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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR PSEUDOEPHEDRINE HYDROCHLORIDE AND FEXOFENADINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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Keywords:

Stability indicating method, Pseudoephedrine Sulphate (PSE), Fexofenadine Hydrochloride (FEX), High performance liquid chromatographic (HPLC), stress degradation conditions.

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ABSTRACT: High performance liquid chromatographic (HPLC) method was described for determination of Pseudoephedrine Hydrochloride (PSE) and Fexofenadine Hydrochloride (FEX). The chromatographic separation was achieved on Kinetex C_{18}, (250 x 4.6 mm i.d), Particle size 5µm. The mobile phase contains a mixture of 0.005 M Dioctyl Sodium Sulfosuccinate Buffer in a mixture of 50 volume of Methanol, 10 volume of Acetonitrile, 40 volume of Water and 1 volume of Glacial Acetic Acid was investigated to separate the drugs from their stressed degradation products. The flow rate was 1.2 ml/min, injection volume of 50 µL, run time of 15 minutes, at column oven temp 50°C. The detector wavelength was 258 nm. PSE and FEX were subjected to stress degradation conditions of hydrolysis (acid and base), oxidation and thermal degradation. Stressed samples were analyzed by the developed procedures. The described method shows excellent linearity over a range of 72 to 720µg/ml and 48 to 480µg/ml PSE and FEX, respectively. Degradation of PSE was observed in oxidative condition but found to be stable in other stress conditions while FEX degradation was observed in oxidative conditions and found to be stable in other stress conditions. This method is capable of complete chromatographic separation of PSE and FEX peaks.

INTRODUCTION: Pseudoephedrine Hydro chloride with Fexofenadine Hydrochloride is found to show antihistaminic effect. Pseudoephedrine Hydrochloride is a Vasoconstrictor and Adrenergic Agent. Its chemical name [1] is (1S, 2S)-2-(methyl amino)-1-phenylpropan-1-ol (**Fig.1**). Fexofenadine Hydrochloride is a Histamine H1 Antagonist and anti-allergic agent. Its chemical name [2] is 2-(4-{1-hydroxy-4-[4-hydroxyldiphenylmethyl) piperidin-1-yl] butyl} phenyl) -2-methyl propanoic acid (**Fig.2**).

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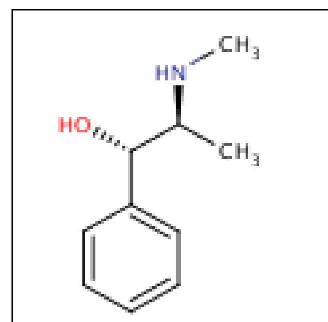

FIG. 1: STRUCTURE OF PSE

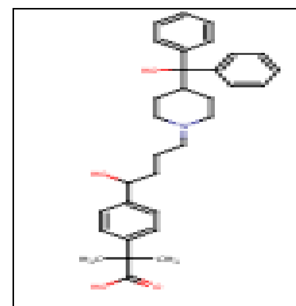

FIG.2: STRUCTURE OF FEX

This combination is available in 120 mg of Fexofenadine Hydrochloride and 180 mg of Pseudoephedrine Hydrochloride dose. Thus it is inevitable to develop such a sensitive, accurate, precise, rapid and economical method for routine analysis of this combination in pharmaceutical dosage form successfully.

MATERIALS AND METHODS:

Instrumentation:

A high performance liquid chromatography system consisting of Agilent technologies 1260 infinity Module with Photo Diode Array detector was used. Chemicals were weighed using Analytical balance Mettler Toledo model MS105DU. All pH measurements were done on pH meter Systronics-model µpH System 361.

Reagents and Chemicals:

HPLC grade solvents methanol, Dioctyl sodium Sulfosuccinate, Acetonitrile and water were obtained from Merck Pvt. Ltd. India. Water was deionised and further purified by means of Milli-Q plus water purification system, Millipore Ltd (U.S.A). Hydrochloride acid AR, sodium hydroxide AR, Hydroxide Peroxide AR and glacial acetic acid AR Grade were obtained from Rankem Pharmaceuticals India Ltd. India.

Chromatographic Conditions and Measurement Procedure:

Preparation of Mobile phase:

Accurately weighed about 2.223 gm of Dioctyl Sodium Sulfosuccinate Buffer (0.005 M) and dissolved in a mixture of 50 volume of Methanol, 10 volume of Acetonitrile, 40 volume of Water and 1 volume of Glacial Acetic Acid and filtered through 0.45 µm filter paper, sonicated for 10 minutes to degas the mixture was used as mobile phase.

