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Research article

Stability-Indicating HPTLC method for the determination of febuxostat in bulk and pharmaceutical formulation

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ABSTRACT

Febuxostat (FEB) is a well-known xanthine oxidase (XO) inhibitor which is preferably employed for treating hyperuricemia (extreme stages of uric acid in the human serum). In all the above, particularly, the high-performance thin layer chromatography (HPTLC) method where the retention factor (Rf) values were found to be quite varying as well as no specific degradation have been yet studied under moisture, sunlight, oxidative stress, acidic environment and alkaline conditions. The present study exclusively focuses on a much optimized stability-indicating HPTLC-based precise, accurate and specific HPTLC method for FEB detection in the existence of its degradation components using densitometric detection. The study hereby opened a new perspective in developing a novel HPTLC method for the chromatographic determination of United States Food and Drug Administration (USFDA)-approved drug FEB in bulk and tablet formulations. The method was properly authenticated by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q2A and guideline Q2B and therefore is found to be accurate, linear, reproducible, precise, robust and economically adequate to execute day after day custom analysis in the pharmaceutical industry scale. The investigation also unties new opportunities for the coherent optimization of HPTLC-based validated analytical methods for other drug products alone as well as simultaneously for frequently available formulations.

Keywords: Febuxostat, HPTLC, Tablet, Estimation, Stability-indicating, Validation.

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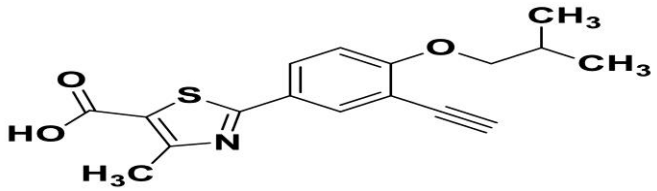
INTRODUCTION

Febuxostat (FEB), chemically known as 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid (*Chemical Formula*: $C_{16}H_{16}N_2O_3S$; *Molecular Weight*: 316.374 g/mol) is a well-known xanthine oxidase (XO) inhibitor which is preferably employed for treating hyperuricemia (extreme stages of uric acid in the human serum) (Figure 1) [1]. It is available in the market as oral tablets (80 mg) in the brand names Adenuric® and Uloric®. It is long-term prescribed often to those patients by the medical practitioners for treating gout that cannot intake allopurinol. This drug is chemically unrelated to allopurinol as its structure does not resemble a purine or a pyrimidine and therefore is well-tolerated [2].

FEB analyses have been reported to be analyzed through spectrophotometric method in bulk [3], simultaneous estimation with diclofenac in tablet [4], and tablet products [5]; high-performance liquid chromatography (HPLC) method in bulk [6], human plasma [7], tablet products [8], metabolites like 67M-1, 67M-2 and 67M-4 [9], simultaneous estimation with ketorolac tromethamine [10], Diclofenac potassium [11], montelukast [12]; High-Performance Liquid Chromatography (HPLC) coupled techniques like HPLC-Diode-Array Detector (DAD) [13], HPLC-Mass Spectroscopy (MS) [14], HPLC-Ultraviolet (UV) [15], HPLC-Fluorescence (FL) [16]; high-performance thin-layer chromatography (HPTLC) method in human plasma [17], simultaneous estimation with diclofenac potassium [18], tablet products [19]; cathodic stripping voltammetric [20]; micellar electro kinetic chromatography [21]; ultrahigh-performance liquid chromatography (UPLC)-tandem mass spectrometry (TMS) [22]; etc.

In all the above, particularly, the HPTLC method where the R_f values were found to be quite varying as well as no specific degradation have been yet studied under moisture, sunlight, oxidative stress, acidic environment and alkaline conditions.

Figure 1: Structure of Febuxostat



MATERIALS AND METHODS

Materials

The pharmaceutical grade (101.1% w/w pure) FEB was obtained from Metrochem API Pvt. Ltd., Visakhapatnam, India as a generous gift sample. Febucid® 40 mg tablets (Orange Biotech Pvt. Ltd., Batch No. FBX4T901, Exp. Date 02/2021) were procured from the local Pharmacy at Wardha. Loba Chemicals Pvt. Ltd., Mumbai, India supplied the analytical grade chemicals, reagents and solvents.

