

## A VALIDATED STABILITY INDICATING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE AND EMPAGLIFLOZIN IN BULK DRUG AND TABLET DOSAGE FORM

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### ABSTRACT

**Objective:** To develop a simple, precise, accurate, method was developed and validated for analysis of metformin hydrochloride (MET) and empagliflozin in (EMPA) in bulk and tablet dosage form.

**Methods:** The method used a reverse phase column, dikma C18 (50×2.1 mm, 1.8 μ), a mobile phase comprising of phosphate buffer (pH-3): methanol (30:70 v/v) flow rate of 1.0 ml/min and a detection wavelength of 240 nm using a photodiode array detector. The proposed method was validated for various parameters like linearity, precision, accuracy, robustness, ruggedness, detection, quantification limits, stability studies, formulation analysis as per International Conference on Harmonization (ICH) guidelines.

**Results:** The retention time was found to be 1.189 min and 1.712 min for MET and EMPA respectively. The proposed method was found to be having linearity in the concentration range of 500-2500 μg/ml for MET ( $r^2=0.989$ ) and 5-25 μg/ml for EMPA ( $r^2=0.994$ ), respectively. The mean % recoveries obtained were found to be 100.35-100.48% for MET and 99.80-101.30% for EMPA respectively. Stress testing which covered acid, base, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to International Conference on Harmonization (ICH) guidelines.

**Conclusion:** Thus, the proposed method can be successfully applied for the stability indicating the simultaneous determination of MET and EMPA in bulk and combined tablet dosage form and in the routine quality control analysis.

**Keywords:** Empagliflozin, Metformin hydrochloride, Reversed-phase ultra-performance liquid chromatography, Validation

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### INTRODUCTION

Metformin (MET) hydrochloride is chemically named as (3-(diamino methylidene)-1, 1-dimethylguanidine hydrochloride. It has a molecular formula of  $C_4H_{12}ClN_5$  and the molecular weight is 165.62 g/mol. MET is an oral anti-hyperglycemic agent (Type 2 diabetes) belongs to a class of biguanides and useful for treating non-insulin dependent diabetes mellitus. MET decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by MET of AMP-activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats [1-5].

Empagliflozin (EMPA) is chemically named as 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene (fig. 2). It has a molecular formula of  $C_{23}H_{27}ClO_7$  and molecular weight is 450.912 g/mol. Empagliflozin is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting the excretion of excess glucose in the urine [6, 8]. The sodium glucose cotransporter 2 (SGLT2), located in the proximal tubule of the nephron, is estimated to facilitate-90% of this reabsorption [9, 10]. It is a potent and selective competitive inhibitor of the SGLT2 protein. Sodium-glucose co-transporter 2 (SGLT2) inhibitors offer an insulin-independent mechanism for improving blood glucose levels since they promote urinary glucose excretion (UGE) by inhibiting glucose reabsorption in the kidney. In addition to glucose control, SGLT2 inhibitors are associated with weight loss and blood pressure reductions and do not increase the risk of hypoglycemia [9].

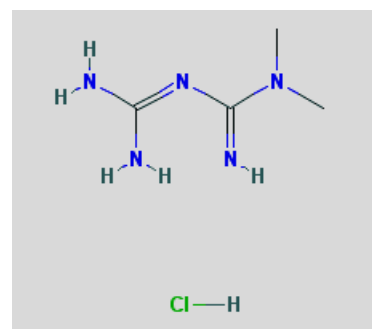


Fig. 1: Metformin (MET) hydrochloride structure from pub chem

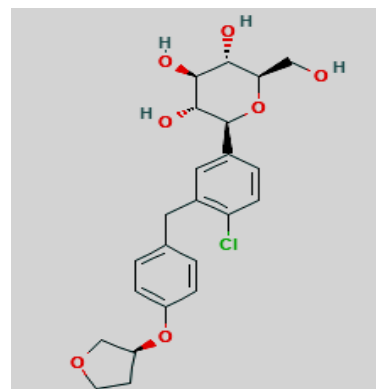


Fig. 2: Empagliflozin (EMPA) structure from pubchem

Literature survey revealed that few analytical methods are reported for analysis of both the drugs alone as well as in combination using ultraviolet spectrophotometry [15, 22], high-pressure liquid chromatography (HPLC) [10, 12, 17-19, 23, 25, 26] and ultra-pressure liquid chromatography [11]. To date, there have been no published reports about the simultaneous estimation of metformin HCl and empagliflozin by reverse phase ultra performance liquid chromatography (RP-UPLC) in bulk drug and in pharmaceutical dosage forms. Hence, an attempt has been made to develop a new method for simultaneous estimation and validation of MET and EMPA in tablet formulation in accordance with the International Conference on Harmonization (ICH) guidelines [26].

