3:1). LOQ was found to be 0.504µg and 0.65µg for CANA and MET, respectively

(signal to noise ratio of 10:1). Accuracy performed on three levels 50%, 100% and 150% and its percentage recoveries along with standard deviation and relative standard deviations for each analytes are given in table 3. Recovery studies showed the method to be highly accurate and suitable for intended use. Intra-day precision was determined by injecting six sample solutions of target concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (%RSD) of the peak area was calculated to represent precision. Results of intra-day and inter-day precision are presented in Table 2. Robustness of the method was performed by slightly varying chromatographic conditions. The results showed that slight variations in chromatographic conditions had a negligible effect on the chromatographic parameters (Tables 5 and 6). Specificity of the developed method was evaluated by applying different stress conditions (acid, base, oxidation, thermal and photolytic) to CANA and MET in combination form.

Table 1: System suitability parameters of CANA and MET

Component	Retention time	Tailing factor	Theoretical plates
MET	1.7	1.4	3423
CANA	5.4	1.1	47935

Table 2: Intra-day and inter-day precision of the proposed UPLC method.

	Intra-day precision measured		Inter-day precision measured	
S.No	MET	CANA	MET	CANA
1	98.2	98.6	98.6	98.5
2	98	99.1	98.3	98.1
3	98.7	98.4	99.3	99.7
4	99.3	98.0	99.7	99.2
5	98.2	99.5	98.6	98.7
6	99.2	98.8	99.2	98.5
Avg	98.6	98.7	99	98.8
%RSD	0.6	0.5	0.5	1.5

Table 3: Accuracy of the proposed UPLC method.

Drugs	Spiked concentration in mg	Synthetic mixtures measured concentration (mg) ± SD; RSD (%)	Synthetic mixtures measured concentration (%Recovery) ± SD; RSD (%)
Metformin	3500	3466.2±37.9,1.1	99.0±1.0,1.1
	10000	9873.3±61.6,0.6	98.7±0.6,0.6
	15000	14960±99.9,0.7	99.7±0.7,0.6
Canagliflozin	350	346.3±3.9,1.1	99.0±1.5,1.5
	1500	1518.9±17.5,1.2	100.9±1.2,1.1
	2250	2228.3±9.4,0.4	99.0±0.4,0.4

Table 4: Stress testing results of CANA and MET

Nature of stress	Storage conditions	Time (h)	MET remaining	CANA remaining
1 M HCl	60 C	24	98.7	99.1
1M NaOH	60°C	3	96.8	98.3
30% H_2O_2	Room Temp	24	98	93.6
Thermal	105°C	24	99.4	95.2
Photolysis	Photolytic chamber	18	100.6	100.4

Table 5: Robustness study of Metformin

Chromatographic conditions	Assay	tR(min)	Theoretical plates	Tailing
Buffer: Methanol(780:220)	98.5	1.5	4125	1.3
Buffer: Methanol(800:200)	99.7	1.7	3418	1.4
Buffer: Methanol(820:180)	98.2	1.9	3652	1.4
Flow rate (0.32 mL/min)	98.6	1.9	3982	1.5
Flow rate (0.3 mL/min)	99.7	1.7	3418	1.4
Flow rate (0.28 mL/min)	98.5	1.4	3325	1.3
Acetonitrile: Methanol(480:520)	99.2	1.6	3865	1.5
Acetonitrile: Methanol(500:500)	99.7	1.7	3418	1.4
Acetonitrile: Methanol(520:480)	98.6	1.5	3125	1.4

Table 6: Robustness study of Canagliflozin

Chromatographic conditions	Assay	tR(min)	Theoretical plates	Tailing
Buffer: Methanol(780:220)	99.2	5.1	46852	1.2
Buffer: Methanol(800:200)	98.6	5.4	47853	1.1
Buffer: Methanol(820:180)	98.5	5.9	51264	1.1
Flow rate (0.32 ml/min)	98.7	5.7	45158	1.3
Flow rate (0.3 ml/min)	98.6	5.4	47853	1.1
Flow rate (0.28 ml/min)	99.2	5.2	42153	1.1
Acetonitrile: Methanol(480:520)	98.7	5.3	48521	1.2
Acetonitrile: Methanol(500:500)	98.6	5.4	47853	1.1
Acetonitrile: Methanol(520:480)	98.5	4.9	48532	1.2

