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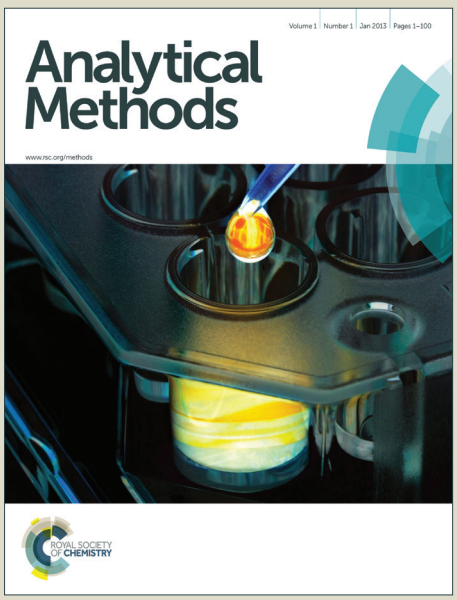

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Validation of a Stability-indicating Micellar Eletrokinectic Capillary Method for the Assessment of Febuxostat and its Correlation with Reversed-phase LC Method

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A stability-indicating Micellar Eletrokinectic Capillary Chromatography (MEKC) method was validated for the analysis of febuxostat in pharmaceutical formulations, using lisinopril as internal standard (IS). A fused-silica capillary (50 µm i.d.; effective length, 40 cm) was used maintained at 30ºC and the applied voltage was 20 kV. The Background electrolyte solution consisted of 15 mmol L^{-1} sodium tetraborate buffer and 25 mmol L^{-1} sodium dodecyl sulphate solution at pH 10. Injections were performed using a pressure mode at 50 mbar for 45 s, with detection by photodiode array detector set at 216 nm. Specificity and stability-indicating capability were established in degradation studies, which also showed that there was no interference of the excipients. The method was linear over the concentration range of 0.10-50 µg mL^{-1} (r^2= 0.9993) and the limit of detection (LOD) and limit of quantitation (LOQ) were 0.05 µg mL^{-1} and 0.10 µg mL^{-1}, respectively. The accuracy was 99.89% with relative error lower than 1.04%. The proposed method was applied to the quantitative analysis of febuxostat in tablet dosage forms and in human plasma, and the results were correlated to those of a validated reversed-phase (RP-LC) method, in attempts to improve quality control of pharmaceutical products.

1. Introduction

Febuxostat (2-(3-cyano-4-(2-methylpropoxy)phenyl-4-methylthiazole-5-carboxylic acid, is a novel potent non-purine, selective inhibitor of xantine oxidase (XO), indicated to treat hyperucemia in patients with gout.[1,2] Its empirical formula is $C_{16}H_{16}N_2O_3S$, with a molecular weight of 316, and it is a weak acid with a pKa of 3.42. [3,4]

A gradient RP-LC-MS/MS method using C_{18} column and detection at 315 nm has been applied to identify the profile of impurities present in the active pharmaceutical ingredient (API) of synthetic febuxostat.[5] An isocratic UPLC method has also been validated for use in forced degradation studies for febuxostat API only, using a C_{18} column and detection at 315 nm.[6] An isocratic RP-LC method has been validated for the analysis of febuxostat in pharmaceutical formulations using a C_{18} column with detection at 316 nm.[7] Bioavailability and pharmacokinetic studies on febuxostat have been performed by isocratic RP-LC method with fluorescence detection at 320-380 nm, using a C_{18} column, with sensitivity of 0.01 µg mL^{-1}.[8] A LC-MS/MS using C_{18} column and electrospray ionization in the positive mode has been validated for the determination of febuxostat in human plasma with the sensitivity of 10.0 ng mL^{-1}.[9] Capillary electrophoresis (CE) has expanded its scope as a powerful analytical technique for pharmaceutical analysis allowing the determination of the active pharmaceutical ingredients and their impurities, with some advantages related to the existing methodologies.[10-15]

Development of stability-indicating method using the approach of stress testing as determined by the ICH guideline,[16] is highly recommended to the quantitative analysis of pharmaceutical formulations. The method must be able to resolve febuxostat from its potential impurities and degradation products, and validated as recommended.[17,18] Although febuxostat has received approval of FDA and EMA, it has not been described in any Pharmacopoeia, and there is no published CE method.