## MATERIALS AND METHODS

### Chemicals and reagents

Metformin HCl and empagliflozin were procured gift sample from pharma train research solutions, Hyderabad. Tablets were procured from a local pharmacy containing 1000 mg (Metformin HCl) and 10 mg (Empagliflozin). All solvents were of HPLC grade and all reagents were of analytical grade. Orthophosphoric acid was obtained from Merck (India), water and methanol for HPLC were obtained from lichrosolv (Merck), potassium dihydrogen orthophosphate was obtained from finer chemical Ltd and acetonitrile was obtained from molychem. All solvents and solutions were filtered through a membrane filter (Millipore Millex-HV filter units, 0.45 µm pore size; nylon) and degassed before use.

### Instrumentation

The UPLC was carried out on the waters with empower 2695 separation module, auto Sampler and photodiode array (PDA) detector were used in the analysis. Ultraviolet-visible spectrophotometer (Labindia), Balance (Afcoset ER-200A) and pH meter (Adwa-AD 1020).

### Preparation of the metformin HCl and empagliflozin standard and sample solution

Accurately weighed 10 tablets crushed in mortar and transferred equivalent to 1000 mg of metformin HCl and 10 mg empagliflozin sample into a 100 ml clean dry volumetric flask added about 70 ml of diluent and sonicated to dissolve it completely and made the volume up to the mark with the same solvent (Stock solution). Pipetted out 1.5 ml of metformin HCl and empagliflozin of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. In a similar manner working standard solutions of metformin HCl and empagliflozin were prepared (150 µg/ml MET and 15 µg/ml of EMPA) [11, 13, 15, 16].

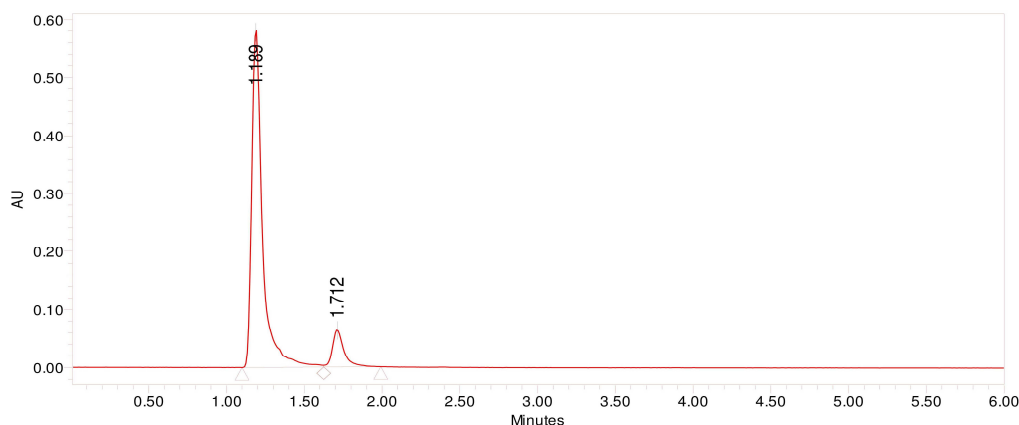
### Chromatographic conditions

Chromatographic conditions the UPLC system consisted of 2695 separation module, autosampler and PDA detector. The wavelength of detection as set at 240 nm. Separation was carried out in dikma C18 (50×2.1 mm, 1.8 µ) and the retention time (RT) of metformin HCl and empagliflozin was found to be 1.189 min and 1.712 min respectively shown in fig. 3, using mobile phase consisting phosphate buffer and methanol, pH 3.0 in the ratio of 30: 70 v/v at a flow rate of 1 ml/min with UV detection at 240 nm. The mobile phase was filtered through 0.45 µ filter under vacuum filtration [18, 19, 21].