Standard Preparation:

Accurately weighed and transferred about 180 mg of PSE and 120 mg of FEX into a 100 ml volumetric flask and dissolved in 50 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1800 µg/ml PSE and 1200 µg/ml FEX. From this solution 5 ml was transferred to 50 ml volumetric flask. The volume was adjusted to the mark with the mobile phase to give a solution containing 360µg/ml PSE and 240µg/ml FEX.

Sample preparation:

Twenty tablets were weighed and finely powdered. The powder equivalent to 180 mg PSE and 120 mg FEX was accurately weighed. These PSE and FEX powder was transferred to volumetric flask of 100 ml capacity and dissolved in 50 ml of mobile phase. The flask was sonicated for 10 minute. The flask was shaken and volume was made up to the mark with mobile phase.

The above solution was filtered through whatmann filter paper (0.45µ). This solution is expected to contain PSE – 1800 µg/ml and FEX – 1200 µg/ml. From this, 10 ml of aliquot was taken and transferred to volumetric flask of 50 ml capacity and volume was made up to the mark with the mobile phase to give a solution containing 360µg/ml PSE and 240 µg/ml FEX. This solution was used for the estimation of PSE and FEX.

Selection of wavelength maxima:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study individual drug solutions of 360 µg/ml PSE and 240 µg/ml FEX were prepared in mobile phase of 0.005 M Dioctyl Sodium Sulfosuccinate Buffer in a mixture of 50 volume of Methanol, 10 volume of Acetonitrile, 40 volume of Water and 1 volume of Glacial Acetic Acid. These drug solutions were than scanned in the UV region of 200-400 nm and the overlay spectrum was recorded (**Fig.3**).

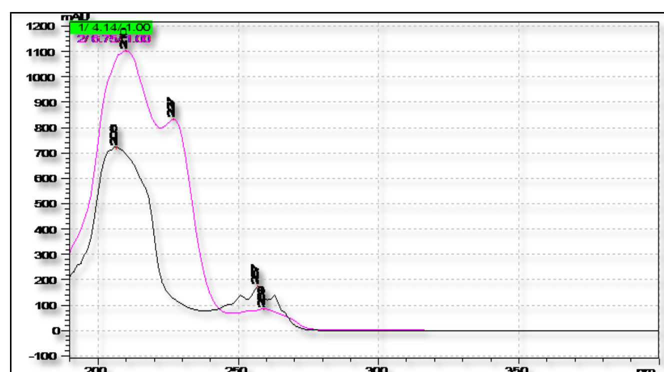

FIG.3: OVERLAIN ZERO ORDER UV SPECTRUM OF PSE AND FEX IN DILUENTS

Method Development:
By using the chromatographic conditions that were used for assay of Anti – Histamine Drugs as reference, various trials were made. Each trial mixture of known components were injected and observed for resolution and tailing factor of the peaks. Various proportions of buffer, Methanol and Acetonitrile were tried as mobile phase and 0.005M Dioctyl Sodium Sulfosuccinate Buffer in a mixture of 50 volume of Methanol, 10 volume of Acetonitrile, 40 volume of Water and 1 volume of Glacial Acetic Acid improved peak symmetry and resolution. Different flow rates of the mobile phase were tried for good resolution. Both the drugs PSE and FEX were found to be soluble and stable in a mixture of 0.005M Dioctyl Sodium Sulfosuccinate Buffer in a mixture of 50 volume of Methanol, 10 volume of Acetonitrile, 40 volume of Water and 1 volume of Glacial Acetic Acid. Finally the chromatographic conditions were optimized at flow rate 1.2 ml/min, injection volume of 50 µL, run time of 15 minutes, at column oven temp 50°C with mobile phase (sonicated and degased) as diluent in a Kinetex C_{18}, (250 x 4.6 mm i.d), Particle size 5 µm. Absorption maximum was found to be 258 nm. And peaks shape was good. The %RSD for both the drugs PSE and FEX were found to be 0.04 and 0.21 respectively and tailing factor was < 1. The retention time for PSE and FEX was found to be 4.12 minutes and 6.73 minutes respectively (**Table 1**).

