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Development and validation of sample simultaneous analysis for ertugliflozin and metformin by reverse phase-high performance liquid chromatography (RP-HPLC) in tablet dosage form

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Abstract

To evaluate simultaneously metformin and ertugliflozin in tablet dosage form, a quick, reliable and consistent method was developed. Ascentis C18 150 x 4.6 mm, 5 µm column was used for chromatogram. 0.01N phosphate buffer mobile phase: acetonitrile pumped by column in the ratio of 60:40 has a flow rate of 0.9 ml/min at 220 nm, metformin and ertugliflozin retention time was observed to be 2.296 min and 2.967 min, respectively. 99.75% and 100.13% for metformin and ertugliflozin, respectively, degree recovery has been reached. The values of LOD, LOQ from regression calculations were 0.75, 2.28 and 0.02, 0.05, respectively. Ertugliflozin regression equation is y = 33635x + 320.6; y = 40672x + 9450 metformin regression equation. Degradation studies were done, in all conditions purity threshold was more than purity angle and within the acceptable range. This approach that can be used in industry in a daily quality assurance process became easy and economical.

1. Introduction

Metformin hydrochloride (Wang *et al.*, 2017), chemically designated as N, N-dimethylimidodicarbonimidic diamide hydrochloride, belongs to a category of anti-hyperglycemic agent (Rena *et al.*, 2017). It is a white to off-white compound with a molecular formula of $C_4H_{11}N_5$.HCl and has a molecular weight of 165.63 g/mol (Madiraju *et al.*, 2018). It is freely soluble in water and practically insoluble in acetone, ether and chloroform and has a pKa value of 12.4. It is used in the treatment of type 2 diabetes mellitus (Madiraju *et al.*, 2014). Ertugliflozin L-pyroglutamic acid, chemically designated as (1S, 2S, 3S, 4R, 5S)-5-(4-chloro-3-(4-ethoxybenzyl) phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1] octane-2,3,4-triol, compound with (2S)-5-oxopyrrolidine-2-carboxylic acid belongs to anti-hyperglycemic agent (FDA, 2017). It has a molecular formula of $C_{27}H_{32}ClNO_{10}$ with a molecular weight of 566 g/mol. It is a white to off-white powder that is soluble in ethanol and acetone, slightly soluble in ethyl acetate and acetonitrile and very slightly soluble in water (Cinti *et al.*, 2017). It is used in the treatment of type 2 diabetes mellitus, acts by inhibiting sodium glucose co-transporter 2 (SGLT2) (Merck, 2017). Literature survey reveals that there were few methods developed for the simultaneous estimation of metformin and ertugliflozin such as RP-HPLC method (Kumari and Bandhakavi, 2020; Sunkara *et al.*, 2021; Harsha Hruditha and Jayachandra Reddy, 2020; Jagadeesh and Annapurna, 2019; Wajahat *et al.*, 2020; China Babu *et al.*, 2019; Nizami *et al.*, 2018). The main objective of the proposed work was to develop and validate a RP-HPLC method for the sample simultaneous analysis for ertugliflozin and metformin in tablet dosage form (Figure 1a, 1b).

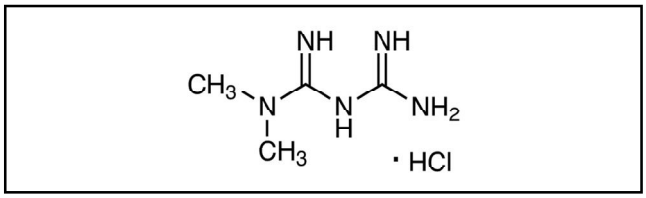

Figure 1(a): Chemical structure of metformin hydrochloride.

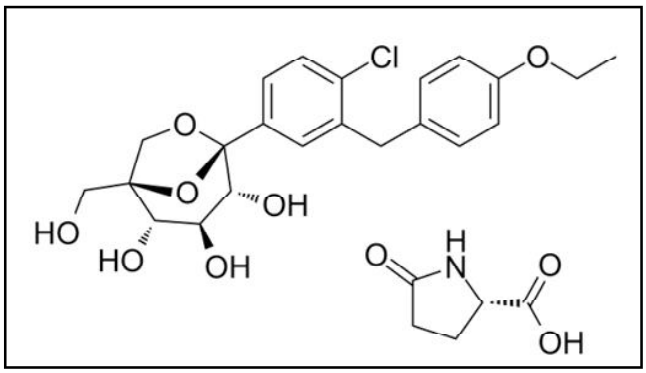

Figure 1(b): Chemical structure of ertugliflozin pyroglutamic acid.

