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Development and Validation of Bioanalytical Method for the Quantification of Febuxostat in Human K2 EDTA Plasma by LC-MS/MS: Pharmacokinetic Studies in Wister Rats

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A specific, linear and accurate LC-MS/MS method was developed for the determination of febuxostat in human K2 EDTA plasma and it was successfully applied for the pharmacokinetic study in wister rats. Chromatographic isolation of febuxostat and febuxostat D9 were attained on Purosphur C18, 100 × 4.6 mm, 5 µ column with 1.0 mL/min flowing rate. The technique was linear over the standard concentrations ranging from 24.995-7001.401 ng/mL and the regression coefficient was perceived to be ≥ 0.9997. All LLOQ samples % RSD of back computed concentrations varied from 1.00 to 4.01. The analysis of plasma from healthy rats was successfully conducted using the validated LC-MS/MS procedure to quantify the presence of febuxostat. From the pharmacokinetic studies, T_{max}, C_{max}, $T_{1/2}$ and $AUC_{0-\infty}$ of the febuxostat tablets were 2.0 ± 0.03 h, 8.85 ± 1.87 ng/mL, 6.34 ± 0.53 h, 95.58 ± 6.37 ng h/mL, respectively.

Keywords: Febuxostat, Gout, LC-MS/MS, Validation, Pharmacokinetics.

INTRODUCTION

In 2009, the US Food and Drug Administration (FDA) approved the use of februxostat, 2-[3-cyano-4-(2-methyl propoxy)phenyl]-4-methyl thiazole-5-carboxylic acid (Fig. 1), a new non-purine selective xanthine oxidase (XO) blocker, for the management of hyperuricemia in gouty adults. Since the first authorization of allopurinol in 1964 [1,2], it is the first substance to have been licensed for the treatment of gout in the USA. A long-term treatment for gout brought on by excessive uric acid levels is febuxostat, which is marketed under the trade names uloric and adenuric among others. Generally speaking, it is only advised for those who cannot take allopurinol. To stop gout flare-ups when first initiated, drugs like NSAIDs are often used [3-5]. Inflammation and continuous crystal formation of urate in joints, organs, tissues and bones are symptoms of gout, a kind of critical arthritis that is defined by the build up of mono-sodium urate and crystals of urate in or around a joint. These symptoms may worsen over time. Since hyperuricemia and abnormal serum uric acid levels are thought to be the bio-chemical abnormality contributing to the pathogenesis of gout, the two conditions are closely related to one another and may

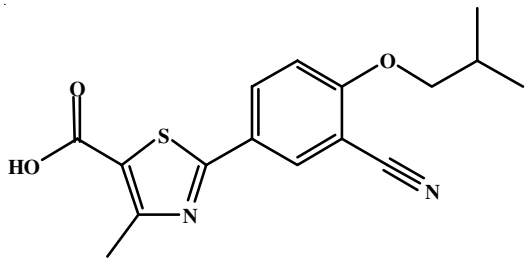

Fig. 1. Chemical structure of febuxostat

exist for many years prior to the first clinical attack of gout. A xanthine oxidase or a xanthine dehydrogenase, respectively, is a xanthine oxidoreductase (XOR) [5]. It is a crucial enzyme for uric acid synthesis in humans [6]. By inhibiting both XOR's oxidase and dehydrogenase functions, febuxostat effectively inhibits this enzyme. Febuxostat links to XOR with great affinity in the molecular channels that connects to the molybdenum-pterin active site, where allopurinol demonstrates relatively weak competitive inhibition [5-7].

Literature on febuxostat revealed that some analytical procedures reported on UV [8], LC [9-12] and LCMS/MS [13]. For the estimate of febuxostat by LC-MS/MS with rat kinetics,

no single analytical approach was devised. Thus, LCMS MS technology is required for the study of biological materials, as it will be helpful in pharmacokinetic, pharmacodynamic and forensic research. A technique based on human K2 EDTA plasma is developed in the current study and used the same way for rat kinetics.