## RESULTS AND DISCUSSION

### Method validation

The developed method was validated as per ICH guidelines for its system suitability, linearity, accuracy, precision, and robustness, limit of detection and limit of quantification by using the following procedures [26].



Peak Results					
Name	RT	Area	Height	USP Tailing	USP Plate Count
1	1.189	2801834	580489	1.90	1859.77
2	1.712	329455	63889	1.40	3117.46

Fig. 3: Chromatogram of metformin HCl and empagliflozin

### Accuracy

Accuracy of the method was established by performing recovery studies of ICH guidelines. Spiked samples were prepared by pre-analyzed sample solutions with the pure drug at three different concentration levels each in triplicate. The % mean recovery of metformin HCl (100.35-100.48%) and empagliflozin (99.80-101.30 %) at each level were within the limits of 98% and 102% indicating that the method is more accurate shown in table 1, when compared with metformin HCl (100.64-100.89%) and empagliflozin (99.95-101.34) by hplc [27] method. The current research work indicates that retention time value is less than 2

min which is a good recovery value of the existing method in comparison with HPLC method.

### Precision

The Intraday precision was demonstrated by injecting standard solutions of metformin HCl and empagliflozin with 1500µg/ml and 15µg/ml, respectively as per the test procedure and recording the chromatograms of six standard solutions. The % relative standard deviation (RSD) of metformin HCl and empagliflozin was found to be 1 and 1.1 respectively shown in table 2. % RSD values were within the limits and the method was found to be precise.

Table 1: Accuracy data of metformin HCl and empagliflozin

Metformin HCl					
% concentration (at specification Level), (n=3)	Area	Amount added (mg)	Amount found (mg)	% recovery	% RSD
50%	2403115	500	501.76	100.35	1.21
100%	4830189	1000	1008.52	100.85	1.29
150%	7218887	1500	1507.27	100.48	0.63
Empagliflozin					
50%	102326	5.0	5.06	101.30	1.16
100%	201600	10.0	9.98	99.80	1.29
150%	304187	15.0	15.07	100.47	0.64

Table 2: Precision data of metformin HCl and empagliflozin

S. No.	Metformin HCl	Empagliflozin
	Area	Area
1	4669547	205555
2	4633682	213714
3	4711857	202403
4	4586290	191233
5	4690109	197507
6	4674377	205189
mean±SD,(n=6)	4660977.0±44751.8	192600.0±13583.0
% RSD	1	1.1

#### Intermediate precision

The intermediate precision of the analytical method was determined by performing method precision on in three successive days by different analysts under same experimental condition by injecting

six replicate standard preparations was determined and the mean % RSD of metformin HCl (1500µg/ml) and empagliflozin (15µg/ml) was found to be 0.9 and 0.8 respectively shown in table 3. % RSD values were within the limits and the method was found to be precise.

Table 3: Intermediate precision data of metformin HCl and empagliflozin

S. No.	Metformin HCl	Empagliflozin
	Area	Area
1	4744291	195724
2	4657794	204293
3	4797415	204813
4	4706370	204018
5	4721262	191618
6	4781858	201052
mean±SD, (n=6)	4768164.9±86790.3	205252.9±16346.1
%RSD	0.9	0.8

#### Linearity

The measurement of linearity was evaluated by analyzing different concentrations of the standard solution of metformin HCl and empagliflozin. For both the drugs, the Beer-Lambert's law was obeyed in the concentration range 500-2500 µg/ml and 5-25 µg/ml for metformin HCl and empagliflozin respectively. The linearity of the proposed UPLC method was constructed by considering concentration (µg/ml) on X-axis and peak area on Y-axis. The regression coefficient was considered to be 0.98 over a concentration range of 500–2500 µg/ml (MET) and 0.99 over a concentration range of 5–25 µg/ml (EMPA). The representative linearity equation was found to be  $y = 2656x + 53186$  (MET) and  $y = 12865x + 27421$  (EMPA) as shown in fig. 4 and 5 and the corresponding data were shown in table 4. For both the methods the % RSD was found to be within the acceptable theoretical limits of ≤ 2%, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs.

#### Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is calculated using the formula  $3.3 \sigma/s$  where " $\sigma$ " is the standard deviation of the intercept obtained for calibration curve and " $s$ " is the slope of the calibration curve. Similarly, LOQ is calculated using the formula  $10 \sigma/s$ . The calculated LOD and LOQ for MET and EMPA using the proposed method were found to be 0.2626 µg/ml, 0.795 µg/ml and 0.02615 µg/ml, 0.0792 µg/ml respectively. The results obtained were presented in table 5.

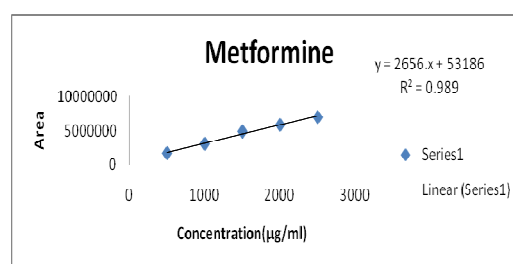


Fig. 4: Linearity graph for metformin HCl

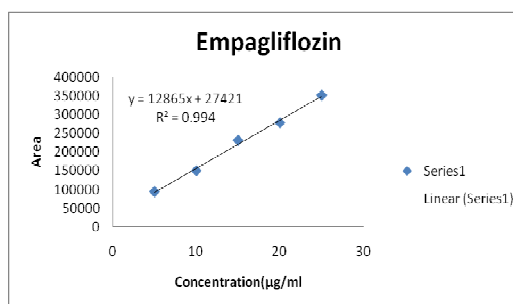


Fig. 5: Linearity graph for Empagliflozin

Table 4: Linearity data of metformin HCl and empagliflozin

S. No.	Metformin HCl		Empagliflozin	
	Concentration( $\mu\text{g/ml}$ )	Area	Concentration( $\mu\text{g/ml}$ )	Area
1	500	1737625	5	93475
2	1000	3100790	10	149362
3	1500	4887985	15	230699
4	2000	5851773	20	277360
5	2500	7002614	25	351109
Correlation coefficient		0.989	0.994	

Table 5: LOD and LOQ data of metformin HCl and empagliflozin

Drug name	Standard deviation( $\sigma$ )	Slope(s)	Intercept	LOD ( $\mu\text{g/ml}$ )	LOQ ( $\mu\text{g/ml}$ )
Metformin. HCl	2111167	2656	53186	0.2623	0.795
Empagliflozin	101970.2	12865	27421	0.02615	0.0792

### Robustness

Robustness were measured by making small, but deliberate changes in flow rate were varied at 0.9 ml/min to 1.1 ml/min and 10% organic

content in mobile phase affected the method significantly. This deliberate change in the above parameters has no significant effect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust and the results were shown in table 6.

Table 6: Robustness data of metformin HCl and empagliflozin

Parameter	Metformin HCl		Empagliflozin	
	USP Plate count	USP tailing	USP Plate count	USP tailing
Less flow rate (0.8 ml/min)	2001.88	1.13	4005.09	1.15
More flow rate (1.2 ml/min)	2796.68	1.09	3430.48	1.21
Mobile phase(10% less)	2041.88	1.13	4025.09	1.15
Mobile phase(10% more)	2796.68	1.09	3430.48	1.21

### Degradation studies

ICH degradation was attempted to various stress conditions such as acid hydrolysis (0.1N HCl), base hydrolysis (0.1 N NaOH), oxidative hydrolysis (3% H<sub>2</sub>O<sub>2</sub>), thermal degradation (heated at 110 °C for 24 h) and photolytic degradation (overall illumination of  $\geq 210\text{W h/m}^2$  at 25 °C for 7 d with UV radiation at 320-400 nm), to evaluate the ability of the proposed method to separate MET and EMPA from its degradation products [25]. It was observed that the drug degrades as shown by the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug,

with additional degradation peaks. Percent degradation was calculated by comparing the areas of the degraded peaks at each degradation condition with the corresponding areas of the peaks of both the drugs under non-degradation condition. Comparatively, more degradation was found with base, peroxide, photo, thermal for EMPA and with thermal for MET. There was no interference in any of the degradation products of the stress conditions tested in the current study. Thus, the developed method of analytical determination of MET and EMPA was established to be specific and stability-indicating. The results are shown in table 7 and fig. 6,7,8,9,10 [20, 22].

