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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RELATED SUBSTANCES OF TRANDOLAPRIL BY RP-HPLC AND ITS DEGRADATION

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ABSTRACT

Objective: A validated stability-indicating RP-HPLC method for Trandolapril was developed by separating its related impurities.

Methods: By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument, the chromatographic separation of Trandolapril and its related impurities was achieved on the column of Agilent eclipse C_{18} (150x4.6 mm, 3.5 µ) using gradient elution with a buffer containing 0.1percent formic acid and acetonitrile as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 213 nm utilizing the PDA detector was given in the instrumental settings. The linearity was studied between the concentration range of 4-60 µg/ml of Trandolapril and 0.5-7.5 µg/ml of imp-E, imp-A, imp-B and 0.7-10.5 µg/ml of imp-D were injected with a run time of 17 min. Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines.

Results: LOD and LOQ for the Trandolapril and its impurities were established with respect to test concentration. The plotted calibration curves were linear with a regression coefficient of R^2>0.999, which indicates that the linearity was within the limit. As a part of method validation, the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit.

Conclusion: The method developed was found to be applicable to routine analysis and to be used for the measurement of active pharmaceutical ingredients (i. e, Trandolapril and its related impurities). Since there is no HPLC method reported in the literature for the estimation of Trandolapril and its related impurities; there is a need to develop quantitative methods under different conditions to achieve improvement in specificity, selectivity etc.

Keywords: Trandolapril, Related impurities, HPLC, Development, Validation

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INTRODUCTION

Trandolapril is an ACE inhibitor [1, 2] used to treat high blood pressure [3, 4]. It may also be used to treat other conditions. Side effects reported for trandolapril include nausea, vomiting, diarrhea, headache, dry cough, dizziness or lightheadedness when sitting up or standing, hypotension [5], or fatigue [6]. Patients also on diuretics [7] may experience an excessive reduction of blood pressure after initiation of therapy with trandolapril. It can reduce potassium loss caused by thiazide [8] diuretics and increase serum potassium when used alone. Therefore, hyperkalemia [9, 10] is a possible risk. Increased serum lithium levels can occur in patients who are also on lithium. Trandolapril is teratogenic [11] (US: pregnancy category D) and can cause birth defects and even death of the developing fetus. The highest risk to the fetus is during the second and third trimesters. When pregnancy is detected, trandolapril should be discontinued as soon as possible. Trandolapril should not be administered to nursing mothers. Combination therapy with paricalcitol and trandolapril has been found to reduce fibrosis [12] in obstructive uropathy [13, 14]. Trandolapril is a prodrug that is de-esterified to trandolaprilat. It is believed to exert its antihypertensive effect through the renin-angiotensin-aldosterone system [15, 16]. Trandolapril has a half-life of about 6 h, and trandolaprilat has a half-life of about 10 h. Trandolaprilat has about eight times the activity of its parent drug. About one-third of trandolapril and its metabolites [17] are excreted in the urine [18, 19], and about two-thirds of trandolapril and its metabolites are excreted in the feces. Serum protein binding of trandolapril is about 80%. Trandolapril acts by competitive inhibition of the angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system, which plays an important role in regulating blood pressure. So, we developed a method for the estimation of Trandolapril by using RP-HPLC.