EXPERIMENTAL

Febuxostat and febuxostat D9 were procured from Novartis, India. The LC-grade methyl alcohol, acetonitrile and GR-grade formic acid and ammonia were purchased from Merck, Mumbai, India. The LC-water was prepared using purified water from the MilliQ-system (Millipores, USA). The Institutional Ethical Committee granted approval for the pharmacokinetic investigation on healthy Wistrar rats with the following ethical no.: 1447/PO/Re/S/11/CPCSEA-67/A. An LC-MS/MS system of Quattros Premier X.E attached with HPLC 2695 isolation module was utilized for this research work. The chromatograms were processed and data was generated using Mass Lynx V 4.1 software.

Preparation of internal standard (IS) solution: To 1.0 mL volumetric flask, 1 mg of febuxostat D9 reference component was added, dissolved and made upto the volume with methanol. The ISTD stock (1 mg/mL) solution of 0.25 mL was pipetted out into a 50 mL volumetric flask using a calibrated pipette, and the volume was then made up with the diluent (5.0 µg/mL). Mixed well, marked the mixture and maintain it between 2 and 8 ºC.

Preparation of calibration standards: Dissolved 10 mg of febuxostat standard in methyl alcohol and then made up to volume and stored at 2-8 ºC. Process the serial dilution method and prepare the solution concentrations in between 24.99-7000 ng/mL with mobile phase. Prepared the spiked calibration standards in the same concentration range by utilizing the human K2 EDTA plasma

Preparation of quality control (QC) samples: Accuraely weighed 10 mg febuxostat was dissolved in methanol dissolved and then made up volume. The QC stock solution was used to prepare LQC (74.47 ng/mL), MQC 2 (696 ng/mL), MQCs 1 (3480 ng/mL) and HQCs (5800 ng/mL) spiking solutions.

Extraction of sample: The plasma samples were thawed and vortexed at room temperature. Except for the standard blank, 50 µL of 5.0 µg/mL IS working solutions were put to prelabeled empty tubings in batch sequence. A 200 µL plasma from Step-1 was vortexed for 05 s in ISTD tubes. Vortexed all tubes with 100 µL extraction buffer for 5 s. All the vials contains 2.5 mL of ethinyl acetate and mixed rigrously at 40 rpm for 25 min. Centrifuged all the vials at 4500 rpm at 4 ºC for 5 min, then 2.0 mL of upper layer was moved to pre-labeled evaporation tubes, dried under nitrogen atmosphere at 40 ± 5 ºC. For 1 min, all tubes were vortexed again with 200 µL reconstitution solution. Utilizing auto-sampler vials that have already been labeled, inject 10 µL of reconstituted solution into the LC-MS/MS.

Optimized chromatographic conditions: A Purosphur C18, 100 × 4.6 mm, 5 µ column contains methyl alcohol/ammonium acetate of 5 mM (90/10, v/v) as mobile phase at 1.0 mL/min, which was utilized for the separation of the components. Then 10 µL was utilized to separate the peaks within 2.30 min at 40 ± 5 ºC of oven temperature. The analyte retention time was 1.75 min, while the retention time of ISTD was 1.73 min.

Mass instrument conditions: Table-1 shows the parameters for mass spectrometry using an electrospray ionization (ESI) source and multiple reaction monitor (MRM). The MRM transitions of febuxostat and the internal standard solutions were *m/z* 315.28/270.98 and 324.28/279.98, respectively.

TABLE-1
MASS SYSTEM PARAMETERS

ES-Source parameters	Values
Capillary	2.50 kV
Extractor	1.00 V
Source temperature (°C)	120
De solvation temperature (°C)	300
Cone flows	100 ± 5 L/h
De solvation flow (L/h)	700 ± 10
Collision cell pressure (mbar)	$3.5e^{-3}$ – $4.5e^{-3}$
Dwell	0.200
Cone voltage (V)	28
Collision energy	23

Validation of method: The developed procedure was validated in accordance with FDA, 2001 and EMA, 2011 [14-18].