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2. Materials and Methods

2.1 Reagents and chemicals

Metformin hydrochloride and ertugliflozin working standards are procured as gift samples from Spectrum labs, Hyderabad, Telangana. The tablet dosage form (Segluromet) were purchased from the local pharmacy. All the chemicals used for the development and validation were of AR grade and procured from Merck, India. And the solvents used were also purchased from Merck, India.

2.2 Instruments and chromatographic conditions

HPLC of waters Alliance 2695 separation module equipped with 2996 PDA detector, with empower 2 software was used as the instrument for the separation of metformin and ertugliflozin. Optimized chromatographic conditions were Ascentis C18 (4.6 × 150 mm, 5 µm particle size) column consisting of mobile phase composition of acetonitrile and phosphate buffer in the ratio of 40:60 %v/v on isocratic mode with flow rate 0.9 ml/min. The detection wavelength used was 220 nm. Other instruments used were pH meter (Lab India), weighing machine (Sartorius) and digital ultra sonicator (Labman). Glassware such as volumetric flasks, pipettes, burettes and beakers made of borosil was used.

2.3 Experimental work

2.3.1 Preparation of potassium dihydrogen phosphate buffer

Dissolved 6.8043 g of potassium dihydrogen phosphate in 1000 ml of HPLC grade water. Filtered and sonicated the solution by vacuum filtration and ultra-sonication.

2.3.2 Preparation of mobile phase

Accurately measured 400 ml (40%) of acetonitrile and 600 ml (60%) of phosphate buffer were mixed and degassed in digital ultra-sonicator for 15 min and then filtered through 0.45µ filter under vacuum filtration.

2.3.3 Preparation of diluent

The mobile phase was used as diluent.

2.3.4 Preparation of standard and sample solution

Accurately weighed and transferred 0.75 mg of ertugliflozin and 50 mg of metformin working standard into two separate 50 ml clean dry volumetric flasks. About 35 ml of diluent was added and dissolved using sonicator and made the volume up to the mark using diluent as solvent in both the volumetric flasks. From the above prepared stock solutions, 1 ml each of the ertugliflozin and metformin was transferred into a 10 ml volumetric flask and diluted up to the mark with diluent.

20 tablets of segluromet were taken and average weight was calculated. The tablets were crushed and an amount equivalent to 50 mg of metformin was weighed and dissolved in 50 ml of diluent using sonicator. The solution was filtered and diluted by pipetting 1ml of solution into 10 ml of volumetric flask and the volume was made up to the mark using diluent.

2.3.5 Method development

Ascentis C18 (4.6 × 150 mm, 5 µm) column as stationary phase, acetonitrile and phosphate buffer in the ration 40:60%v/v as mobile phase on isocratic mode at a flow rate of 0.9 ml/min with detection wavelength 220 nm gave better separation for the drugs. The standard and sample solution prepared as mentioned above were injected in to the chromatographic system and the chromatograms were shown in the Figures 2a and 2b, respectively. The retention time for metformin and ertugliflozin were found to be 2.296 min and 2.967 min, respectively, and their % assay was calculated.

2.3.6 Method validation

(ICH, 2005; ICH, 2003; ICH, 1996)

2.3.6.1 System suitability

The standard solution prepared was injected for five times and measured the area for all five injections in HPLC. The system suitability parameters such as plate count, tailing factor, resolution and % RSD for the area of five replicate injections were evaluated.

2.3.6.2 Specificity

Prepared standard solution and placebo solution were injected individually in to the chromatographic system and interference of drug peaks with excipient peaks were determined.

2.3.6.3 Linearity

For the determination of linearity of the developed method, five serial dilutions of concentration ranging from 25 µg/ml to 150 µg/ml for metformin and from 0.375 µg/ml to 2.25 µg/ml for ertugliflozin were prepared and injected in to the HPLC.

2.3.6.4 Precision

2.3.6.4.1 Repeatability

Repeatability of the method was determined by calculating the % RSD of the peak areas obtained from the five replicates of standard solution injected into the system.