TABLE 1: SYSTEM SUITABILITY TEST PARAMETER FOR PSE AND FEX

System Suitability Parameters	Proposed Method	
	PSE	FEX
Retention times (R_t) (min)	4.127 ± 0.003	6.736 ± 0.0027
Theoretical plates (N)	8262 ± 99.22	7530 ± 22.33
Resolution (R_S)	10.6 ± 0.03	
Tailing factor (A_S)	0.97 ± 0.001	1.89 ± 0.01
RSD (%) of all Replicates area of Standard Solution	0.04 %	0.21 %

The method was further validated under the chromatographic conditions.

Method Validation:
Once chromatographic conditions were established, the method was validated in compliance with ICH guidelines. The following parameters like system suitability along with specificity, linearity, precision and accuracy, limits of detection and limit of quantification were performed for validation. The specificity of the method was described as the ability to discriminate the analyte from all potential interfering substances (i.e. excipients) in the tablet dosage form. This test was performed by recording chromatograms of placebo blank solution and drug mixture spiked in the placebo solution. The placebo blank solution was prepared by mixing the corresponding tablet excipients such as microcrystalline cellulose, magnesium stearate, starch, lactose, Tween 20, polysorbate 80, and sodium starch glycolate (SSG). It can be seen from the chromatogram, that no peaks were observed in the placebo blank solution and percentage recovery of drugs spiked in placebo blank solution indicating that no interference due the excipients for the recovery of the analytes occurred.

A study to evaluate the interference of placebo was conducted. Samples were prepared in duplicate by taking placebo equivalent to the weight present in portion of test preparation as per the test method and injected into the HPLC system. It was observed that there were no peaks interfering with the analyte peak. The chromatogram indicates that the peak is homogeneous, there is no interference from the excipients at the retention time of analyte peak and has no co-eluting peaks indicating specificity of the method. For the analytical method, determination of assay specificity was also demonstrated by performing force degradation study of placebo and drug product under various stress conditions like Acid degradation, Alkali degradation, Oxidative degradation, Photolytic degradation and Thermal degradation.

Forced degradation studies:

Degradation with 3% H_2O_2:
Weighed and transferred about 570 mg of sample solution in to 100 mL volumetric flask Add 5.0 mL 3% v/v Hydrogen peroxide to the flask. Reflux the solution for 1 hour at 80ºC. Allow to cool solution at room temperature and dilute to the volume with diluent and mix. Dilute 10 ml of this solution to 50

ml with the mobile phase (360µg/ml PSE and 240µg/ml FEX) (**Fig.4**).

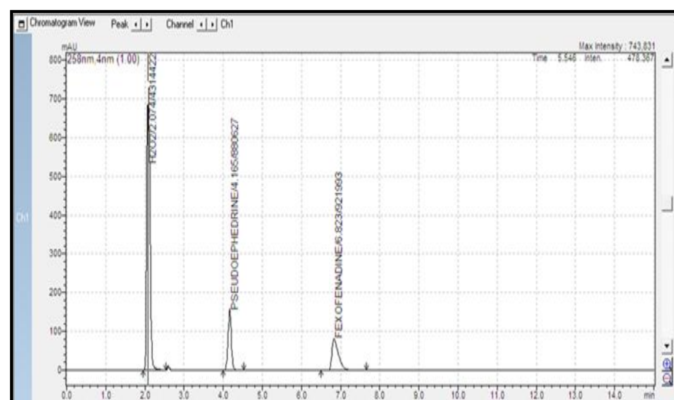

FIG.4: CHROMATOGRAPH OF FORCED DEGRADATION STUDY ON SAMPLE SOLUTION CONTAINING PSE AND FEX USING 3 % H_2O_2 SOLUTION

Degradation with 0.1M HCl:

Weighed and transferred about 570 mg of sample solution in to 100 mL volumetric flask. Add 5.0 mL 0.1 M Hydrochloric acid to the flask. Reflux the solution for 1 hour at 80ºC. Allow to cool solution at room temperature and add 5.0 mL 0.1 M Sodium hydroxide. And dilute to the volume with diluent and mix. Dilute 10 ml of this solution to 50 ml with the mobile phase (360µg/ml PSE and 240µg/ml FEX) (**Fig.5**).