The aim of the present study was to develop and validate a stability-indicating MEKC method to determine febuxostat in solid dosage forms; to correlate the results with a RP-LC method; thus contributing to the development of alternative method to monitor stability, improve quality control, and thereby assure the therapeutic efficacy of the pharmaceutical formulations.

2. Experimental

2.1 Chemicals and reagents

Febuxostat reference substance (FRS) and lisinopril (IS) were purchased from Sequoia Research Products (Oxford, UK). A total of eight batches of Uloric® (Takeda Pharmaceuticals, USA) tablets containing 40 and 80 mg of febuxostat were obtained from commercial sources within their shelf life period and were identified from 1 to 8. Analytical grade disodium tetraborate decahydrate and ultrapure sodium dodecyl sulphate (SDS) were acquired from Merck (Darmstadt, Germany) and Bio-Rad Labs (Hercules, CA, USA), respectively. HPLC-grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). All chemicals used were of pharmaceutical or special analytical grade. For all of the analyses, ultrapure water was obtained using an Elix 3 coupled to a Milli-Q Gradient A10 system Millipore (Bedford, MA, USA).

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All solutions were filtered through a 0.22 µm Millex filter Millipore (Bedford, MA, USA).

2.2 Apparatus

MEKC experiments were performed on an Agilent ^{3D}CE apparatus Agilent Technologies (Waldbronn, Germany) consisting of a photodiode array (PDA) detector, a temperature controlling system (4–60ºC) and a power supply able to deliver up to 30 kV. CE ChemStation software was used for instrument control, data acquisition and data analysis. The pH of the solutions was measured using a pH-meter Thermo Orion Model 420, (Beverly, MA, USA).

The LC method was carried out on a Shimadzu LC system (Kyoto, Japan) equipped with a SCL-10A$_{VP}$ system controller, a LC-10 AD$_{VP}$ pump, a DGU-14A degasser, a CTO-10A$_{VP}$ column oven, a SIL-10AD$_{VP}$ autosampler, and a SPD-M10A$_{VP}$ PDA detector set at 316 nm. Peak areas were automatically integrated by computer using a Shimadzu Class VP® V 6.14 software program.

2.3 Solutions

2.3.1 Background electrolyte solution (BGE). The optimized BGE solution consisted of 15 mmol L^{-1} disodium tetraborate decahydrate and 25 mmol L^{-1} SDS, at pH 10 adjusted by adding 1 mol L^{-1} sodium hydroxide.

2.3.2 Reference substance and Internal Standard solutions. The stock solutions were prepared by accurately weighting 10 mg of FRS and 0.25 mg of IS, transferred to individual 25 mL volumetric flask and diluted to volume with acetonitrile, to obtain final concentrations of 400 µg mL^{-1} and 10 µg mL^{-1}, respectively. The stock solutions were stored at 2–8°C, protected from light and daily diluted with background electrolyte solution (BGE) to an appropriate concentration, and filtered though a 0.22 µm membrane filter Milex Millipore (Bedford, MA, USA).

2.3.3 Samples solutions. To prepare the sample stock solutions, tablets containing respectively, 40 and 80 mg of febuxostat were accurately weighed and crushed to a fine powder. An appropriated amount was transferred into an individual 10 mL volumetric flask and diluted to volume with acetonitrile to obtain a concentration of 400 µg mL^{-1}. The stock solution was stored at 2–8ºC, protected from light, daily diluted with BGE to an appropriate concentration and filtered through a 0.22 µm membrane filter.

2.4 Electrophoresis method

All experiments were carried out on a fused-silica capillary with 50 µm i.d. and 48.5 cm of total length (effective length 40 cm), thermostatized at 30ºC, and detection with PDA set at 216 nm. Before the first use, the fused-silica capillary was sequentially rinsed with 100 mmoL^{-1} sodium hydroxide for 30 min, followed by water for 15 min and BGE by 15 min. At the beginning of each working day, the capillary was conditioned by rinsing with 1 mol L^{-1} sodium hydroxide for 20 min, followed by water for 10 min, and then with running electrolyte solution for 20 min. To improve the reproducibility of the migration time between injections, the capillary was conditioned again with water (1 min), and running BGE solution (3 min). Samples were injected using the pressure mode at 50 mbar for 45 s with a constant voltage of 20 kV (current about 41.5 µA) applied during the analysis.