Table 7: Degradation data of metformin HCl and empagliflozin

Stress condition	Metformin HCl		Empagliflozin	
	% assay	% degraded	% assay	% degraded
Standard	100	-	100	-
Acid	97.6	2.40	94.03	5.97
Base	94.56	5.44	91.3	8.70
Peroxide	95.04	4.96	92.14	7.86
Thermal	92.52	7.48	89.51	10.49
Photo	95.15	4.85	91.04	8.96

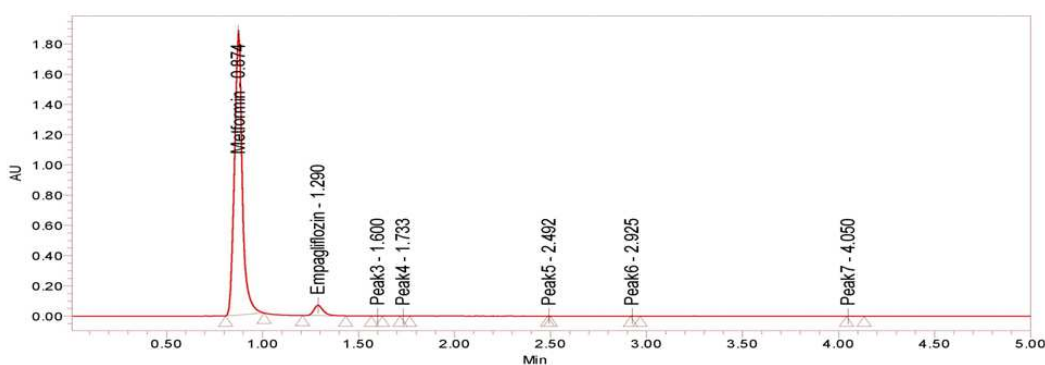


Fig. 6: Chromatogram of acid degradation

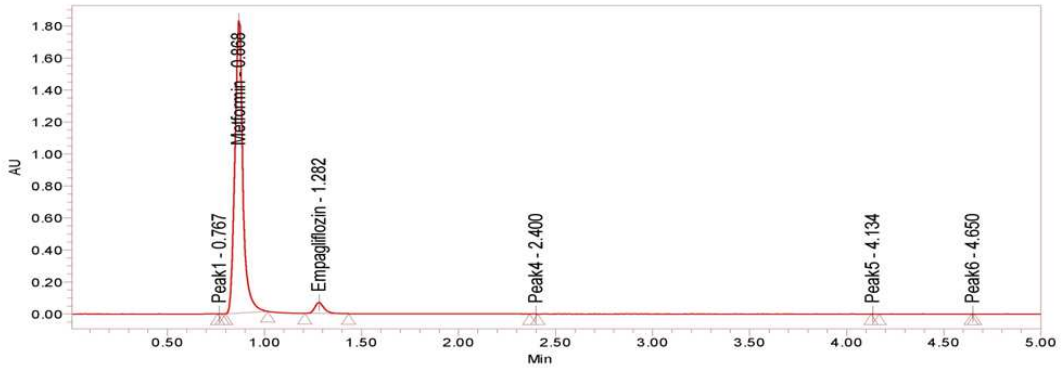


Fig. 7: Chromatogram of base degradation

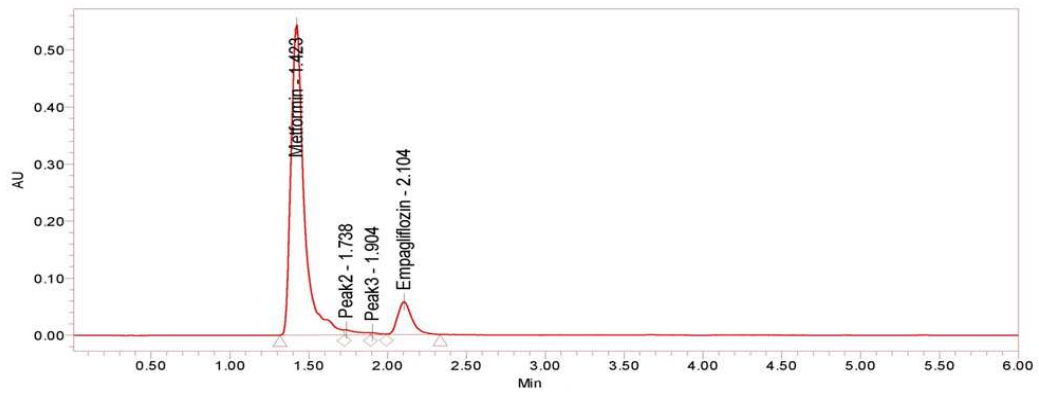


Fig. 8: Chromatogram of peroxide degradation

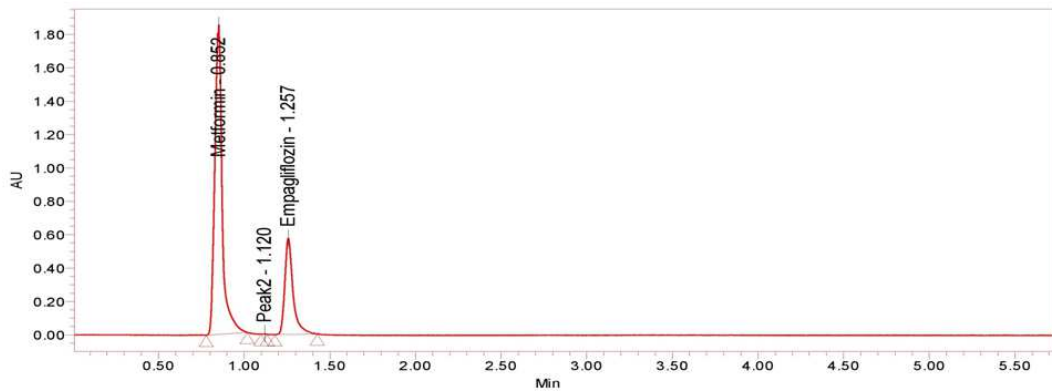


Fig. 9: Chromatogram of photo degradation

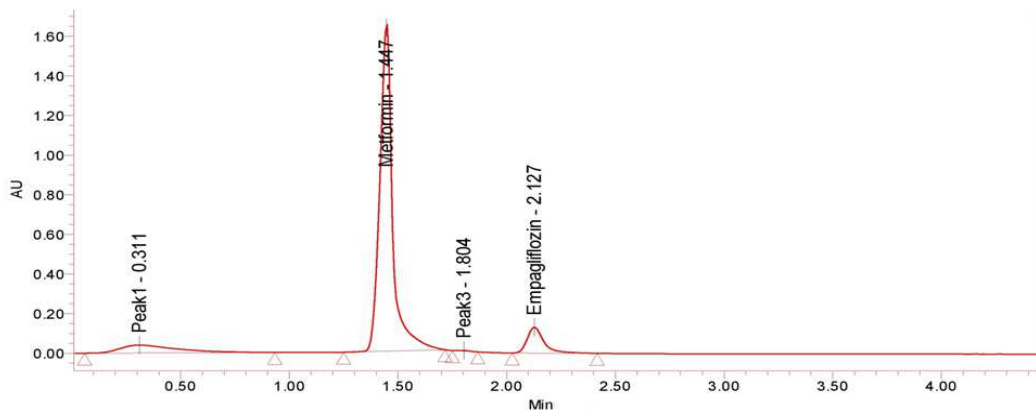


Fig. 10: Chromatogram of thermal degradation

## CONCLUSION

The newly developed UPLC method of metformin HCl and empagliflozin assay determination was found to provide faster retention time with a good resolution in the present study. A good linearity was obtained for both drugs (500-2500 µg/ml and 5-25 µg/ml) with a correlation coefficient of 0.994 and 0.998 for metformin HCl and empagliflozin respectively. The results of precision, recovery and ruggedness were within limits. Hence the method was successfully applied for degradation studies. The UPLC method of simultaneous estimation of metformin HCl and empagliflozin was found to be a novel, simple, precise, accurate and effective method. There were no UPLC methods reported till now on the above-mentioned combination drugs. Hence the developed method is suitable for the routine analysis and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

## CONFLICTS OF INTERESTS

Declare none

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