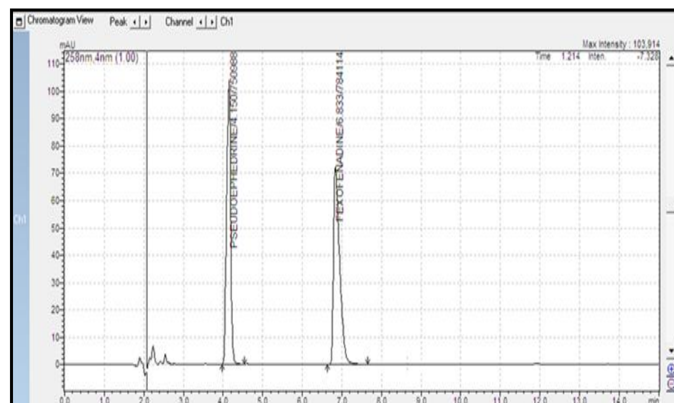

FIG.5: CHROMATOGRAPH OF FORCED DEGRADATION STUDY ON SAMPLE SOLUTION CONTAINING PSE AND FEX USING 0.1 M HCL SOLUTION

Degradation with 0.1M NaOH:

Weighed and transferred about 570 mg of sample solution in to 100 mL volumetric flask Add 5.0 mL 0.1 M Sodium hydroxide to the flask. Reflux the solution for 1 hour at 80ºC. Allow to cool solution at room temperature and add 5.0mL 0.1 M Hydrochloric acid. And dilute to the volume with diluent and mix. Dilute 10 ml of this solution to 50 ml with the mobile phase (360µg/ml PSE and 240µg/ml FEX) (**Fig.6**).

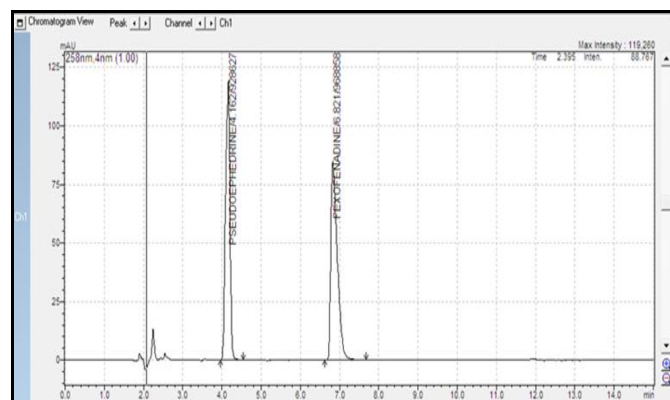

FIG.6: CHROMATOGRAPH OF FORCED DEGRADATION STUDY ON SAMPLE SOLUTION CONTAINING PSE AND FEX USING 0.1 M NAOH SOLUTION

Exposed to Heat:

Weighed and transferred about 570 mg of sample exposed under heat at 80ºC for 1 hour in 100 ml volumetric flask, Add about 50 ml of mobile phase and sonicated to dissolve it completely and make volume up to the mark with mobile Phase. Dilute 10 ml of this solution to 50 ml with the mobile phase (360µg/ml PSE and 240µg/ml FEX) (**Fig.7**).

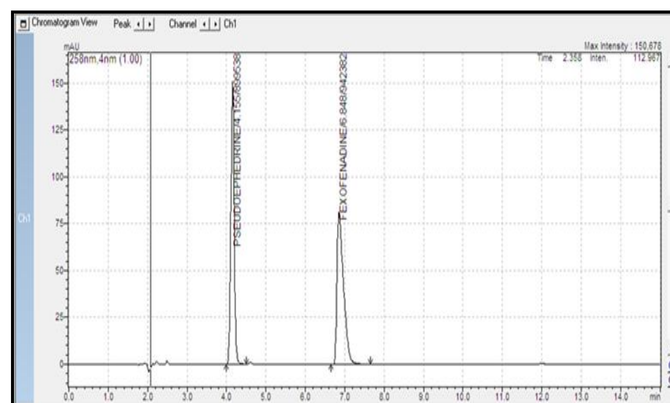

FIG.7: CHROMATOGRAPH OF FORCED DEGRADATION STUDY ON SAMPLE SOLUTION CONTAINING PSE AND FEX USING HEAT AT 80°C FOR 1 HOUR

Exposed to UV light:

Weighed and transferred about 570 mg of sample exposed UV radiation for 12 hour in 100 ml volumetric flask, Add about 50 ml of mobile phase and sonicated to dissolve it completely and make volume up to the mark with mobile Phase. Dilute 10 ml of this solution to 50 ml with the mobile phase (360µg/ml PSE and 240µg/ml FEX) (**Fig.8**).