2.3.6.4.2 Intermediate precision

For the estimation of intermediate precision, standard solution prepared above was analyzed on two different days maintaining same conditions.

2.3.6.5 Accuracy

Accuracy was determined in terms of % recovery, where three levels of concentrations 50%, 100% and 150% were prepared by spike method and analyzed.

2.3.6.6 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined on the basis of standard deviation and slope of the regression equation.

2.3.6.7 Robustness

Robustness of the method was analyzed by varying the optimized chromatographic conditions slightly such as ± 0.1 ml/min flow rate, ± 5% organic phase in mobile phase composition and ± 5°C column oven temperature.

2.3.6.8 Forced degradation studies

The forced degradation study (ICH, 2003; Jahnavi Bandla and Ashok Gorja, 2021) was performed to determine the specificity and stability indicating property of developed method. The drug was

deliberately subjected to stress conditions such as acidic condition, alkaline condition, oxidation condition, photolytic condition and thermal condition. All the solutions for degradation were prepared by dissolving drug in diluent to get an initial concentration and filtered. Acid decomposition was carried out in 1N hydrochloric acid and alkaline degradation was conducted using 1N sodium hydroxide and kept aside for 24 h. Solutions for oxidative degradation were prepared using 3% hydrogen peroxide at a concentration of 100 µg/ml of metformin and 1.5 µg/ml of ertugliflozin and kept aside for 24 h. To assess the stress testing for the photolytic conditions (Jahnavi Bandla and Ganapaty, 2018), the drug solution was exposed to UV light by keeping in UV chamber for 7 d or 200 Watt h/m^2 in photo stability chamber. Then, the resultant solutions were diluted to obtain concentration of standard solution. For thermal degradation study, the drug solution was heated in calibrated oven at 80ºC for 8 h, cooled and used. These solutions are injected into the HPLC system and values were noted.

3. Results

The prepared standard and sample solutions were injected into the HPLC system and their chromatograms were shown in Figures 2a, 2b.

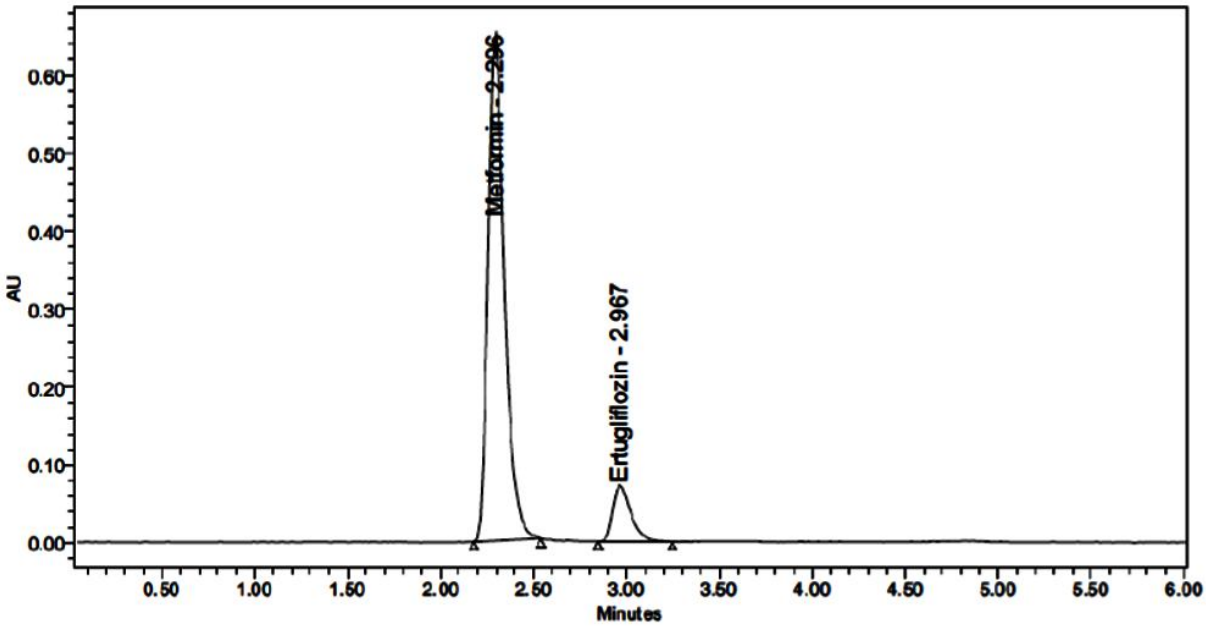

Figure 2(a): Chromatogram of standard solution.