4.2 Force degradation study

The chromatograms under acidic, basic, peroxide, thermal and photolytic stress conditions are shown in Figures. 5 to 9 for both the active ingredients. The results of stress studies are given in Table 4. All the stress conditions applied were enough to degrade both the drugs. Comparison of the two drugs showed that MET is more stable as compared to CANA. Under acidic condition CANA was stable and MET was degraded up to 1.3%. Under basic stress CANA was degraded up to 1.7% and MET was degraded up to 3.2%. Under oxidative stress Canagliflozin was degraded up to 6.4% and MET was degraded up to 2%. Under thermal stress studies, CANA was degraded up to 4.8% and MET was found to be stable. Under photolytic stress CANA and MET both were stable. From these stress studies it is thus concluded that CANA was not stable in basic, peroxide and thermal conditions whereas MET was unstable in basic condition. The developed method effectively separated the degradation products or impurities. Therefore, the developed method is considered to be highly specific and selective for intended use.

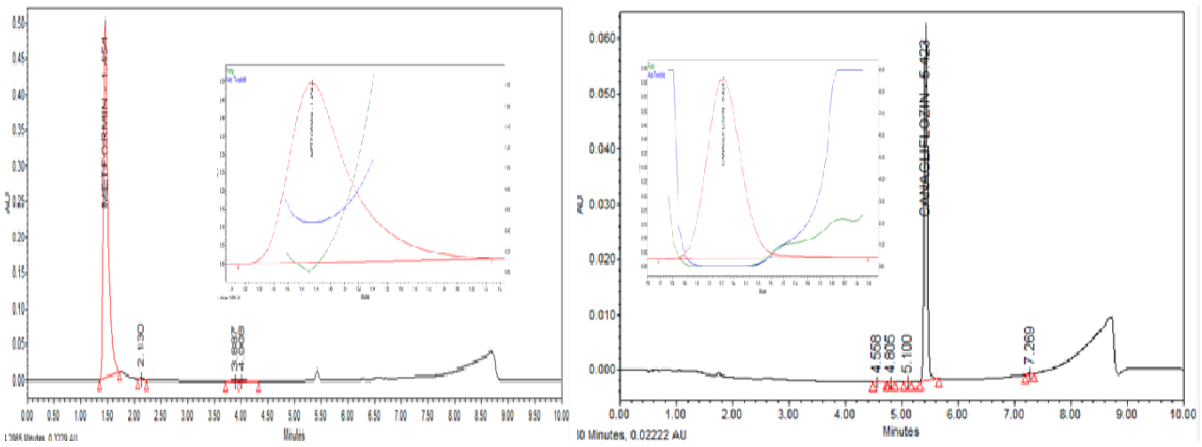


Fig. 5 Chromatograms of CANA and MET under acid stress

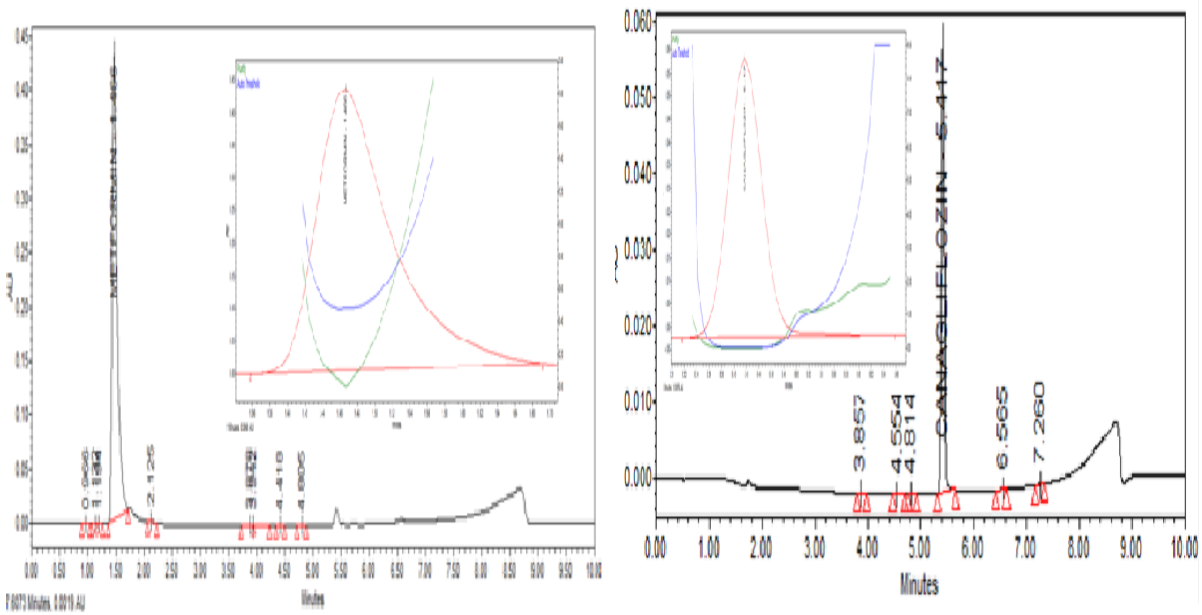


Fig. 6 Chromatograms of CANA and MET under basic stress

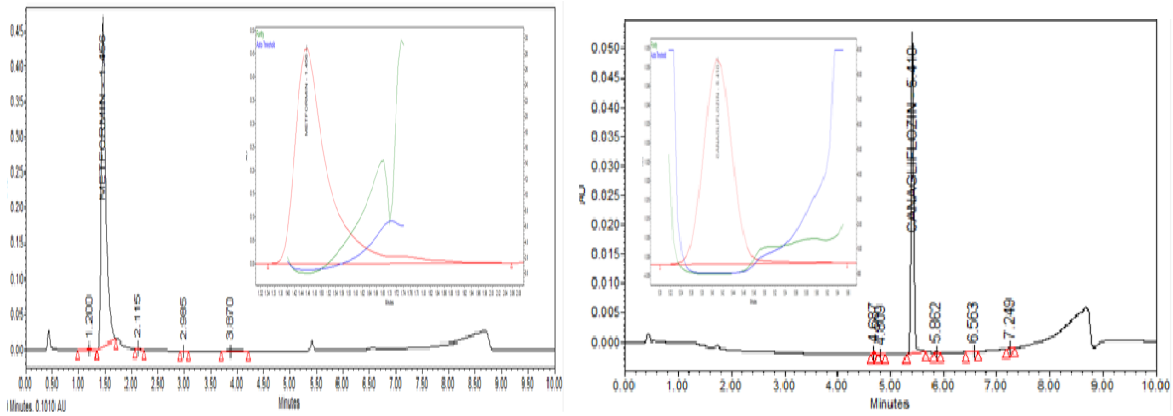


Fig. 7 Chromatograms of CANA and MET under peroxide stress

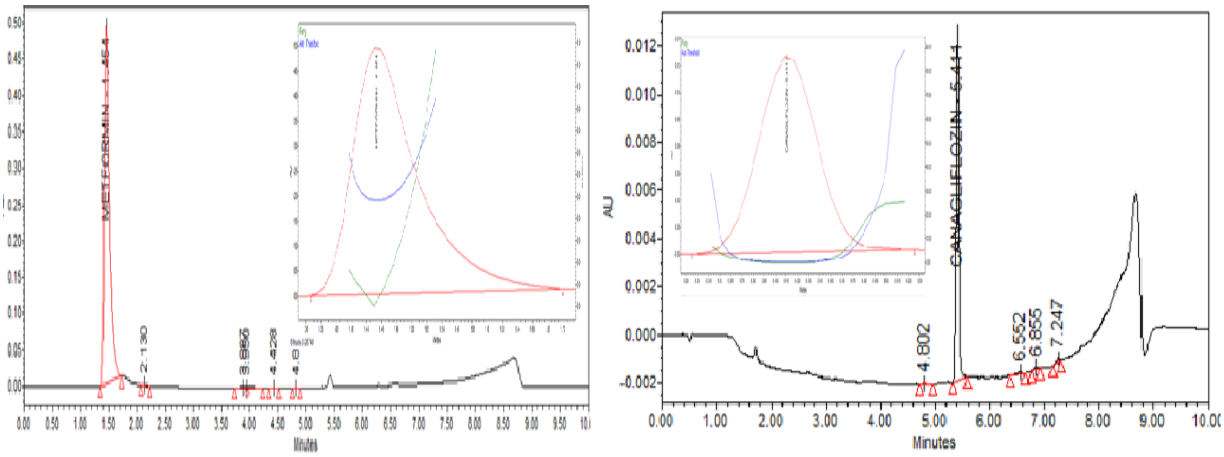


Fig. 8 Chromatograms of CANA and MET under thermal stress

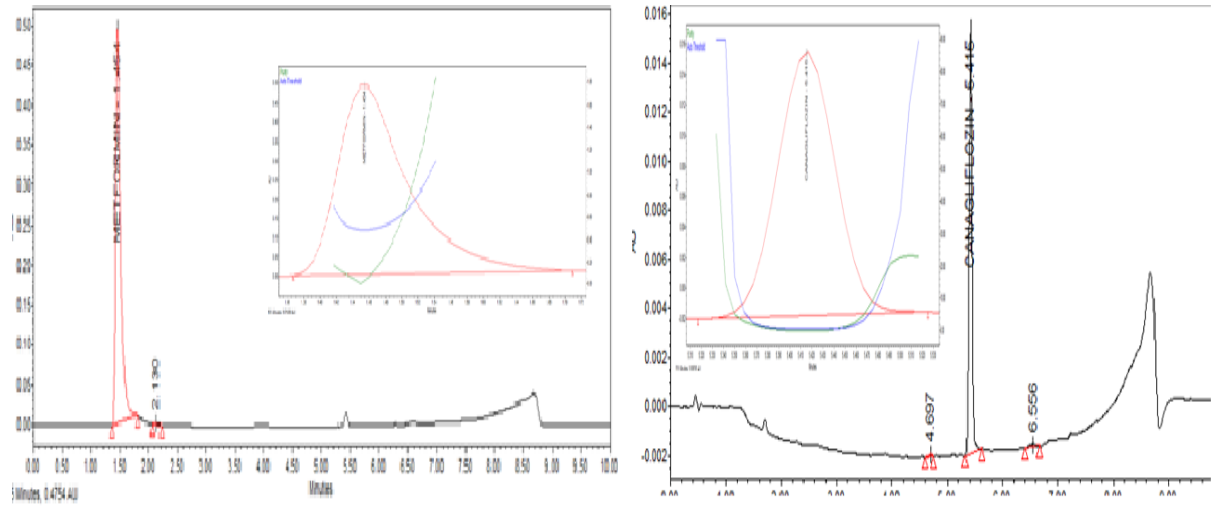


Fig. 9 Chromatograms of CANA and MET under photolytic stress

4. CONCLUSION

The novel, simultaneous stability indicating method developed for quantitative analysis of CANA and MET in fixed dose pharmaceutical dosage form. The developed method was validated by testing its linearity, accuracy, precision, specificity, limits of detection and quantification. The shorter run time enables rapid determination of the drugs individually or in combination in in-process testing and finished product testing. The method was found to be specific and stability indicating. This method exhibited an excellent performance in terms of sensitivity and speed hence method can be applicable for dissolution studies for finished dosage forms. The method is more economical and suitable for laboratory use as solvent consumption is very less. Conventional reported HPLC methods may be replaced by the proposed UPLC method because of its superiority.