Instrumentations

Methanol was utilized for pre-washing of HPTLC plates and was activated at 110ºC±1ºC temperature for 5 min duration aforementioned to the chromatography process. The spotting of the FEB was performed in bandwidths of 6 mm with Camag 100L sample syringe (Hamilton, Switzerland) on the silica gel G60 F_{254} pre-coated HPTLC aluminum plate of dimension 20 cm × 10 cm with 250 µm thickness [E Merck® KG, Germany, provided by Anchrom Enterprises India Pvt. Ltd., Mumbai] using a Camag® Linomat - IV applicator (Switzerland). 0.1 µL/s was the application rate with 6 mm spacing between the two bands. Linear ascending development was executed in a 20 cm × 10 cm twin trough glass chamber (Camag®, Switzerland) and was further saturated with the mobile phase (ethyl acetate: n-hexane: formic acid). The saturation time required for the mobile phase in the chamber was 20 min at room temperature (25ºC±2ºC) using the saturation pads. 8 cm was the chromatogram run length. Camag® TLC Scanner-III equipped with Camag® vision CATS software was employed for densitometric scanning in the reflectance absorbance mode, keeping the slit dimension 5 mm × 0.45 mm with a scanning speed of 10 mm/s. 190 nm and 400 nm UV radiation source were taken into application using a deuterium lamp. The intensity of the diffused light was applied for estimating FEB and peak areas with linear regression was utilized for the evaluation.

Preparation of Solutions

Stock Solution

FEB; 10 mg was carefully measured and relocated into a volumetric flask of 10 mL volume. Methanol was employed as the solvent and was added upto the mark to produce 1000 µg/mL concentration. Further, dilutions produced 100 µg/mL concentration.

Mobile Phase

7 mL of ethyl acetate and 3 mL of n-hexane were mixed carefully and 0.1% formic acid was added into it to form the mobile phase composition ethyl acetate: n-hexane: formic acid in the ratio of 7:3:0.1 v/v/v. 20 mL volume of the prepared above solution was used per chromatography run.

HPTLC-based precise, accurate and specific HPTLC method for FEB detection in the existence of degradation components using densitometric detection.

Analysis of Marketed Formulation

Through the HPTLC method, FEB containing marketed product Febucid® was analyzed thoroughly. The marketed dosage units (20 in number) were weighed in a weighing balance and then powdered to the smallest extent. Equivalent quantity (~40 mg) was taken in a volumetric flask (100 mL volume). Methanol (50 mL quantity) was added, solicited for half an hour, and ultimately diluted with rest of the content. For 5 min, centrifugation was executed and the supernatant served as a source to estimate the drug content. 1 mL of the mentioned content was taken in a volumetric flask (20 mL) and 100 µg/mL of final strength was achieved by appropriate dilution with methanol. FEB was spotted at concentration of 1 µL for 6-times to accomplish the desired concentration of 100 ng/spot. The HPTLC plate was eluted using the developed chromatographic condition. The prospects of excipient intervention during the HPTLC analysis were investigated as well. 315 nm was the detecting wavelength for estimating the spot peak areas. Employing the multi-level calibration curve, the concentrations in the samples were estimated on the equivalent plate under the identical conditions by means of a linear regression equation.

Method validation

The developed HPTLC method was properly corroborated in agreement with the Q2A guideline and Q2B guideline of International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), in assent with the United States Pharmacopoeia (USP) and with the guidance of the United States Food and Drugs Administration (USFDA).

Linearity and range

FEB; 10 mg was placed in volumetric flask (10 mL volume) and consequently dissolved in methanol. The solvent was filled upto the mark, 5 min sonication was performed, and filtered further to achieve 1000 µg/mL concentration. Then, 100 µg/mL concentration was produced in a similar way. Further, dilutions were made in 10-60 µg/mL range by taking 1 mL to 6 mL solution from the standard solution. 10 µL content was applied over the HPTLC plate as a band and the chromatography was done. The average peak area versus concentration was utilized for plotting the calibration curve. Later, the regression equation was generated [23].

Accuracy

The accuracy (or the recovery parameter) of this HPTLC method was determined through spiking the standard drug solution at the concentrations of 50% of the target concentration, 100% of the target concentration, and 150% of the target concentration (standard addition method). The process was accomplished in a triplicate way and the acquired mean data were articulated in the form of % recovery ± confidence interval with calculated % relative error, on the basis of the specific concentrations [24].