2.5 Validation of the MEKC method

The method was validated using samples of a pharmaceutical formulation of febuxostat with the label claim of 400 µg mL^{-1}. The following parameters were determined: specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, stability, and system suitability test, according to the ICH guidelines.[17] Lisinopril (IS) was used to compensate for any injection errors and minor fluctuations of the migration time, thus improving the reproducibility of the MEKC method.

2.6 Forced degradation studies

The stability-indicating capability of the MEKC method was determined by subjecting sample solution of pharmaceutical formulation (400 µg mL^{-1}) to accelerated degradation by different acidic, basic, neutral, oxidative, and photolytic conditions. Working solutions prepared in 1 mol L^{-1} hydrochloric acid was used for acidic hydrolysis, and working solutions in 0.1 mol L^{-1}sodium hydroxide was employed for basic hydrolysis evaluation. Both solutions were refluxed at 100ºC for 60 min and 15 min, respectively, cooled and neutralized with acid or base, as necessary. To conduct the study under neutral condition, the sample solution was diluted in acetonitrile and heated at 80ºC for 2 h. Oxidative degradation was induced by storing the samples in 20% hydrogen peroxide, at ambient temperature for 24 h, protected from light. Photodegradation was induced by exposing the sample in a photostability chamber to 200 W h m^{-2} of near ultraviolet light for 2 h. For the analysis, the solutions were diluted with the electrolyte solution to final concentrations of 20 µg mL^{-1}. The interference of the excipients of the pharmaceutical formulation was determined by injecting a sample containing only a placebo (in-house mixture of all the tablet excipients), and by the standard addition method, where a calibration curve was constructed by the addition of known amounts of the reference substance to the placebo.[16] Then, the specificity of the method was established by determining the peak purity of febuxostat in the samples using a PDA detector.

2.7 RP-LC method

The validated RP-LC method was described elsewhere.[7] Briefly, the elution was carried out on a reversed-phase Waters (Dublin, Ireland) XTerra C$_{18}$ column (150 x 3.9 mm i.d., with a particle size of 5 µm and pore size of 100 Å), maintained at 25ºC . A security guard holder was used to protect the analytical column. The LC system was operated isocratically using a mobile phase consisting of water (pH 3.5) - acetonitrile (40:60, v/v)) and run at a constant flow rate of 0.8 mL min^{-1} and using photodiode array (PDA) detection at 316 nm. The mobile phase was filtered

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through a 0.45 μm membrane filter Millipore (Bedford,MA, USA). The injection volume was 20 μL for both the reference substance and the samples.

2.8 Analysis of febuxostat in pharmaceutical formulations

For the quantitation of febuxostat in tablet formulations, the respective stock solutions were diluted to an appropriate concentration of 20 μg mL^{-1} with a BGE solution or a mobile phase, respectively, for the electrophoretic or chromatographic methods, injected in triplicate and the percentage recoveries calculated against the reference substance.

2.9 Determination of febuxostat in human plasma

Plasma samples were spiked with FRS (5 μg mL^{-1}) and lisinopril (10 μg mL^{-1}), as internal standard (IS). Then, a 4 mL aliquot of tert-butyl methyl ether, was added and vortex-mixed for 90 s. The tubes were centrifuged for 15 min at 2700 rpm and the organic layer was filtered through a Millex GV 0.45 μm filter unit Millipore (Bedford, MA, USA) into 15 mL conical tubes and evaporated under nitrogen stream while immersed in a 40°C water bath. The residues were reconstituted with 300 μL of BGE solution or mobile phase, respectively.