Pharmacokinetic studies: Wistar rats weighing between 150 and 180 g were chosen for this investigation and maintained a good health throughout. The animals were housed with 100% fresh air exchange, constant power and supply and regulated climatic conditions (relative humidity of 45%, temperature of 25 ºC and 12 h of alternating dark light cycles). The rats were fed daily and had access to water all times. The rats were starved for 24 h before to the experiments. Animals were given a single oral dosage of febuxostat equivalent to the animal dose and 0.5 mL of blood were collected from the retro-orbital puncher at 0, 0.5, 1.0, 1.50, 2, 2.50, 3, 4, 6, 8, 12, 16, 20 and 24 h later [19].

RESULTS AND DISCUSSION

Validation of the method

System suitability: It was processed with six successive injections of an aqueous standard mixture at MQC1 concentration (Fig. 2). System suitability was tested daily throughout method validation [20]. In this process, the retention time %CVs were ≤ 0.30 for the analyte and ISTD and the results are summarized in Table-2.

Auto sampler carryover effect: The auto sampler carryover impact analysis was conducted by infusing an unextracted sample solutions of mobile solvent, LLOQ, ULOQ and extracted solutions of standard blank, ULOQ, blank and LLOQ. The findings indicated that there was no carryover effect [21-23].

Specificity and screening of biological matrix: For the estimation of the specificity, 10 different lots of plasma were examined. Seven of the ten samples were intended to contain anticoagulant plasma, one contained hemolytic, one contained lipidemic and one contained anticoagulant (heparin) plasma. All the examined human plasma lots were found to be devoid

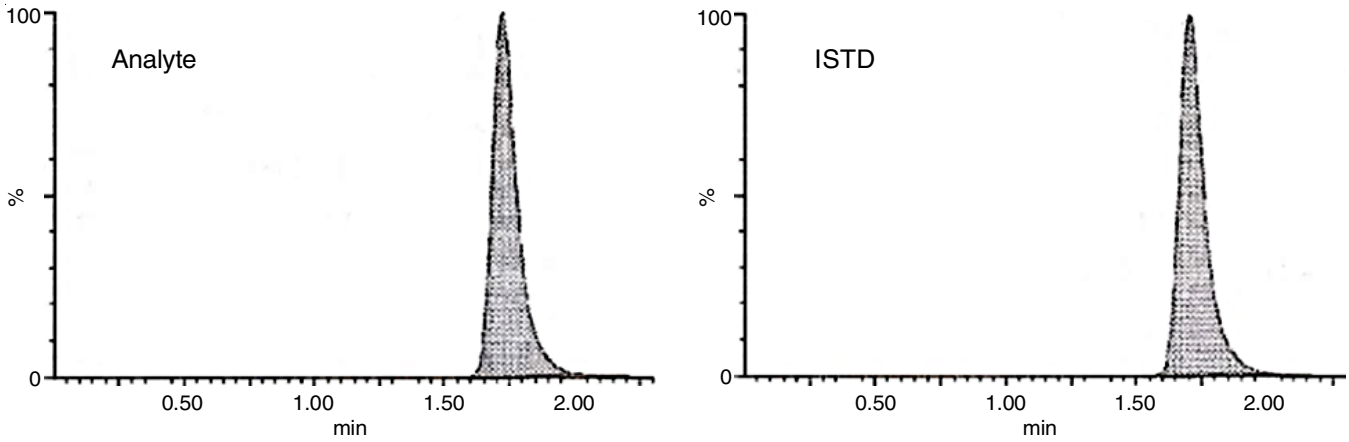

Fig. 2. Representative chromatogram of aqueous MQC1

TABLE-2
FEBUXOSTAT SYSTEM SUITABILITY (ANALYTE: FEBUXOSTAT; ISTD: FEBUXOSTAT D9)

Name of sample	Area of analyte	RT of drug (min)	Response of ISTD	RT of IS	Ratio response
AQ MQC1	230850	1.72	86948	1.70	2.6550
AQ MQC1	232523	1.72	87785	1.70	2.6488
AQ MQC1	234987	1.72	88345	1.71	2.6599
AQ MQC1	235277	1.73	88065	1.71	2.6716
AQ MQC1	240164	1.72	89709	1.71	2.6772
AQ MQC1	240856	1.73	90197	1.71	2.6703
MEAN		1.723		1.707	2.66380
SD		0.0052		0.0052	0.010957
%CV		0.30		0.30	0.41

of substantial interferences at the drug's retention timings and ISTD (Fig. 3).