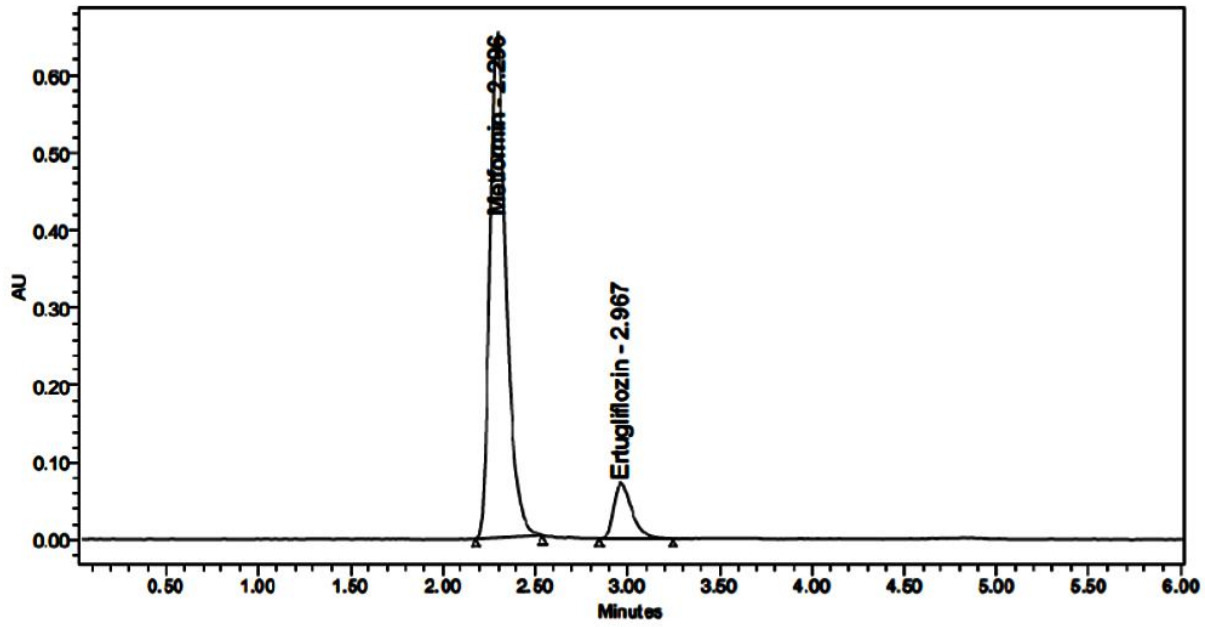

Figure 2(b): Chromatogram of sample solution.

The system suitability parameters like plate count, tailing factor, resolution and % RSD for the five replicates of standard solution were found to be within the acceptance criteria and the results were summarized in Table 1.

Table 1: Validation parameter results

Parameters			Metformin	Ertugliflozin
Specificity			Specific, no interference	Specific, no interference
Linearity	Regression equation, y=mx+c		y = 40672x+9450	y =33635x+320.6
	Correlation coefficient (r)		0.9999	0.9998
Accuracy (recovery) n=3	Level I (50%)		99.01%	99.76%
	Level II (100%)		100.22%	100.68%
	Level III (150%)		100.01%	99.95%
Precision, repeatability (%RSD) n=5			1.1	0.7
Intermediate precision (%RSD) n=5		Day 1	0.7	0.8
		Day 2	1.1	1.0
Limit of detection (LOD)			0.75 µg/ml	0.02 µg/ml
Limit of quantitation (LOQ)			2.28 µg/ml	0.05 µg/ml
System suitability	USP plate count		4464	3249
	USP tailing		1.48	1.40
	Resolution		3.9	