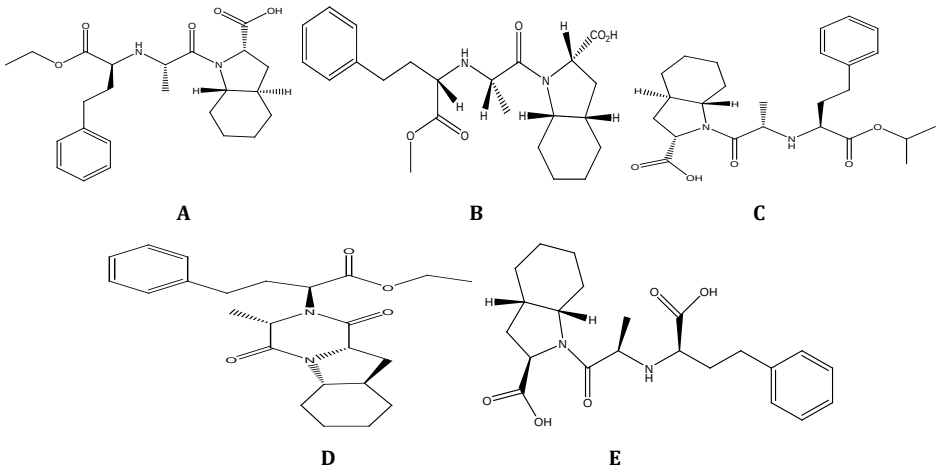

Fig. 1: Chemical structures of (A) Trandolapril (B) Impurity-A (C) Impurity-B (D) Impurity-D (E) Impurity-E

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade orthophosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. Candila health care ltd, Ahmedabad, India provided the reference criteria for Trandolapril and its related impurities.

The ınstrumentation

Waters alliance liquid chromatography (model e-2695) monitored with empower 2.0 data handling system and a detector of photodiode array (model 2998) [20] was used for this study.

Preparation of mobile phase-A: 1 ml of formic acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Mobile Phase-B: Acetonitrile

Optimization of mobile phase

Different trails have been done, different buffers and different mobile phases were used to develop the method. In all trails, peaks are not separated properly. Finally, for the proposed method, all the peaks are separated and the entire suitability conditions are within the limit.

Table 1: Gradient program

Time (min)	Mobile phase-a	Mobile phase-b
0.00	80	20
5	50	50
7	20	80
10	20	80
12	80	20
17	80	20

Till today there are no HPLC methods reported in the literature, So, it has more interested to develop a novel and reliable HPLC strategy for the establishment of Trandolapril and its related impurities.

Chromatographic conditions

The HPLC analysis was performed on a reverse-phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid and Agilent eclipse C_{18} (150x4.6 mm, 3.5 µ) column with a flow rate of 1 ml/min.

Diluent

Mobile phase was used as a diluent.

Validation procedure

The analytical parameters [21-25] such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines.

Standard stock solution

Weigh accurately 40 mg of Trandolapril and transferred into a 100 ml volumetric flask; add 70 ml of diluent sonicated for 10 min to dissolve the contents make up to the mark with diluent.

Sample stock solution

Transfer 740 mg (equivalent to 40 mg of Trandolapril and each tablet contains 4 mg of Trandolapril) of sample into a 100 ml volumetric flask diluted to volume with diluent. Filter through 0.45µ nylon syringe filter.

Impurity standard stock solution

Weigh accurately 5 mg each of imp-E, imp-A, imp-B and 7 mg of imp-D into a 100 ml volumetric flask. Add 70 ml of diluent, sonicated to dissolve and make up.

Spiked standard solution

Transfer 5 ml of standard stock into a 50 ml volumetric flask, add 40 ml of diluent and also add 5 ml of impurity standard stock solution and makeup to the mark with diluent. Filter through 0.45µ syringe filter.

Spiked sample solution

Transfer 5 ml of sample stock into a 50 ml volumetric flask, add 40 ml of diluent and also add 5 ml of impurity standard stock solution and makeup to the mark with diluent. Filter through 0.45µ syringe filter.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized.

Method validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

System suitability

Device suitability was performed by injecting a spiked standard solution containing 40 µg/ml of Trandolapril, 5 µg/ml each of imp-E, imp-A, imp-B and 7 µg/ml of imp-D in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH [26]. The results were shown below.

Table 2: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name trandolapril
USP Plate count	NLT 2000	51863
USP Tailing	NMT 2.0	1.04
USP Resolution	NLT 2.0	22.69
% RSD	NMT 2.0	0.82
Retention Time	NLT 2.0	9.894