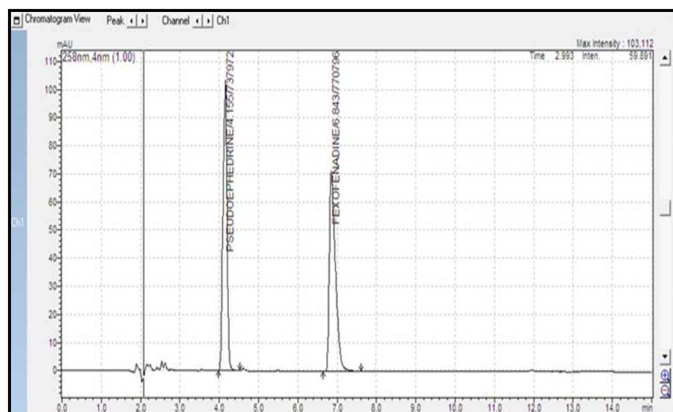

FIG.8 CHROMATOGRAPH OF FORCED DEGRADATION STUDY ON SAMPLE SOLUTION CONTAINING PSE AND FEX USING UV LIGHT FOR 12 HOURS

RESULTS AND DISCUSSION:

System Suitability:

The standard solution was prepared by using working standard as per the method. For six replicate injections system suitability parameters like number of theoretical plates, USP Tailing and % RSD were found to be within specified limits.

Specificity:

A study was carried out by determining peak purity. It observed that there were no peaks interfering with the analyte which was evident from the purity data.

Linearity:

Linearity of detector response was established by plotting graph between concentrations versus average area counts of the analytes. The prepared sample solution was chromatographed for 15 minutes using mobile phase at a flow rate of 1.2 ml/min. Data shown (**Table 2**) and represented graphically (**Fig.9** and **Fig. 10**) indicate that the response is linear over the specified range.

TABLE 2: LINEARITY LEVEL FOR PSE AND FEX

Linearity Level (%)	Volume taken from Stock (ml)	Dilute to (ml)	Final conc. (µg/mL)		Mean Area (n = 3) ± SD	
			PSE	FEX	PSE	FEX
20	2	50	72	48	182384 ± 204.73	191617 ± 187.25
50	5	50	180	120	434687 ± 221.28	455643 ± 289.44
75	7.5	50	288	180	655085 ± 322.93	687387 ± 364.26
100	10	50	360	240	892742 ± 432.02	937941 ± 531.56
150	15	50	540	360	1289858 ± 667.63	1353262 ± 794.27
200	20	50	720	480	1781041 ± 881.73	1862138 ± 997.59

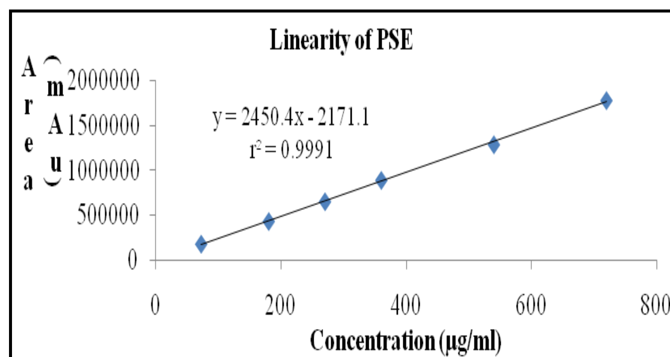

FIG.9: CALIBRATION CURVE OF PSE

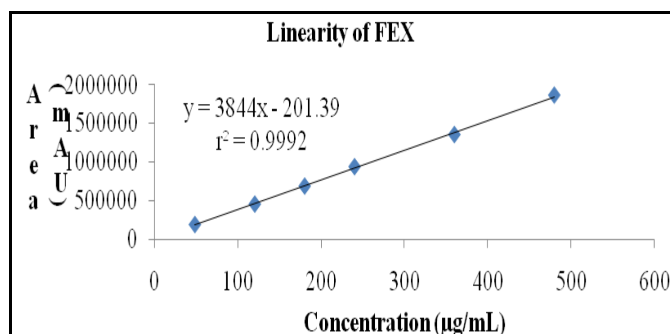

FIG.10: CALIBRATION CURVE OF FEX

Accuracy:

A study of accuracy (recovery) was performed on known amount of placebo by spiking active pharmaceutical ingredient. Samples were prepared as per the proposed method at 80% to 120% of the sample concentration. Data shown indicate that the method has an acceptable level of accuracy (**Table 3**).