Precision

The precision (variability) of this HPTLC method was estimated through spiking the standard drug solution at the concentrations of 80% of the target concentration, 100% of the target

concentration, and 120% of the target concentration (standard addition method), three-times in a single day (intra-day variability) and three-times on three different days (inter-day variability). The level of precision was estimated through the acquired relative standard deviation (RSD) data [25].

Robustness

The planned discrepancy in the systems suitability parameters like the composition of mobile phase (6:4:0.1 v/v/v and 8:2:0.1 v/v/v), time taken from spotting to commencement of chromatography and from commencement of chromatography to time taken from scanning was diverged by ±10 min and spotting of FEB at 50 ng concentration for 6-times, keeping all the other aspects (factors) constant and uniform [26].

Systems suitability parameters

The reproducibility features of the developed HPTLC method were comprehensively estimated by spiking the standard solution five-times and further estimating the parameters like peak area, retention time, tailing factor and theoretical plates [27].

Limit of detection

Limit of detection (LOD) defines the smallest available concentration that can easily be sensed by this HPTLC method but not always necessary to quantify in exact value [28]. LOD was estimated from formula:

$$\mathbf{LOD = 3.3\ (\sigma/S)}$$

Where, *S* signifies the calibration curve slope determined from the analyte calibration curve and σ referred to standard deviation of response.

Limit of quantification

Limit of quantification (LOQ) defines the smallest available concentration which can perhaps quantified consistently with a meticulous level of precision and accuracy [29]. LOQ was estimated from formula:

$$\mathbf{LOQ = 10\ (\sigma/S)}$$

Where, S signifies the calibration curve slope determined from the analyte calibration curve and σ referred to standard deviation of response.

Forced degradation studies

Alkali degradation studies

0.5 mg equivalent of FEB was taken in a volumetric flask (100 mL) and diluent (methanol) was added to exactly the half, sonication was done for 15 min duration and further, the volume was filled upto desired level. The above content was mixed thoroughly employing the magnetic stirrer for 30 min duration and then at 3,000 rpm centrifugation was done for a period of 5 min. 5 mL of the above content was taken in a volumetric flask, 0.1 N NaOH solution (10 mL) was added and the above content was heated at 80°C temperature for the duration of 8 hours under dark condition. The content was neutralized with the same quantity of HCl and the desired volume was achieved with the mobile phase. The final content was filtered employing the nylon membrane (0.45 µm pore size) [30].

Acid degradation studies

0.5 mg equivalent of FEB was taken in a volumetric flask (100 mL) and diluent (methanol) was added to exactly the half, sonication was done for 15 min duration and further, the desired volume was achieved. The content was stirred using the magnetic stirrer for 30 min duration and then at 3,000 rpm centrifugation was done for a period of 5 min. 5 mL of the above content was taken in a volumetric flask, 0.1 N HCl solution (10 mL) was added and the above content was heated at 80°C temperature for the duration of 8 hours under dark condition. The content was neutralized with the same quantity of NaOH and the desired volume was achieved with the mobile phase. The final content was filtered employing the nylon membrane (0.45 µm pore size) [31].

Oxidation degradation studies

The oxidative stress (hydrogen peroxide method) tendered to FEB was determined by dissolving 0.5 mg equivalent weight in H_2O_2 (3% v/v) (10 mL volume) in a volumetric flask and further boiling for the duration of 1 hr. The above content was kept at the room temperature to commence the process of degradation. Then, the content was suitably diluted, further sonicated and the desired volume was achieved. The final content was filtered employing the nylon membrane (0.45 µm pore size) [32].

Photo-stability studies

In photo stability studies, the compound absorbs photon and leads to overlapping of the absorption band which results in valence electron reaching the excited state. 0.5 mg equivalent of FEB was taken in a petri dish and exposed to sunlight for the duration of 12 hours for a single day. The exposed material was subsequently placed in a volumetric flask (100 mL), the mobile phase was added, sonication was performed for a period of 15 min and the desired volume was achieved. The final content was further filtered employing the nylon membrane (0.45 µm pore size). The radiations of 254 nm wavelength commenced the degradation process and the primary degradation products were determined by HPTLC [33].