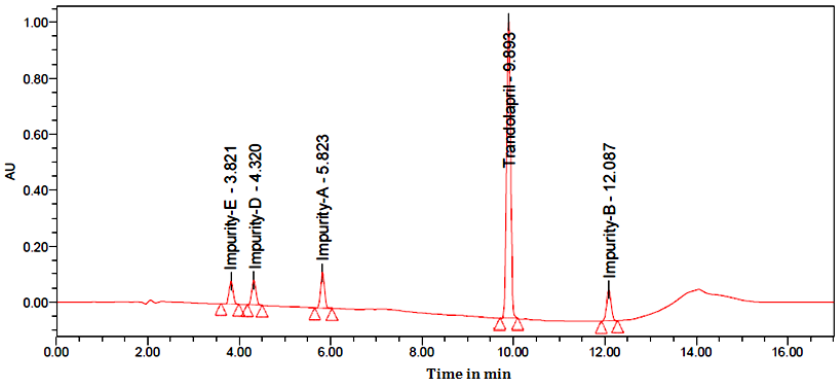

Fig. 2: Chromatogram of standard

Specificity

In this test method, placebo, sample and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredient and its related substances were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

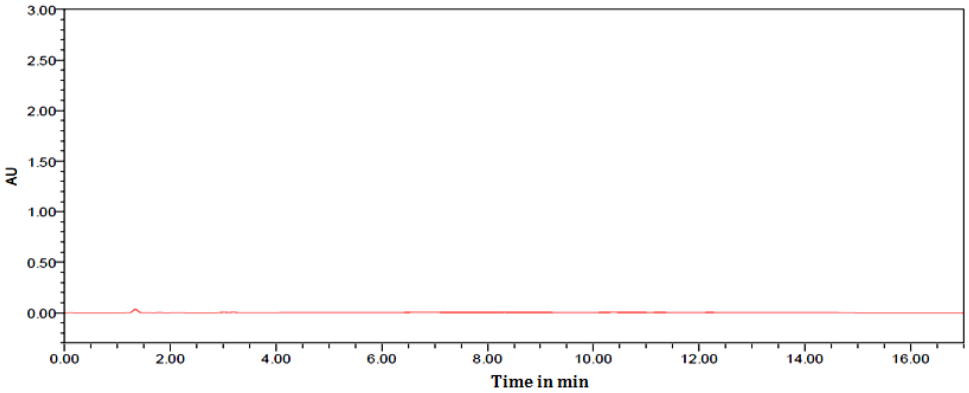

Fig. 3: Chromatogram of blank

Linearity

Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was noticed that the curve was linear between the range of 4-60µg/ml of Trandolapril and 0.5-7.5µg/ml each of imp-E, imp-A, imp-B and 0.7-10.5 µg/ml of imp-D. The regression equations for calibration curve was Y=147215.89x+159148.18 (R^2=0.9996) for Trandolapril and Y= 9503.01x+1281.27 (R^2=0.9993) for imp-E and Y=7488.75x+397.6 (R^2=0.9998) for imp-D and Y=14689.54x+1046.22 (R^2=0.9993) for imp-A and Y=14478.22x+389.98 (R^2=0.9995) for imp-B respectively.

Table 3: Linearity results of trandolapril and its impurities

A

Linearity	Imp-E		Imp-D		Imp-A	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	0.50	6035	0.70	6484	0.50	9046
Linearity-2	1.25	13458	1.75	13311	1.25	19198
Linearity-3	2.50	25824	3.50	25892	2.50	37293
Linearity-4	5.00	50245	7.00	53572	5.00	77891
Linearity-5	6.25	60822	8.75	65900	6.25	91630
Linearity-6	7.50	71154	10.50	78762	7.50	110125
Slope	9503.01		7488.75		14689.54	
Intercept	1281.27		397.6		1046.22	
CC	0.9993		0.9998		0.9993	

B

Linearity	Trandolapril		Imp-B	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	4.00	781724	0.50	8168
Linearity-2	10.00	1702135	1.25	18324
Linearity-3	20.00	3226387	2.50	36182
Linearity-4	40.00	6049599	5.00	74780
Linearity-5	50.00	7596589	6.25	88636
Linearity-6	60.00	8885327	7.50	109639
Slope	147215.89		14478.22	
Intercept	159148.18		389.98	
CC	0.9996		0.9995	

Accuracy

The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of 20, 40 and 60µg/ml of Trandolapril and 125, 250 and 375µg/ml of Permethrin were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100% and also the RSD values were less than±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in table 4.

Precision

The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The accuracy of the process of the drugs was calculated by injection of six individual determinations of Trandolapril and its related substances. Method precision results were shown in table 4 and sample chromatogram was shown in fig. 5.