TABLE 3: ACCURACY FOR PSE AND FEX

Accuracy Level	Theoretical amount (ppm)		Practical amount (ppm)		% Recovery		Mean	
	PSE	FEX	PSE	FEX	PSE	FEX	PSE	FEX
80 % Set - 1	288.60	8.04	288.20	7.99	99.90	99.40		
80 % Set - 2	288.40	8.24	288.00	8.24	99.90	99.90	99.90	99.80
80 % Set - 3	288.10	8.02	288.30	8.02	100.10	100.00		
100 % Set - 1	360.16	10.28	360.40	10.18	100.10	99.00		
100 % Set - 2	360.32	10.14	360.40	10.18	100.00	100.40	100.00	100.00
100 % Set - 3	360.36	10.06	360.40	10.12	100.00	100.60		
120 % Set - 1	432.20	12.12	432.40	12.12	100.00	100.00		
120 % Set - 2	432.60	12.13	432.70	12.10	100.00	99.80	100.00	100.00
120 % Set - 3	432.40	12.13	432.70	12.16	100.10	100.30		

Precision:

System precision:

Six replicate injections of standard solution were injected into the HPLC system. The %RSD for six replicated injections was found to be in the limits.

Method precision:

The precision of test method was evaluated by analysing assay for six individual samples prepared from same batch by the proposed method. The average %Assay and the relative standard deviation for the six sample preparation were found to be in the specified limits (**Table 4**).

TABLE 4: METHOD PRECISION FOR PSE AND FEX

	Injections	Mean Area ± SD		% Assay	
		PSE	FEX	PSE	FEX
Concentration PSE (360 ppm) FEX (240 ppm)	1	853741 ± 101.02	892949 ± 203.47	98.50	98.10
	2	871888 ± 172.35	913397 ± 267.28	100.60	100.30
	3	863415 ± 152.27	904205 ± 188.38	99.60	99.30
	4	863840 ± 221.19	905310 ± 198.22	99.60	99.40
	5	873698 ± 203.49	914949 ± 284.09	100.80	100.50
	6	866511 ± 198.35	908025 ± 234.20	99.90	99.70
Mean				99.80	99.60
% RSD				0.83	0.86

Intermediate precision (Ruggedness):

The ruggedness of method was verified by conducting the precision study by using different HPLC, different columns of same make by different analyst on different days. Six samples of same batch were prepared and analysed by the proposed method. The mean, standard deviation, and %RSD for the two sets of data are shown (**Table 5**). Ruggedness of the method is indicated by the overall RSD between the two sets of data.

TABLE 5: INTERMEDIATE PRECISION FOR PSE AND FEX

	Injections	Mean Area ± SD		% Assay	
		PSE	FEX	PSE	FEX
Concentration PSE (360 ppm) FEX (240 ppm)	1	875204 ± 156.44	915133 ± 293.26	100.9	100.5
	2	873745 ± 209.29	926042 ± 309.46	100.8	101.8
	3	879652 ± 183.02	914264 ± 244.28	101.4	100.4
	4	875040 ± 273.25	916292 ± 264.47	100.9	100.7
	5	877404 ± 240.93	918490 ± 320.92	101.2	100.9
	6	868396 ± 193.45	909326 ± 276.38	100.1	99.90
Mean				100.9	100.7
% RSD				0.44	0.63
% Difference between Method Precision and Intermediate Precision				1.08	1.10

Robustness: Robustness of the method was investigated (**Table 6**) by varying the instrumental conditions such as flow rate, column oven temperature and organic content in mobile phase. Standard solution was prepared and analysed as per the test procedure monitored the system suitability results.