Sensitivity: By assessing six LLOQ, the developed method's sensitivity was determined to be 24.995 ng/mL for febuxostat. At LLOQ level, the precision and accuracy were found to be 8.80% and 103.97%, respectively.

Matrix effect: To evaluate the impact of matrix on LC-MS/MS spectroscopy, six lots of plasma were chromatographically screened. Each batch of plasma was produced in batches and administered in triplicate at each stage, with febuxostat concentrations matching the LQC and HQC [16,21]. The overall % RSD was 1.28 and 1.63 for high and low QC solutions of all batches. The mean accuracy values were 97.34 and 96.13 for HQC and LQC samples of all lots, respectively (Table-3).

Calibration curve: During validation, all the four calibration curves were linear for standards concentrations from 24.995 to 7001.401 ng/mL having r = 0.9997 (Table-4).

Precision: The accuracy of the LC-MSMS method was assessed throughout the validation process utilizing the % CV at different concentrations of LQC, MQC1, LLOQ, MQC2 and HQC. The %CV of back computed concentration solutions was varied from 0.57 to 3.24. The %CV of the back-calculated concentrations for all of the quality control samples was between 1.5% and 2.9%, well within the permissible range of

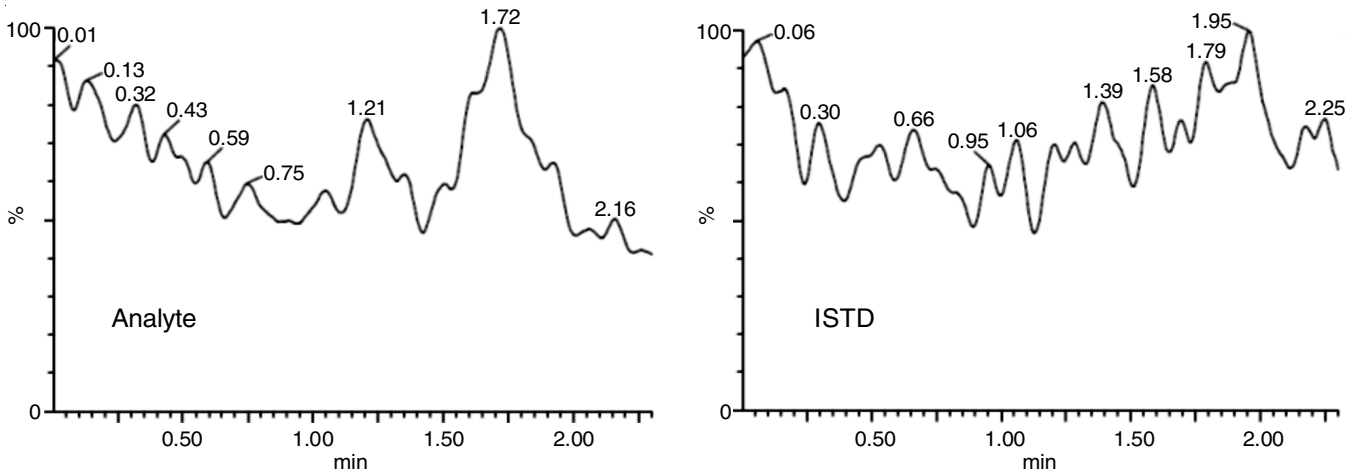

Fig. 3. Chromatogram of blank solution

TABLE-3
MATRIX EFFECT FOR ANALYTE
(ANALYTE: FEBUXOSTAT; ISTD: FEBUXOSTAT D9)