3 Results and discussion

3.1 Optimization of the electrophoretic conditions

To develop the MEKC method, some electrolyte solutions containing TRIS (hydroxymethyl) aminomethane, sodium dihydrogen phosphate, MES [2-(N-morpholino) ethanesulfonic acid], boric acid, respectively, were tested by adding the surfactant SDS in the pH range from 6-11, selecting disodium tetraborate decahydrate. The optimum pH of a BGE solution containing 20 mmol L^{-1} disodium tetraborate was investigated in the range of 6–11, and pH 10 was selected since it showed better peak symmetry (about 1.04), since lower pHs resulted in an increase of the migration time and peak width. The disodium tetraborate concentration was evaluated at concentrations from 10–35 mmol L^{-1}, and constant 25 mmol L^{-1} SDS concentration at pH 10, which demonstrated a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. A 15 mmol L^{-1} solution was selected due to its low effect on current and non-significant increase on the migration time. The influence of SDS was also investigated at the concentration range from 10 to 35 mmol L^{-1} and constant 15 mmol L^{-1} disodium tetraborate decahydrate buffer concentration at pH 10. The migration time of febuxostat increased with the increase of the concentrations, giving better efficiency combined with short analysis time with 25 mmol L^{-1} SDS. The effects of organic modifiers, acetonitrile or methanol, in the concentration range of 5–10%, were also evaluated, but no improvement on the electrophoretic conditions was achieved. The temperature effect on the separation was investigated in the range of 15–36ºC, and a temperature of 30ºC was chosen due to short run time and acceptable current. The effect of the voltage was studied through changes from 14 to 30 kV, showing that a potential of 20 kV yielded a short analysis time with an acceptable current (about 41.5 μA). Sample solutions were injected using a pressure mode at 50 mbar for 45 s, equivalent to a injection volume of 85 nL. Wavelength detection was evaluated in the range of 190-400 nm, and a wavelength of 216 nm was chosen due to better sensitivity and signal-to-noise ratio.

3.2 Validation of the electrophoresis method

The MEKC method was validated for the analysis of febuxostat in pharmaceutical formulations with a migration time of about 3.70 min, as shown in the typical electrophrograms (Figs. 1a, 1b). The stability-indicating capability of the MEKC method[18,19] was evaluated under acidic condition which resulted in a decrease of the area (21.3%) and two additional peaks at 3.42 and 4.10 min (Fig. 1c). The basic condition exhibited decrease of the area (12.21%), and only one peak was detected at 2.77 min (Fig. 1d). The neutral condition exhibited a decrease of the area (19.54%) without any additional peak, indicating that the degradation products were not detected by UV (Fig. 1e). The forced oxidative degradation studies exhibited decrease of the area (14.52%) with one additional peak at 3.12 min (Fig. 1f). The photolytic condition showed decrease of the area (15.87%) with an additional peak at 3.29 min (Fig. 1g). Specificity of the method was established by determining the peak purity of the analyte and the IS in the working reference substance solution, by overlaying the spectra captured at the apex, upslope and downslope using a PDA detector. Additionally the standard addition method was applied to evaluate the interference from formulation excipients. Non-significant difference ($p>0.05$) was found between the slopes calculated for the calibration curve and the standard addition method. The data, together with the peak purity index in the range of 0.9999 – 1, showed that the peak was free from any co-migrating peak, with no interference of excipients as also demonstrated by the injection of the placebo (Fig. h), thus confirming that the proposed method is specific for the analysis of febuxostat.

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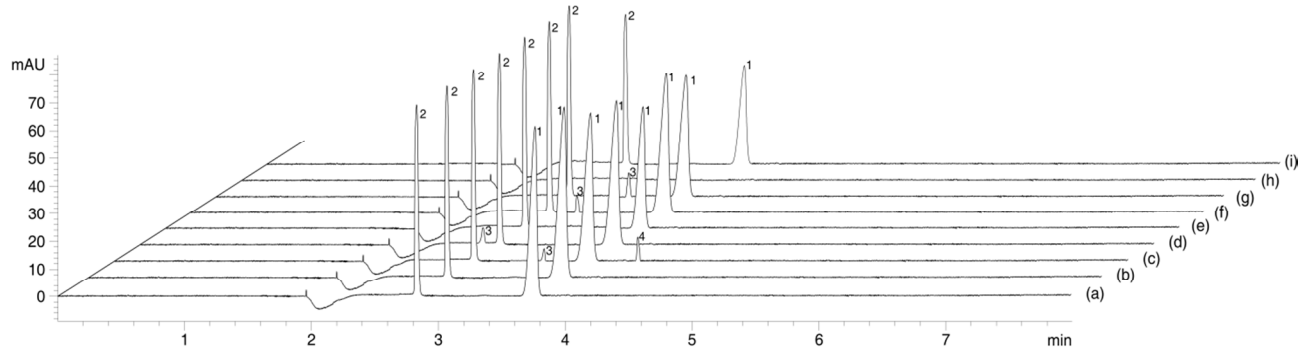