Humidity conditions

0.5 mg equivalent of FEB was taken in a PVC bottle and subjected to accelerated conditions of temperature (40°C±2°C) and humidity (75%±5%) for the duration of 90 days. On the 91$^{st\ day}$, the material was placed carefully in a volumetric flask (100 mL), addition of mobile phase was done, sonication was performed for a period of 15 min and finally the desired volume was achieved. The final content was carefully filtered employing the nylon membrane (0.45 µm pore size) [34].

RESULTS AND DISCUSSION

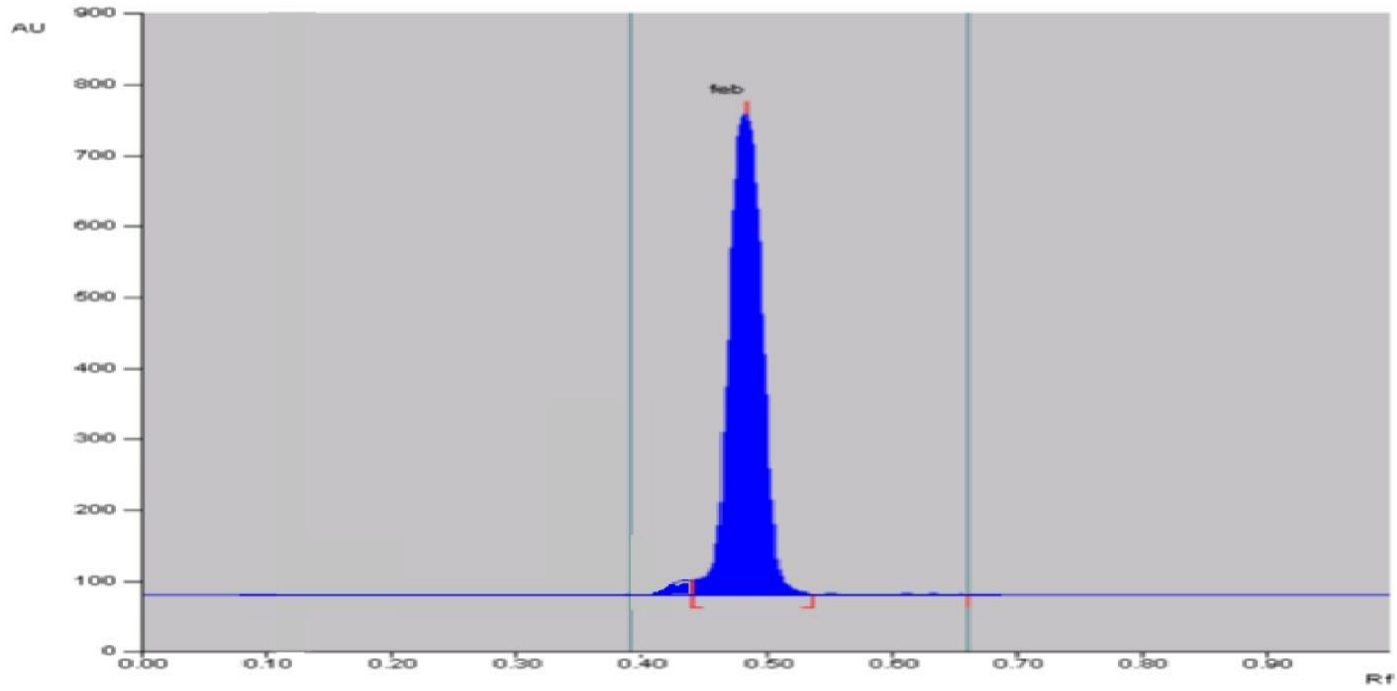
Chromatographic condition

The optimum mobile phase was ethyl acetate: n-hexane:

formic acid (7:3:0.1 v/v/v) which resolved the degraded elements from the standard peak. In the existence of degradation components, the method pleasingly resolute the drug material with R_f value of 0.56 (Figure 2).

The detection UV was selected at 315 nm as FEB was appreciably absorbed at this wavelength. As the developed analytical HPTLC method completely separated the API from the other excipients, it was considered quite specific.

Figure 2: Chromatogram depicting the retention time of Febuxostat.



Method validation

Linearity and range

Over the preferred range of the target analytical concentration (10 µg/mL to 60 µg/mL), an excellent level of linearity was detected for FEB. The linear regression equation was scrutinized to be y1 = 27167x + 2564.2 with a regression coefficient value (r^2) of 0.9978 which signifies a highly acceptable degree of linearity.