Intraday precision

Six replicates of a sample solution containing Trandolapril and is related substances were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

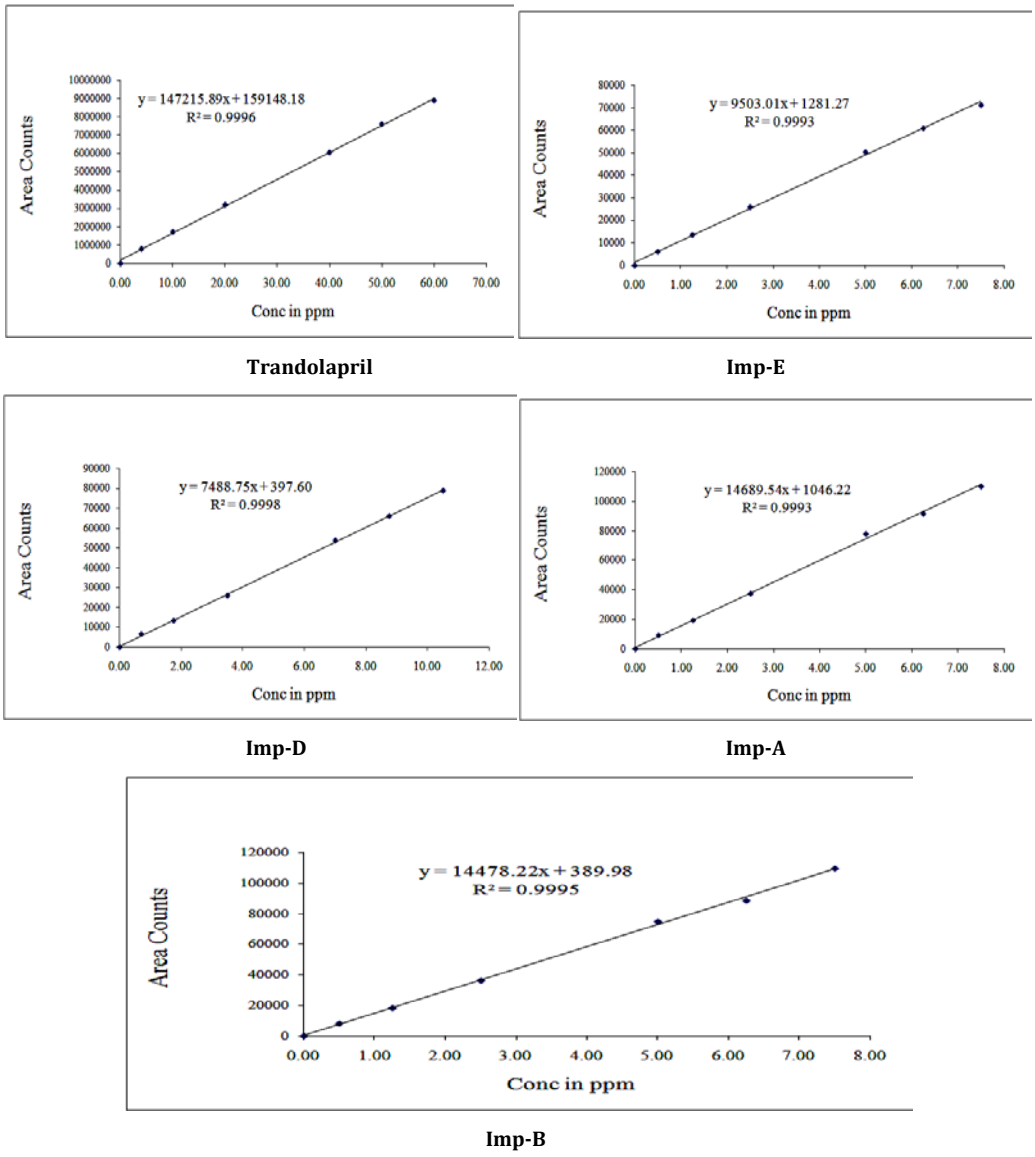

Fig. 4: Calibration plots of trandolapril and its related impurities

Table 4: Results of accuracy

S. No.	% Level	Trandolapril % recovery
1	50	99.82
2	100	100.05
3	150	99.76
Mean		99.88
Std Dev		0.153

mean±SD (n=3)