Fig. 1 Representative MEKC electropherograms showing peak 1= febuxostat, peak 2= internal standard, peaks 3-4= degraded forms. (a) Febuxostat reference substance solution; (b) Sample of pharmaceutical formulation. febuxostat reference substance solution and IS after degradation under conditions: (c) acidic hydrolysis, (d) basic hydrolysis, (e) neutral, (f) oxidative, (g) photolytic, (h) Placebo, (i) Blank plasma spiked with IS and febuxostat.

The linearity was determined by constructing three calibration curves, each one with eight concentrations of febuxostat reference solution in the 0.1–50 μg mL^{-1} range, spiked with IS at 10 μg mL^{-1}. The value of the determination coefficient calculated by the least squares regression analysis (r^2 = 0.9993, n=8, y = (0.0213± 0.0022) x + (0.0074 ± 0.0067), where, x is concentration in μg mL^{-1} , and y is the peak-area ratio of febuxostat to IS, indicated the linearity of the calibration curve for the method.

The precision of the method, evaluated as the repeatability of the method, was studied by calculating the relative standard deviation (RSD%) of the migration time and the peak-area ratio, for eight determinations at a concentration of 20 μg mL^{-1}, performed on the same day, under the same experimental conditions. The obtained RSD values were 0.39 and 1.22% for the migration time and peak-area ratio, respectively. The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (inter-days) giving RSD values of 0.73 and 0.52%, respectively. The between-analysts precision was determined by calculating the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 0.77 and 0.69%, respectively, as given in Table 1.

Table 1 Inter-day and between-analysts precision data of MEKC for febuxostat in samples of tablet dosage forms

	Inter-day			Between-analysts		
Sample	Day	Concentration found [a] (%)	RSD (%)	Analysts	Concentration found[a] (%)	RSD (%)
	1	99.11		A	99.27	
1	2	98.82	0.73	B	97.74	0.77
	3	97.73		C	98.41	
	1	99.42		A	99.32	
2	2	99.46	0.52	B	100.02	0.69
	3	100.34		C	100.72	

[a] Mean of three replicates.

The accuracy was assessed from three replicate determinations of three solutions of in-house mixtures of the excipients with known amounts of the drug, containing 10, 20, and 30 μg mL^{-1}, corresponding to 80, 100, and 120% of the analytical concentrations, respectively. The absolute means obtained with a mean value of 99.89% and a relative error lower than 1.04% (Table 2), show that the method is accurate within the desired range.[20]

Table 2 Accuracy of MEKC for febuxostat in the formulations

Nominal concentration (μg mL^{-1})	Mean concentration found[a] (μg mL^{-1})	RSD[b] (%)	Accuracy (%)	RE[c] (%)
10	9.90	0.36	98.97	-0.17
20	19.98	0.21	99.91	-0.09
30	30.23	0.48	100.78	1.04

[a] Mean of three replicates. [b] RSD, relative standard deviation. [c] RE, relative error, [(measured concentration - nominal concentration)/nominal concentration] x 100.