TABLE 6: ROBUSTNESS STUDY FOR PSE AND FEX

Factors		Retention time (min)		Asymmetry (A_s)		Resolution
		PSE	FEX	PSE	FEX	
Flow rate	1.0	5.153	8.477	1.100	1.755	10.580
	1.2	4.127	6.733	0.968	2.017	11.688
	1.4	3.698	6.206	0.916	2.298	9.194
	Mean ± SD	4.326 ± 0.75	7.139 ± 1.19	0.995 ± 0.09	2.023 ± 0.27	10.487 ± 1.25
Temp (°C)	48	4.220	6.789	1.136	1.668	9.664
	50	4.127	6.733	0.968	2.017	11.688
	52	4.491	7.438	1.265	1.583	9.155
	Mean ± SD	4.279 ± 0.19	6.987 ± 0. 39	1.123 ± 0.15	1.756 ± 0.23	10.169 ± 1.34
Mobile Phase Ratio (Methanol: ACN: Water: GAA)	(47.5:10:42.5:1)	4.358	7.432	0.904	2.389	10.723
	(50:10:40:1)	4.127	6.733	0.968	2.017	11.688
	(52.5:10:37.5:1)	4.256	7.056	0.881	2.361	10.146
	Mean ± SD	4.247 ± 0.12	7.074 ± 0.35	0.918 ± 0.05	2.255 ± 0.21	10.852 ± 0.78
Mobile Phase Ratio (Methanol: ACN: Water: GAA)	(50:9.5:40.5:1)	4.372	7.496	0.888	2.378	10.821
	(50:10:40:1)	4.127	6.733	0.968	2.017	11.688
	(50:10.5:39.5:1)	4.226	6.983	0.888	2.296	10.003
	Mean ± SD	4.241 ± 0.12	7.071 ± 0.39	0.915 ± 0.05	2.230 ± 0.19	10.837 ± 0.84

Stability of sample solution: The sample solution was stable up to 72 hours and did not show any appreciable change in sample area (**Table 7**).

TABLE 7: SOLVENT SUITABILITY FOR PSE AND FEX

Time points (hour)	PSE % Difference		FEX % Difference	
	Standard Preparation	Test Preparation	Standard Preparation	Test Preparation
0 Hours	NA	NA	NA	NA
24 Hours	0.19	1.42	0.12	1.28
48 Hours	0.26	1.51	0.31	1.42
72 Hours	0.27	1.57	0.34	1.51

Forced degradation study:

The Data for Forced degradation are tabulated (**Table 8).** There was no interference of any peak at the retention time of analyte peaks from blank and placebo, Peak purity of all forced degradation treated samples were passed.

TABLE 8: FORCED DEGRADATION STUDY FOR PSE AND FEX

Sr. No.	Condition applied	Area		% Assay		% Degradation	
		PSE	FEX	PSE	FEX	PSE	FEX
1	Untreated Sample	871888	913397	100.6	100.3	---	---
2	0.1 M HCl	790988	884114	91.2	97.1	8.8	2.9
3	0.1 M NaOH	828627	878858	95.5	96.6	4.5	3.4
4	3 % H_2O_2	780627	821993	90.0	90.3	10.0	9.7
5	Heat Treatment	839538	902382	96.8	99.1	3.2	0.9
6	UV light treatment	837972	870796	96.6	95.7	3.4	4.3

Summary of validation parameter are tabulated (**Table 9**).

TABLE 9: SUMMARY OF VALIDATION PARAMETERS OF RP-HPLC FOR PSE AND FEX

Parameters	PSE	FEX
Recovery %	99.9 – 100.0	99.0 – 100.0
Method precision	0.83	0.86
Intermediate precision	0.97	0.60
Specificity	Specific	Specific
Solvent suitability	Solvent suitable for 72 hours	Solvent suitable for 72 hours

Estimation of marketed formulation:
Estimation of marketed formulation was carried out (**Table 10**). From this study it has been concluded that the proposed method is specific and stability indicating for the estimation of PSE and FEX in the tablet dosage form.

TABLE 10: ASSAY RESULTS OF MARKETED FORMULATION FOR PSE AND FEX

Formulation	Drug	Label claim (mg)	Amount Taken (µg/ml) (n = 3)	Amount Found (µg/ml) (n = 3)	% Label claim ± SD
	PSE	180	360	359.89	99.90 ± 0.06
Alvita -D	FEX	120	240	240.24	100.10 ± 0.15

CONCLUSION: This intended study can be concluded as the proposed method is simple, highly fast, economical, sensitive and reliable and is found to be more precise, accurate, specific, stability indicating, rugged and robust. Hence it can be employed for routine estimation of tablets containing PSE and FEX. Conventional reported chromatographic methods may be replaced by the proposed stability indicating HPLC method because of its superiority in cost effectiveness, short analysis time per sample and better detection. For faster samples testing routinely in QC lab the validated method may be used.

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