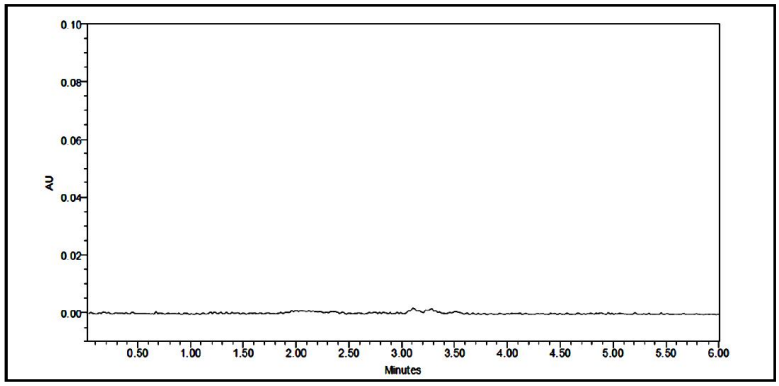

Figure 3: Chromatogram of placebo solution.

The linearity plot was presented in Figure 4 and the results were summarized in Table 1.

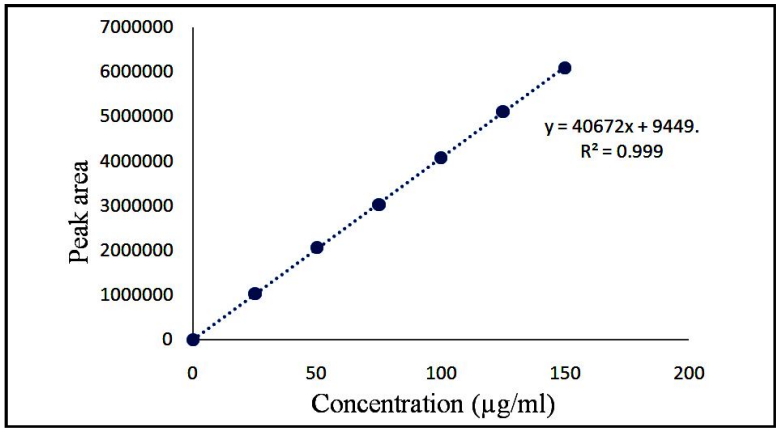

Figure 4 (a): Linearity plot of metformin hydrochloride.

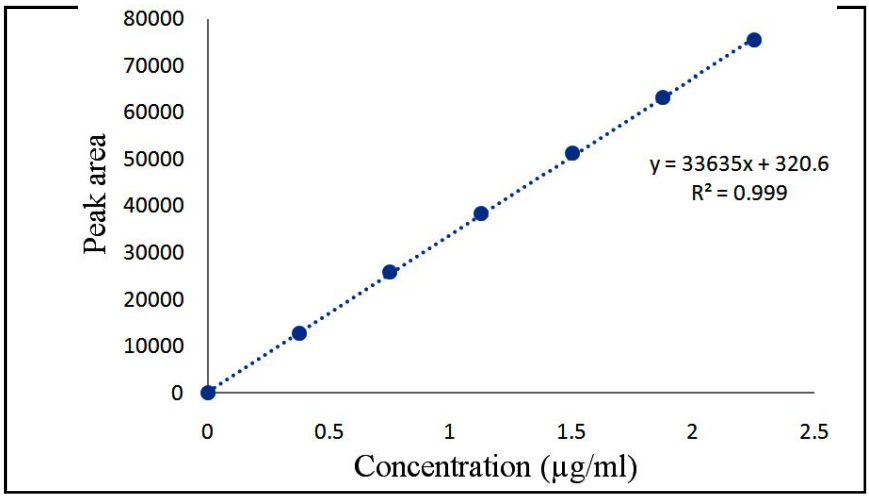

Figure 4(b): Linearity plot of ertugliflozin.

The robustness results were summarized in Table 2.

Table 2: Robustness results

S.No.	Condition	%RSD of metformin	%RSD of ertugliflozin
1	Flow rate (-) 0.8 ml/min	0.6	0.6
2	Flow rate (+) 1.0 ml/min	1.0	0.1
3	Mobile phase (-) Buffer: Acetonitrile (65:35)	0.7	0.5
4	Mobile phase (+) Buffer: Acetonitrile (55:45)	0.5	0.2
5	Temperature (-) 25°C	0.7	0.8
6	Temperature (+) 35°C	0.6	1.1

The forced degradation studies results were summarized in Table 3 and chromatograms were shown in Figure 5.