The LOD and the LOQ were calculated from the slope and the standard deviation of the intercept determined by a linear-regression model, by using the mean values of the three independent calibration curves. The obtained values were 0.05 and 0.14 μg mL^{-1}, respectively. The evaluated experimental LOQ with a precision lower than 5% and an accuracy within ± 5%,[21,22] was determined as 0.10 μg mL^{-1}, suitable as an alternative for quality control analysis, also compared to the RP-LC method which showed 0.25 μg mL^{-1}.[7]

The robustness of the analytical procedure[23] was determined by analyzing samples of the FRS solution of febuxostat containing 20 μg mL^{-1}, in triplicate, by the one-variable-at-a-time (OVAT) approach. The results and the experimental range of the selected variables evaluated are given in Table 3, together with the optimized values. Additionally, the robustness was also carried out and compared by the multi-variable-at-a-time (MVAT) approach[24] at three levels (1 unit per parameter up or down around optimized values). This procedure gives results for minimum changing of maximum number of parameters at a time, and is a very useful, rapid and efficient approach for robustness determination. The results for OVAT and MVAT procedures were within the acceptable deviation (RSD<2%), and an analysis of variance showed non-significant differences (p>0.05) for the dosage of the sample solutions. Analyses performed with wider level of variations of the solution pH, temperature and voltage, showed changes of the migration time related to the optimized conditions. Moreover, the peak symmetry values were also evaluated showing non-significant differences (p>0.05). The electropherogram pattern was not altered and different capillary

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batches also indicated robustness under the conditions tested.

Table 3 MECK conditions and range investigated during robustness testing with the one-variable-at-a-time (OVAT) procedure

Variable	Values	Febuxostat[a](%)	RSD(%)	Migration time (min)	RSD(%)	Symmetry	RSD(%)	Optimized condition
Electrolyte solution pH	9.6	103.12	1.12	3.70	1.32	1.73	1.55	10
	9.8	101.23	0.98	3.73	1.64	1.26	0.89	
	10	100.31	0.25	3.74	0.74	1.13	0.21	
	10.2	100.69	1.01	3.91	0.92	1.29	1.37	
	10.4	100.44	0.73	4.02	1.05	1.86	1.67	
Electrolyte solution concentration (mmol L^{-1})	11	98.17	0.81	3.75	0.51	1.26	1.61	15
	13	99.14	0.32	3.99	0.41	1.23	0.94	
	15	99.71	0.18	3.73	0.36	1.15	0.51	
	17	98.25	0.34	3.15	0.47	1.19	0.78	
	19	99.54	0.78	3.17	0.65	1.22	0.81	
Temperature (°C)	24	99.48	1.26	3.78	0.58	1.29	1.18	30
	27	98.16	0.95	3.76	0.67	1.24	1.03	
	30	99.85	0.31	3.69	0.25	1.12	0.78	
	33	100.54	0.67	3.75	0.41	1.26	1.12	
	36	100.73	0.53	3.89	0.36	1.23	1.54	
Voltage (kV)	14	101.27	0.51	4.12	0.77	1.33	0.71	20
	17	101.63	1.12	3.95	0.56	1.26	0.64	
	20	100.91	0.43	3.72	0.12	1.09	0.16	
	23	102.69	0.84	3.70	0.28	1.15	0.38	
	26	103.04	1.06	3.66	0.23	1.16	0.76	
Time injection (s)	41	99.48	0.66	3.85	0.88	1.19	0.89	45
	43	100.58	0.28	3.81	0.75	1.15	0.81	
	45	100.45	0.12	3.71	0.39	1.05	0.74	
	47	100.44	0.77	3.84	0.58	1.10	1.06	
	49	100.92	0.31	3.73	0.61	1.13	1.08	
Wavelength (nm)	212	98.13	0.96	3.94	0.96	1.24	1.05	216
	214	98.56	0.33	3.86	0.91	1.26	0.81	
	216	99.79	0.21	3.77	0.42	1.14	0.72	
	218	98.51	0.54	3.75	0.95	1.19	1.09	
	220	97.42	0.52	3.78	0.73	1.25	1.00	

[a] Mean of three replicates.

The stability of febuxostat in BGE was assessed after the storage of the samples for 48 h at 2–8°C, and also placed into the autosampler for 24 h at room temperature, showing non-significant changes (< 2%) relative to freshly prepared samples as suggested.[25]

A system suitability test was carried out to evaluate the resolution and the repeatability of the system for the analysis to be performed, using five replicates injections of a FRS solution containing 20 µg mL^{-1} of febuxostat. The obtained RSD values for the migration time, peak area, peak symmetry and peak width, were 0.36, 0.93, 1.21 and 1.10%, respectively. The number of theoretical plates was approximately, 43730, with RSD of 1.33%. The parameters tested were within acceptable range (RSD < 2.0%).