Table 5: Intraday precision results of allantoin and permethrin

Sample No.	% of related substances		
	Spiked impurities	Total impurities	% Purity (100-total impurities)
1	1.15	0.58	99.42
2	1.16	0.62	99.38
3	1.12	0.64	99.36
4	1.24	0.69	99.31
5	1.22	0.63	99.37
6	1.28	0.61	99.39
Average	1.20	0.63	99.37
% RSD	5.12	5.82	0.04

mean±SD (n=6)

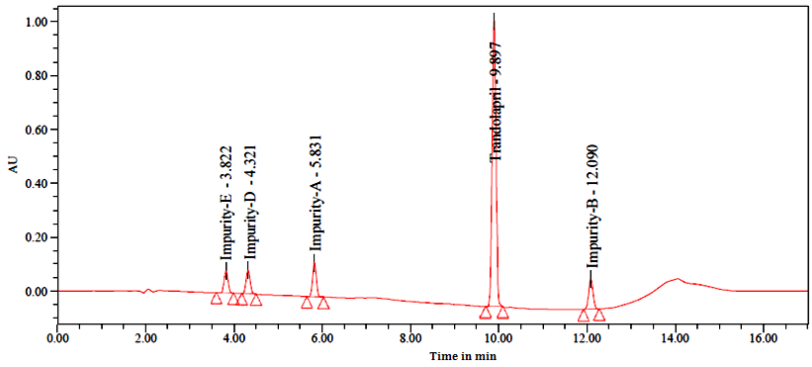

Fig. 5: Chromatogram of sample

Intermediate precision

Six replicates of the sample solution were analyzed by different researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the table below.

Interday precision

Six replicates of a sample solution containing Trandolapril and its related substances were analysed on a different day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also, the percentage assay values were close to be 100%. The results are given in table 6 [27].

LOD and LOQ

LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their s/n values of Allantoin and Permethrin were represented in the following table. This method is validated as per the ICH guidelines [28, 29].