Table 3: Forced degradation studies results

S.No.	Degradation condition	% metformin degraded	% ertugliflozin degraded
1	Acid	3.60	4.52
2	Alkali	4.09	6.75
3	Oxidation	4.69	8.39
4	Thermal	2.05	3.18
5	UV	1.91	0.57

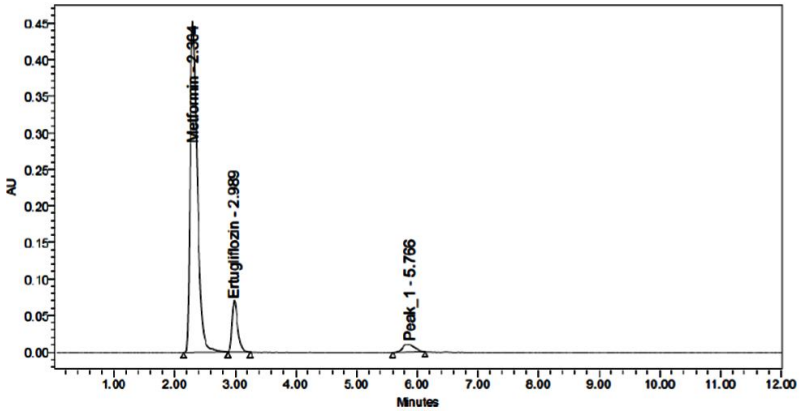

Figure 5(a): Acid degradation chromatogram.

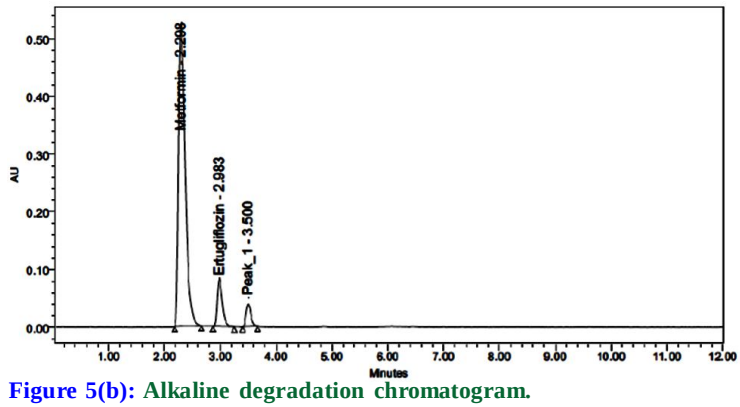

Figure 5(b): Alkaline degradation chromatogram.

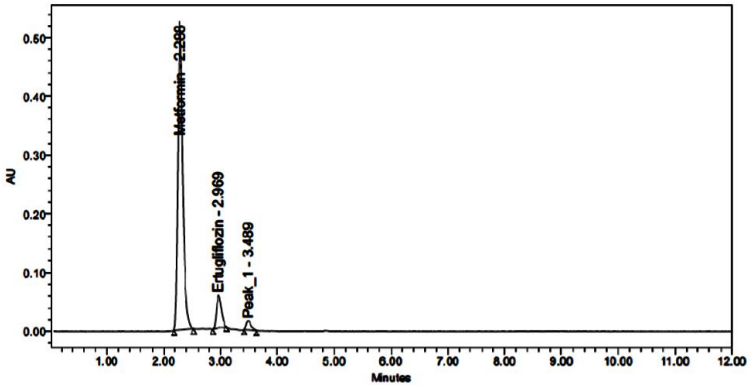

Figure 5(c): Oxidative degradation chromatogram.

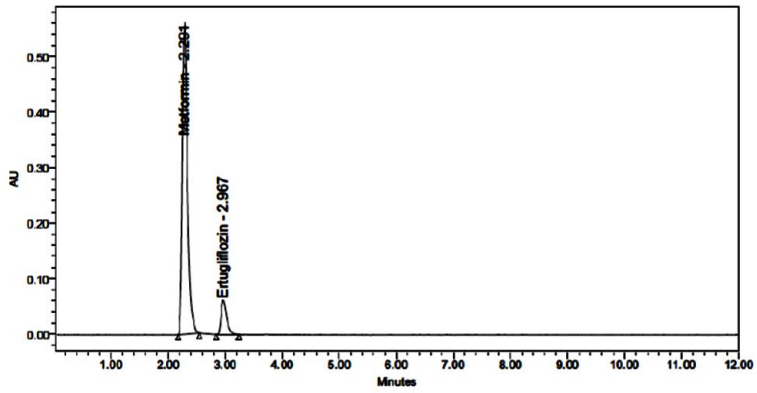

Figure 5(d): Photolytic degradation chromatogram.