Accuracy

The accuracy aspects of the analytical HPTLC method were estimated for the recovery data by utilizing the calibration curve where the Y-intercept and the slope represented a fundamental function in determining the % recovery. The result of the recovery study for FEB was observed to be 99.27% w/w. The perceived % RSD were <2% at three specific concentrations; 50%, 100% and 150% of the target concentration which was in accordance to the prescribed pharmacopeia acceptance limit of ±2% that indicated brilliant accurateness of this novel analytical method.

Precision

Over the 50%, 75% and 150% of the target concentration, the % RSD values were detected to be <2% at both intra-day (n = 3) variability as well as under the inter-day variability (n = 3) which represents that the developed HPTLC method possess high precision attributes with reduced variability in the determining FEB. The % RSD were observed lie in the recommended pharmacopeia acceptance limit of ±2%. The exact values of % RSD values under both conditions are described in Table 1.

Table 1: Validation and Systems Suitability in HPTLC study of Febuxostat

Parameters (Units)	Data observed
Linearity range (µg/mL)	10-60
r^2 (Correlation of Coefficient)	0.9978
Slope	27167
Intercept	2564.2
Limit of Detection (LOD) (µg/mL)	0.20
Limit of Quantification (LOQ) (µg/mL)	0.25
Recovery (%)	99.75
Precision (% RSD)	0.3085
Inter-day variability (n=3)	0.2461
Intra-day variability (n=3)	0.2565
Robustness	Robust
Rf value	0.56

Robustness

After intended adjustments of chromatographic conditions; the composition of the mobile phase (6:4:0.1 v/v/v and 8:2:0.1 v/v/v), time required from spotting to chromatography and from chromatography to scanning was adjusted by ±10 min and spotting of FEB at 50 ng concentration for 6-times, a minuscule variation in the retention value (±0.2) were observed which reflected that the chromatographic method is robust enough to detect FEB even after modifications in the chromatographic environment.

Systems suitability parameters

The system suitability parameters revealed that the developed analytical method has reproducible features, the potential for habitual analysis and competent adequate to be functional. The

average retention time (R_t) of FEB was noticed at 0.567 min from the chromatogram. The number of theoretical plates was distinguished to be much higher than the prescribed minimum USP limits; *i.e.* 2,000, that may be deciphered into superior column efficacy, enhanced resolution and higher separation of the method. The high peak area reflected association with high theoretical separation capability of the developed method [35]. The tailing factor was remarked to have a good symmetry (ideal Gaussian peak characteristics) as the factor was found to be nearly 1 which is nearly equal to the asymmetric factor in the magnitude. The developed new analytical method fulfilled the compulsory requirements (minimum prescribed limit) as suggested in the USP monograph and has perspectives to express reproducible results.

Forced degradation studies

The degradation study indicated that FEB was highly susceptible to alkaline hydrolysis while it was completely stable under the environments of acid, H_2O_2, direct sunlight and humidity conditions (Table 2). Under alkaline hydrolysis, the drug degradation occurred (68.48%) as observed from the lessening drug peak area, when compared with the non-degraded drug component peak area at the identical strength. Two supplementary peaks depicting degradation products were perceived at the R_f values 0.18 and 0.40, respectively (Figure 3). Conversely, the degradation products characterization was not successfully performed. By measuring up the degraded peaks area, the % degradation was estimated for each and every degradation state relating to the drug peak corresponding areas under non-degradation condition.