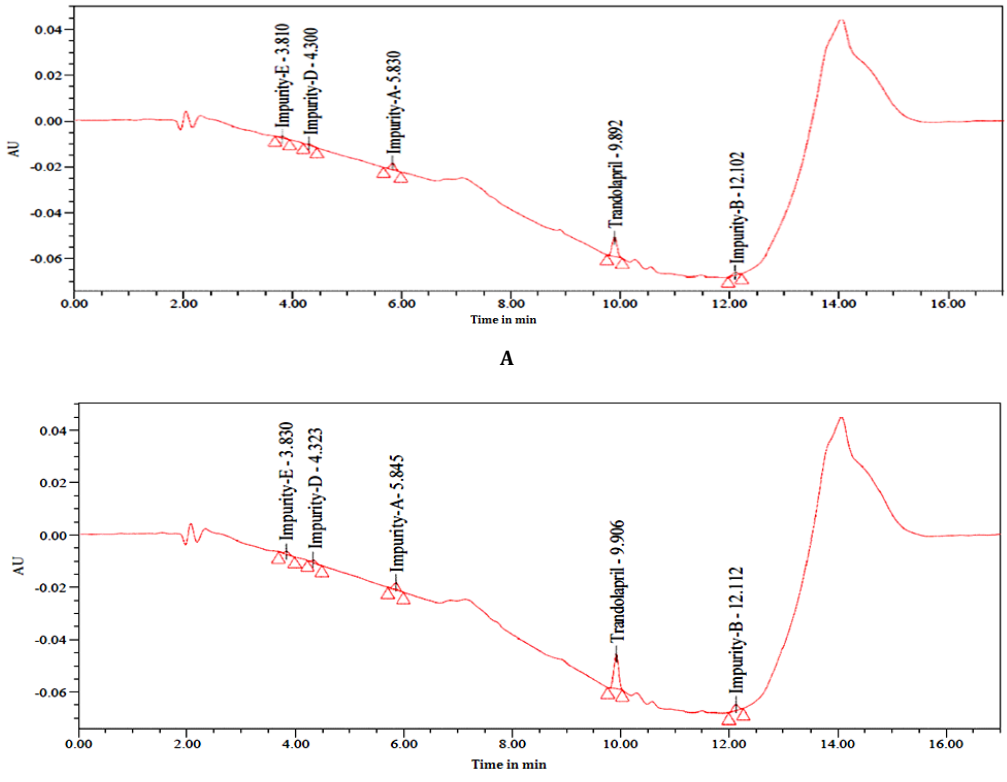

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Robustness

The conditions of the experiment were designed to measure the robustness of the intentionally changed conditions such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for Trandolapril and its impurities were found to be within the limit and results were tabulated in table 8 [30].

Table 6: Inter-day precision results

Sample No.	% of related substances		
	Spiked impurities	Total impurities	% Purity (100-Total impurities)
1	1.19	0.68	99.32
2	1.25	0.66	99.34
3	1.22	0.59	99.41
4	1.17	0.72	99.28
5	1.13	0.55	99.45
6	1.20	0.62	99.38
Average	1.19	0.64	99.36
% RSD	3.46	9.77	0.06

mean±SD (n=6)

Table 7: Results of LOD and LOQ

Name	LOD conc. (µg/ml)	S/N	LOQ conc. (µg/ml)	S/N
Trandolapril	0.05	8	0.165	37
Imp-E	0.0063	4	0.0218	33
Imp-D	0.0088	5	0.029	334
Imp-A	0.0062	4	0.0218	33
Imp-B	0.0061	4	0.0218	33

Table 8: Robustness results of allantoin and permethrin

Parameter name	% RSD
	Trandolapril
Flow rate (0.8 ml/min)	0.35
Flow rate (1.2 ml/min)	0.98
Org Plus (66:34) (+10%)	1.01
Org Minus (54:46) (-10%)	0.86

Table 9: Stability results of trandolapril

Stability	Trandolapril	
	% Purity	% deviation
Initial	99.98	0.01
6 h	99.36	0.24
12 h	98.86	1.14
18 h	98.53	1.47
24 h	98.21	1.79

Stability

Normal solution was kept at room temperature and 2-8 ° C for up to 24 h. These solutions were then pumped into the system and the percent deviation from the initial to 24 h was measured [31]. No major variations were found and verified that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Trandolapril and its related impurities. Stability results were tabulated in table 9.

Degradation studies

Trandolapril and its related substances were subjected to various conditions of forced degradation [32, 33] in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials [34, 35]. In addition, the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities [36].

Acid degradation

Acid degradation was done by using 1N HCl and 15.3% Trandolapril degradation was observed.

Alkali degradation

Alkali degradation was done by using 1N NaOH and 15.1% of Trandolapril degradation was observed.

Peroxide degradation

Peroxide degradation was done by using 30% peroxide and 14.7% of Trandolapril degradation was observed.

Reduction degradation

Reduction degradation was done by using 30% sodium bisulphate solution and 12.4% Trandolapril degradation was observed.

Thermal degradation

Thermal degradation was done at 105 °C and 11.9% of Trandolapril degradation was observed.

Hydrolysis degradation

Hydrolysis degradation was done by using HPLC water and 10.7% Trandolapril degradation was observed.

Table 10: Forced degradation results of allantoin and permethrin

Degradation condition	Trandolapril
	% purity
Acid deg	15.3
Alkali deg	15.1
Peroxide deg	14.7
Reduction deg	12.4
Thermal deg	11.9
Hydrolysis deg	10.7

CONCLUSION

The developed method gave good results between Torsemide and its four impurities with a run time of 17 min, high efficiency and

complies with modified SST specifications of USP. The utilization of Agilent eclipse C_{18} column within the present work has shown better elution of analytes with good resolution, improved plate count and tailing. Therefore the C_{18} columns are often wont to achieve high specificity in a shorter time of study of Trandolapril as per ICH Q 3A (R_2) guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Trandolapril and its impurities. The sample recovery was in good agreement with their respective label claims suggested non-interference within the estimation. Hence, the technique is often easily and conveniently adopted for routine analysis of Trandolapril in the combined dosage form.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Author declares that there have been no conflicts of interest.

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