The validated MEKC method was applied to the determination of febuxostat in tablet dosage forms and the results compared to those obtained using a validated RP-LC method, giving mean differences of 1.45% lower, for the MEKC method, as shown in Table 4. The experimental values were compared statistically by the Student's t-test, showing non-significant differences (p > 0.05). The capability demonstrated for the proposed method can be useful to the determination of febuxostat in pharmaceutical formulations. Moreover, the method was applied to the determination of febuxostat in human plasma, after extraction procedure which showed recoveries of 96.41% and 96.10%, respectively for febuxostat and IS. The LLOQ evaluated in an experimental assay was found to be 0.25 µg mL^{-1}, showed lower sensitivity compared to the existing methods due to the low injection volume and the short optical path-length.

3.3 Method application

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Table 4 Comparative content determination of febuxostat in tablet dosage forms, by electrophoretic and chromatographic methods.

Theoretical amount		Experimental amount					
		MEKC[a]			RP-LC[a]		
Sample	mg per tablet	mg	%	RSD[b] (%)	mg	%	RSD[b] (%)
1	40	39.73	99.32	0.44	40.39	100.97	0.43
2	40	39.19	97.98	0.58	39.74	99.35	0.32
3	40	39.57	98.92	1.12	40.06	100.14	0.71
4	40	39.56	98.84	0.83	40.12	100.31	0.65
5	40	39.87	99.67	0.65	40.49	101.23	0.98
6	40	39.65	99.12	0.42	40.30	100.74	0.67
7	80	80.03	100.04	0.51	81.35	101.69	0.36
8	80	79.65	99.56	0.32	80.67	100.84	0.44
Mean	-	-	99.18	-	-	100.63	-
SD[c]	-	-	0.63	-	-	0.77	-

[a] Mean of three replicates. [b] RSD, Relative standard deviation. [c] SD, Standard deviation.

4. Conclusions

The results of the validation studies show that the MECK method is sensitive with a LOQ of 0.10 μg mL^{-1}, accurate with a mean value of 99.89%, economic and stability-indicating. It possesses significant linearity (r^2 = 0.9993) and precision characteristics without any interference from the excipients. Therefore, the method can be applied as an alternative to the quantitative analysis of febuxostat in tablet dosage forms without prior separation of the excipients of the formulation, with the added advantages of small sample volumes without the consumption of organic solvents, and a short analysis time.

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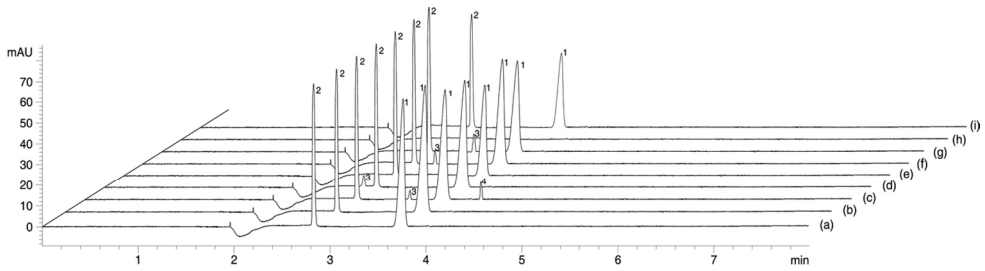

Representative MEKC electropherograms showing peak 1= febuxostat, peak 2= internal standard, peaks 3-4= degraded forms. (a) Febuxostat reference substance solution; (b) Sample of pharmaceutical formulation. febuxostat reference substance solution and IS after degradation under conditions: (c) acidic hydrolysis, (d) basic hydrolysis, (e) neutral, (f) oxidative, (g) photolytic, (h) Placebo, (i) Blank plasma spiked with IS and febuxostat.
123x32mm (300 x 300 DPI)

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