5. REFERENCES

1. Oneil MJ., Smith A., Heckelman PE., The Merck Index: 2001, 13: pp. 349.
2. R.S.Satoskar., S.D.Bhandarkar and S. S. Ainapure., "Pharmacology and Pharmacotherapeutics", Popular Prakashan: 2001, 13th edition, Mumbai, India.
3. C.J.Bailey, M.R.C. Path., R.C.Turner. Metformin New*Engand Journalof medicinal chemistry*, 1996, 334: 574-579.
4. Burger's Medicinal Chemistry and drug discovery, Wiley Interscience: 2007, New Jersey (6 th edition).
5. Edward Chao, C., *Drugs of the Future*, 2011, 36:351–357.
6. Hitt E., Uses of metformin may extend beyond patients with type 2 diabetes. *Drugs*. 1995, 63:1879–94.
7. Madukar A., Prince.A., Vijay kumar R., Sanjeeva Y., Jagadeeshwar .K., D. Raghupratap., Simple and sensitive analytical method development and validation of metformin hydrochloride by RP-HPLC. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011, 3(3):117-120.
8. Cijo M Xavier., Kanakapura Basavaiah., RP-UPLC development and validation of metformin hydrochloride in pure drug and pharmaceutical formulations. *World journal of pharmacy and pharmaceutical sciences*. 2015, 4(4): 1649-1668.
9. Maddu Suma., K.Manasa., Ch.Rajakumari., and B. Lakshmaiah., RP-HPLC method development and validation for the estimation of canagliflozin in tablet dosage form. *International Journal of Pharmacy*. 2014, 5(4):1288-1292.
10. Ishpreet Kaur., Sharad Wakode., Harsharan Pal Singh., Satish Manchand., Development and Validation of a Stability-Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Forms. *Pharmaceutical methods*. 2016, 7(1):54-62.
11. Uttam Prasad Panigrahy., Sunil Kumar Reddy., A novel validated RP-HPLC-DAD method for the simultaneous estimation of metformin hydrochloride and canagliflozin in bulk and pharamaceutical tablet dosage form with forced degradation studies. *International research journal of pure & Applied chemistry*. 2015, 31(3): 1489-1507.
12. Nareddy Preethi Reddy.,Naga Thirumalesh Chevela., A validated

stability indicating RP-HPLC method for simultaneous determination of metformin and canagliflozin in pharmaceutical formulation. *World journal of pharmacy and pharmaceutical sciences*. 2015, 4(12): 631-640.

13. PS Reddy, S Sait, KK Hotha., Estimation of Naproxen Related substances in Sumatriptin Succcinate and Naproxen Sodium Tablets by UPLC, Asian journal of Chemistry. 25(17): 9717-9721.
14. ICH – Q2 (R1), (2005). Guideline on Validation of Analytical Procedure: Text and Methodology.
15. Vijay Kumar. R , Vinay U Rao A., Novel, Rapid, and Validated StabilityIndicating UPLC Method for the Estimation of Drotaverine Hydrochloride and Ibuprofen Impurities in Oral Solid Dosage Form. *Scientia Pharmceutica*. 2015; 83:567–581
16. ICH – Q1A (R2), Stability Testing of New Drug Substances and Products, 2003.
17. ICH – Q1B, Photo Stability Testing of New Drug Substances and Products, 1996.

How to cite this article:
Vijay Kumar et al., A Novel Simultaneous Stability-Indicating UPLC Assay Method for Metformin Hydrochloride and Canagliflozin in Oral Solid Dosage Form. *Int. J. Adv. Pharm. Biotech.*, 2018; 4(1): 10-22.