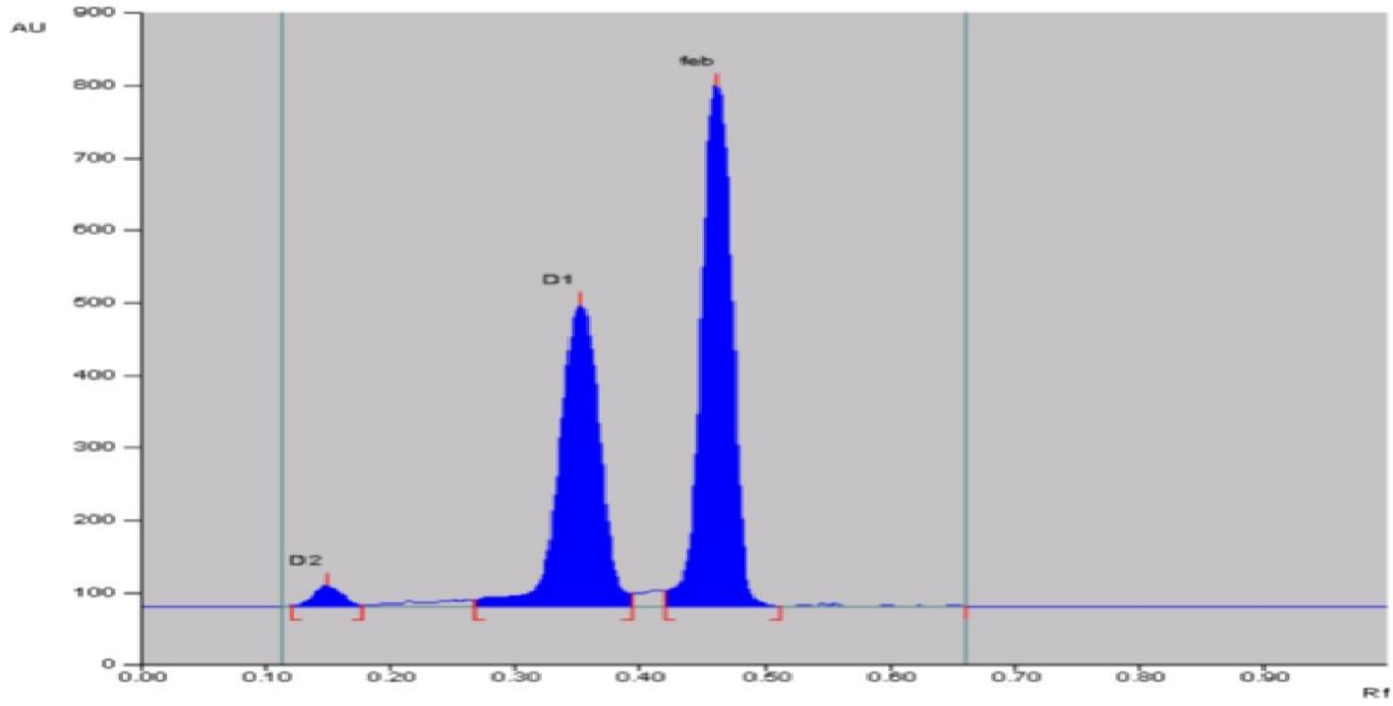
Table 2: Summary of degradation studies for Febuxostat.

Degradation Condition	Time (hr or day)	% Degradation	Rf value of degradation product
Base, 0.1 N NaOH (heated 80ºC)	8 hour	68.48	0.18, 0.40
Acid, 0.1 N HCl (heated 80ºC)	8 hour	-	-
Oxidative, 3% v/v (ambient, dark)	7 days	-	-
Photolytic	12 hour	-	-
Humidity	90 days	-	-

Analysis of the Marketed Product

The developed analytical technique was productively utilized for determining FEB in pharmaceutical formulation. The data obtained after analyzing the FEB tablets through this method exhibited a recovery of 99.75±0.55% which concluded the appropriateness of this analytical technique in determining FEB in the product, without detecting the excipients. The analytical method effectively separated FEB, the API from the various degradation components and it may be utilized regularly for analyzing the FEB in tablets.

Figure 3: Chromatogram of Febuxostat describing the degraded product on alkaline hydrolysis



CONCLUSIONS

This novel HPTLC method can successfully determine the USFDA-approved drug FEB in bulk and tablet formulations. The optimum mobile phase consisting of ethyl acetate: n-hexane: formic acid (7:3:0.1 v/v/v) which resolved Febuxostat at R_f value of 0.56 using UV absorbance at 315 nm. Under alkaline hydrolysis, the drug degradation occurred (68.48%) with degradation peaks observed at R_f values of 0.18 and 0.40. The drug is completely stable under the environments of acid, H_2O_2, direct sunlight and humidity conditions. The study hereby opened a new perspective in developing a novel

HPTLC technique for the chromatographic determination of USFDA-approved drug FEB in bulk and tablet formulations. The method was properly authenticated with accordance to the prescribed ICH guideline Q2A and guideline Q2B and therefore is found to be accurate, linear, reproducible, precise, robust and economically adequate to execute customized analysis at industrial scale. The investigation also unties new opportunities for the coherent optimization of HPTLC-based validated analytical methods for other drug products alone as well as simultaneously for frequently available formulations.

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CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this article.

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