Plasma Lot No.	HQC	LQC
	Nominal concentration (ng/mL)	
	5738.770	74.489
	Nominal concentration range (ng/mL)	
	(4,877.955-6,599.586)	(63.316-85.662)
	Back calculated concentration (ng/mL)	
P-883	5731.082	71.936
	5544.607	70.540
	5563.246	72.302
P-884	5547.387	70.571
	5630.762	70.609
	5669.764	70.226
P-885	5546.670	72.615
	5581.513	71.139
	5556.702	71.323
P-887	5487.645	70.528
	5585.560	71.212
	5701.238	73.509
(P-795)-Lipemic	5637.778	72.296
	5538.249	73.247
	5650.728	71.681
(P-886)-Hemolyzed	5597.449	69.465
	5480.248	73.178
	5499.901	72.536
n	18	18
Mean	5586.1405	71.6063
SD	71.55748	1.16439
%CV	1.28	1.63
%Mean accuracy	97.34	96.13

15%. All LLOQ samples %CV of back computed concentrations were determined to be 3.59, falling within the acceptable range of 20.00%. The findings have been compiled and summarized in Table-5.

Accuracy: The fraction of the estimated average readings of quality controls to their related nominal findings was used to determine the assay's accuracy. The %average accuracies of back calculated concentration levels for all the control solutions were in between 91.41-100.82 [22] (Table-5).

Recovery: The %average recoveries were obtained by comparing extracted plasma quality control solutions to unextracted ones at MQC 01, HQC, MQC 02 and LQC concentrations. At MQC 01, HQC, MQC 02 and LQC concentrations, febuxostat had 88.94, 90.29, 88.33 and 94.48% mean recovery, while the QC levels had 90.51 mean recovery and 3.06 %CV (Table-6).

Integrity of dilution: By diluting 1/5th and 1/10th times to 3 × ULOQ, the dilution integrity of the developed method

TABLE-4
CALCULATED ANALYTE CONCENTRATIONS OF LINEARITY STANDARDS

Conc.	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8	STD9	STD10
Mean	25.1843	50.5737	67.4123	138.6603	354.7933	709.7947	1785.6453	3452.4167	5439.2847	7161.8687
Area ratio	0.0197	0.0405	0.0535	0.1062	0.2656	0.5353	1.3518	2.6118	4.1152	5.3076

Slope	Intercept	r
0.00075574	0.00114445	0.9997
0.000762119	0.00061669	0.9992
0.000778567	0.00119084	0.9997

TABLE-5
ACCURACY AND PRECISION OF FEBUXOSTAT (ANALYTE: FEBUXOSTAT; ISTD: FEBUXOSTAT D9)

	HQC	MQC 01	MQC 02	LQC	LLOQQC
Precision and accuracy					
I					
Mean	5498.8455	3413.4997	661.1462	70.9208	25.8267
SD	136.56995	66.36549	10.00339	2.29688	0.76294
CV (%)	2.48	1.94	1.51	3.24	2.95
Mean accuracy (%)	95.82	100.82	97.63	95.21	103.33
II					
Mean	5559.4380	3403.8082	665.9403	71.4135	26.1328
SD	58.17873	32.56006	3.79061	1.03132	0.26027
CV (%)	1.05	0.96	0.57	1.44	1.00
Mean accuracy (%)	96.88	100.53	98.34	95.87	104.55
III					
Mean	5562.5562	3338.5130	656.0267	68.0922	24.7732
SD	96.26200	40.51542	13.25305	0.48247	0.99409
CV (%)	1.73	1.21	2.02	0.71	4.01
Mean accuracy (%)	96.93	98.60	96.88	91.41	99.11
Between batch precision and accuracy					
n	18	18	18	18	18
Mean	5540.2799	3385.2736	661.0377	70.1422	25.5776
SD	100.58444	57.13294	10.13250	2.04955	0.91699
CV (%)	1.82	1.69	1.53	2.92	3.59
Mean accuracy (%)	96.54	99.98	97.62	94.16	102.33