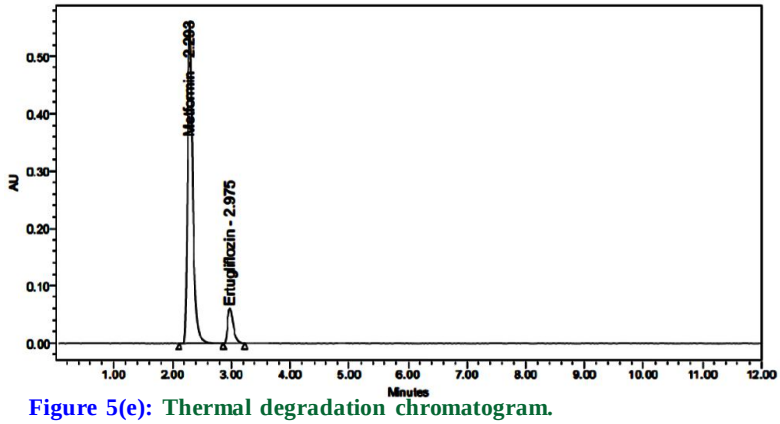

Figure 5(e): Thermal degradation chromatogram.

4. Discussion

Initially various mobile phase compositions such as methanol: water, water: acetonitrile and methanol: phosphate buffer: acetonitrile in varying proportions were tried. Finally, the mobile phase was optimized to phosphate buffer and acetonitrile in the proportion of 60:40 % v/v, respectively.

Similarly for the separation of drugs in the formulation, various columns like symmetry C18 column, ascentis C18 column or zodiac column were tried, but finalized column was ascentis C18 (4.6 × 150 mm, 5 µm) which gave good peak shape and resolution at 0.9 ml/min flow and 220 nm detection wavelength.

After the development of HPLC method for the simultaneous estimation of metformin and ertugliflozin, the method was validated as per the ICH guidelines. The system suitability parameters like plate count, tailing factor, resolution and % RSD for the five replicates of standard solution were found to be within the acceptance criteria.

The specificity of the method was determined by injecting the placebo solution in to the HPLC system and there was no interference of excipients with the peaks of drugs.

For the linearity study, the serial dilutions in the concentration range of 25 µg/ml to 150 µg/ml for metformin and from 0.375 µg/ml to 2.25 µg/ml for ertugliflozin were prepared and injected in to the HPLC. A graph was plotted between peak area (on y-axis) and concentration level (on x-axis) and the correlation coefficient was found to be 0.999.

The %RSD for the repeatability was found to be 1.1 for metformin and 0.7 for ertugliflozin. And for the determination of intermediate precision, the % RSD for day 1 was found to be 0.7 for metformin and 0.8 for ertugliflozin and for day 2; it was found to be 1.1 and 1.0 for metformin and ertugliflozin, respectively. The mean % recovery for metformin was found to be 99.75% and for ertugliflozin it was found to be 100.13%, indicating that the developed method was precise and accurate.

The LOD and LOQ for metformin were found to be 0.75 µg/ml and 2.28 µg/ml, respectively. The LOD and LOQ for ertugliflozin were found to be 0.02 µg/ml and 0.05 µg/ml, respectively. The developed method was found to be robust, as by altering the optimized chromatographic conditions slightly the results were still found to be within the limits.

The forced degradation studies were conducted by exposing the standard solution to the various stress conditions. The net degradation was found to be within the limits, indicates that the drugs are stable at various stress conditions.

5. Conclusion

A new precise, accurate method was developed for the simultaneous estimation of metformin and ertugliflozin in combined tablet dosage form using RP-HPLC. The developed method was validated in accordance to ICH guidelines and the method was found to be specific, precise, accurate, linear and robust. From the forced degradation studies, it was concluded that the drugs were stable when exposed to different stress conditions such as acidic, basic, oxidative, photolytic and thermal conditions. This method can be used for the quality control and routine analysis of the metformin and ertugliflozin in pharmaceutical dosage form.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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