TABLE-6
RECOVERY FOR ANALYTE (ANALYTE: FEBUXOSTAT; ISTD: FEBUXOSTAT D9)

Replicate No.	HQC		MQC1		MQC2		LQC	
	Un extracted response	Extracted response	Un extracted response	Extracted response	Un extracted response	Extracted response	Un extracted response	Extracted response
1	583209	483836	364920	316504	73403	64664	8088	7365
2	575553	508224	364801	311471	72739	67376	7770	6870
3	569361	530213	356052	323689	71667	47457	7792	7124
4	561704	500459	353972	330366	70394	65804	7855	7430
5	553626	499300	353370	324595	71088	67304	7636	7367
6	557816	503174	350414	328686	69813	66413	7592	7995
n	6	6	6	6	6	6	6	6
Mean	566878.2	504201.0	357254.8	322551.8	71517.3	63169.7	7788.8	7358.5
SD	11248.36	15143.44	6161.37	7259.72	1372.70	7763.58	176.83	375.23
CV (%)	1.98	3.00	1.72	2.25	1.92	12.29	2.27	5.10
Mean recovery (%)	88.94		90.29		88.33		94.48	
Overall mean recovery (%)	90.51							
Overall SD	2.770							
Overall CV (%)	3.06							

TABLE-7
STABILITY DATA OF FEBUXOSTAT

Stabilities level	Comparison samples area mean		Stability samples area mean		Mean stability (%)	
	HQC	LQC	HQC	LQC	HQC	LQC
Short-terms	5560.71	70.8407	5612.6615	71.2053	99.43	99.07
Long-terms	4284862	328.500	4234747	318.000	98.93	100.18
Freeze thaws at -28 ± 5 °C	5560.713	70.8407	5600.124	71.8688	101.15	100.73
Freeze thaws at -70 ± 10 °C	5560.71	70.8407	5549.997	71.6262	100.24	100.39
Bench top stability	5560.71	70.8407	5612.6615	71.2053	101.37	99.80
Auto sampler stability	5583.468	71.2515	5612.0973	72.1512	100.95	100.54
Wet extract stability RT	5560.713	70.8407	5580.7807	71.1133	100.80	99.67
Wet extract stability (2-8 °C)	5583.468	71.2515	5590.441	72.1022	100.56	100.47
Dry extract stability	5583.468	71.2515	5558.916	71.4350	99.99	99.54

was also assessed. It was found that the accuracies for the dilutions integrity of 1/5th and 1/10th was 1.22 and 1.56%, respectively.

Stability study: Storing analytes and internal standard (IS) at room temperature for 8 h provided a short-term stability. HQC and LQC monitored the drug and IS stability at 2.0-8.0 ºC for 10 days, 16 h and 20 min for long term stability. Three freeze-thaw cycles were performed at -28 ± 5 ºC and -70 ± 10 ºC. Benchtop stability of spiking quality control sample solutions were measured for 17 h and 28 min at room temperature [24]. The prepared controls were stored in an autosampler at 5 ± 3 ºC for 2 days, 20 h and 27 min to verify their stability. The stability of the wet extract was tested by storing the spiked quality control samples at room temperature for 23 h and 42 min. The wet extract's stability at 2-8 ºC was 2 days, 20 h and 23 min. Dry extracts stabilities of spike controls were also tested at -28 ± 5 ºC for 2 days, 20 h and 2 min. Table-7 showed that all of the investigations were conducted within acceptable limits.

Pharmacokinetic studies: The mean plasma concentration–time curve of wister rats after a single oral dose of febuxostat tablets is shown in Fig. 4, whereas the results of the pharmacokinetic parameters after oral administration in Wister rats are shown in Table-8. The C_{max}, T_{max}, $T_{1/2}$ and $AUC_{0-\infty}$ of febuxostat tablets are 8.85 ± 1.87 ng/mL, 2.0 ± 0.03 h, 6.34 ± 0.53 h and 95.58 ± 6.37ng h/mL respectively.

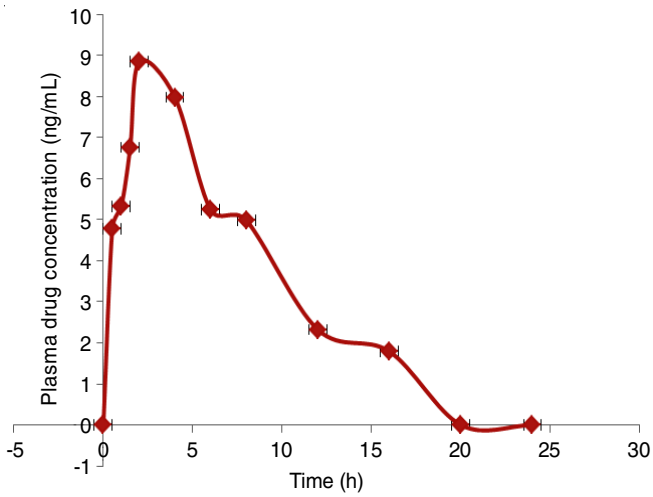

Fig. 4. Average concentration of plasma-time profiles for febuxostat tablets in rats (n = 6)

TABLE-8
AVERAGE PHARMACOKINETICS OF FEBUXOSTAT TABLETS

Pharmacokinetic parameters	Febuxostat tablets
C_{max} (ng/mL)	8.85 ± 1.87
AUC_{0-inf}	95.58 ± 6.37 ng. h/mL
AUC_{0-t}	78.45 ± 5.76 ng. h/mL
$t_{1/2}$ (h)	6.34 ± 0.53
T_{max} (h)	2.0 ± 0.03

Conclusion

An accurate, linear and sensitive LC-MS/MS method is developed for the quantitation of febuxostat drug in human K2EDTA plasma. Chromatographic isolation of febuxostat and febuxostat D9 were attained on Purosphur C_{18}, 100 × 4.6 mm, 5 µ column with 1.0 mL/min flowing rate and coupled triple quadrupole mass system in MRM mode by applying mass transitions *m/z* 315.28/270.98 for febuxostat and *m/z* 324.28/279.98 for febuxostat D9. The % average recovery for febuxostat at MQC1, HQC, MQC2 and LQC levels was found to be 90.29, 88.94, 88.33 and 94.48, respectively. The developed method was also subjected for the pharmacokinetic examination in wistar rats by oral administration of the tablet dose.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P.K. Gandhi, W.M. Gentry and M.B. Bottorff, *Semin. Arthritis Rheum.*, **42**, 562 (2013); https://doi.org/10.1016/j.semarthrit.2012.11.002
2. J. Saban-Ruiz, A. Alonso-Pacho, M. Fabregate-Fuente and C. de la Puerta Gonzalez-Quevedo, *Antiinflamm. Antiallergy Agents Med. Chem.*, **12**, 94 (2013); https://doi.org/10.2174/1871523011312010011
3. H. Tsuda, N. Kawada, J.Y. Kaimori, H. Kitamura, T. Moriyama, H. Rakugi, S. Takahara and Y. Isaka, *Biochem. Biophys. Res. Commun.*, **427**, 266 (2012); https://doi.org/10.1016/j.bbrc.2012.09.032
4. N.L. Edwards, *Rheumatology*, **48(suppl 2)**, 15 (2009); https://doi.org/10.1093/rheumatology/kep088
5. B. Tomlinson, *Ther. Clin. Risk Manag.*, **4**, 1209 (2008); https://doi.org/10.2147/TCRM.S3310
6. T. Nishino and K. Okamoto, *J. Biol. Inorg. Chem.*, **20**, 195 (2015); https://doi.org/10.1007/s00775-014-1210-x
7. B.A. Grabowski, R. Khosravan, L. Vernillet and D.J. Mulford, *J. Clin. Pharmacol.*, **51**, 189 (2011); https://doi.org/10.1177/0091270010365549
8. P. Bagga, M. Salman, H.H. Siddiqui, A.M. Ansari, T. Mehmood and K. Singh, *Int. J. Pharm. Sci. Res.*, **14**, 2655 (2011); https://doi.org/10.13040/IJPSR.0975-8232.2(10).2655-59
9. K.M. Younes, E.F. El-Kady and E.S. Elzanfaly, *J. Chromatogr. Sci.*, **54**, 1022 (2016); https://doi.org/10.1093/chromsci/bmw040
10. K. Nageswara Rao, S. Ganapaty and A. Lakshmana Rao, *Int. J. Res. Pharm. Chem.*, **2**, 1104 (2012).
11. K.C. prasad, B.N. Angothu, T.M. Latha and M. Nagulu, *Int. J. Pharm. Biol. Sci.*, **7**, 1 (2017); https://doi.org/10.21276/ijpbs.2017.7.1.1
12. M.A. Mukthinuthalapati, S.P. Bandaru, V. Bukkapatnam and C. Mohapatro, *J. Chromatogr. Sci.*, **51**, 931 (2013); https://doi.org/10.1093/chromsci/bms192
13. Y. Xu, J. Chen, D. Yang, Y. Hu, X. Hu, B. Jiang, Z. Ruan and H. Lou, *J. Clin. Pharm. Ther.*, **46**, 333 (2021); https://doi.org/10.1111/jcpt.13285
14. C. Zhou, J. Tian, P. Lin, T. Liu, A. He, L. Fang and L. Sun, *Bioanalysis*, **12**, 285 (2020); https://doi.org/10.4155/bio-2020-0011
15. ICH Guidelines for Validation of Analytical Procedures: Text and Methodology. Q2 (R1) ICH, Geneva, pp. 1-14 (2005).
16. US FDA, Guidance for Industry Bioanalytical Method Validation, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville, Maryland, USA (2001).
17. U. Glaenzel, Y. Jin, R. Hansen, K. Schroer, G. Rahmanzadeh, U. Pfaar, J.J. van Lier, H. Borell, A. Meissner, G. Camenisch and S. Zhao, *Drug Metabol. Disp.*, **48**, 873 (2020); https://doi.org/10.1124/dmd.119.090324
18. J.V. Shah, P.A. Shah, P.V. Shah, M. Sanyal and P.S. Shrivastav, *J. Pharm. Anal.*, **7**, 163 (2017); https://doi.org/10.1016/j.jpha.2016.11.004
19. S. Lolla, K.S. Gubbiyappa, S. Cheruku and D.V.R.N. Bhikshapathi, *J. Pharmacol. Toxicol. Methods*, **120**, 107254 (2023); https://doi.org/10.1016/j.vascn.2023.107254
20. K.B. Pasupuleti, A. Venkatachalam and B.R. Kesavan, *Int. J. App. Pharm.*, **13**, 144 (2021); https://doi.org/10.22159/ijap.2021v13i6.42607
21. I. Drapak, B. Zimenkovsky, L. Perekhoda, S. Kovalenko and L. Logoyda, *Int. J. App. Pharm.*, **11**, 125 (2019); https://doi.org/10.22159/ijap.2019v11i5.33873
22. G.S.U. Kiran, S. Cheruku, S. Cheruku, D.V.R.N. Bhikshapathi and M. Palanati, *J. Appl. Pharm. Sci.*, **13**, 180 (2023); https://doi.org/10.7324/JAPS.2023.130117
23. H. Suryadi, A.H. Rasyid and Harmita, *Int. J. Appl. Pharm.*, **10**, 19 (2018); https://doi.org/10.22159/ijap.2018.v10s1.05
24. L. Logoyda, D. Korobko, O. Oleshchuk, T. Proniv and M. Dmutriv, *Int. J. Appl. Pharm.*, **10**, 31 (2018); https://doi.org/10.22159/ijap.2018v10i2.23195