Salting-Out Assisted Liquid–Liquid Extraction for Quantification of Febuxostat in Plasma Using RP-HPLC and Its Pharmacokinetic Application

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Abstract

A rapid and sensitive reversed-phase high-performance liquid chromatography (HPLC) method using novel salting-out assisted liquid–liquid extraction technique has been developed for the quantitative determination of febuxostat (FEB), used for the treatment of gout, in rat plasma. The method was validated according to US FDA guideline. Separation was achieved using a Phenomenex Luna-C18 (250 × 4.60 mm, 5 µm) column and mobile phase composed of potassium dihydrogen orthophosphate buffer 25 mM, adjusted to pH 6.8 with triethylamine:methanol in a ratio of 35:65 (v/v) showing retention time 5.56 and 8.86 min for FEB and internal standard, respectively. The optimal salting-out parameters; 1 mL of acetonitrile and 200 µL of 2 M ammonium acetate salt showed extraction recovery >90% for FEB from plasma. This extraction procedure afforded clear samples resulting in convenient and cost-saving procedure and showed good linear relationship (r > 0.9997) between peak area ratio and concentration from 0.3 to 20 µg/mL. The results of pharmacokinetic study showed that absorption profile of spherical agglomerate of FEB compared to marketed formulation was higher indicating greater systemic absorption. In conclusion, the developed SALLE-HPLC method with simple ultraviolet detection offered a number of advantages including good quantitative ability, wide linear range, high recovery, short analysis time as well as low cost.

Introduction

Febuxostat (FEB) [2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1, 3-thiazole-5-carboxylic acid] is a non-purine, selective inhibitor of xanthine oxidase, used for the management of hyperuricemia in patients with gout (Figure 1) (1).

Literature reviewed reveals a few quantitative bioanalytical methods available for the estimation of FEB in biological fluids. These methods include liquid chromatography–tandem mass spectrometry (LC–MS) in rat plasma using protein precipitation extraction (PPE) method (2); ultra performance liquid chromatography–tandem mass spectrometry in human plasma (LC–MS–MS) (3), LC–MS–MS in human plasma (4) and high-performance liquid chromatography (HPLC)-MS/MS-ESI in human plasma using liquid–liquid extraction (LLE) technique (5) that requires an expensive instrumentation, not available in conventional analytical laboratory. Thus, a simple HPLC method with ultraviolet (UV) detection based on salting-out assisted liquid–liquid extraction (SALLE) for the sample preparation was developed for the quantification of FEB from plasma.

Sample preparation is an integral part of optimization of bioanalytical method (6), and various offline sample clean up procedures like PPE, LLE and solid-phase extraction (SPE) are most widely used (7–12). Protein precipitation is simple and applicable to both hydrophilic and hydrophobic compounds, but the supernatant is relatively...
unclean since it still contains a significant amount of unprecipitated soluble plasma components that could affect chromatographic separation or suppress the ionization of the target analyte (7–12). SPE works with most compounds but suffers from having a complicated and time-consuming procedure, relatively poor reproducibility and high cost (7–12). LLE is simple and the extracts are clean, but the recovery percentage of drug is less and it is generally unsuitable for the hydrophilic and thermolabile substances (7–12).

Nowadays, a fast high-throughput SALLE technique established has a number of advantages over well-established methods such as PPE, LLE, SPE and online SPE (7–17). In this study, during extraction of FEB from the plasma, the samples underwent double protein precipitation by ammonium acetate and acetonitrile sequentially to remove plasma proteins completely. Ammonium acetate was concentrated enough to force the supernatant to form two phases. FEB followed organic phase, and the extract was filtered through nylon membrane filter and injected into reversed-phase high-performance liquid chromatography (RP-HPLC).

Moreover, FEB is a poorly water-soluble Biopharmaceutical Classification System (BCS) Class II drug having bioavailability of only 49%. Therefore, the improvement of solubility and dissolution rate of FEB is an important research concern for enhancing its bioavailability and therapeutic efficacy. Hence, the spherical agglomerates of FEB were developed in our research laboratory using HPMC E3LV polymer by spherical crystallization technique. Furthermore, a novel bioanalytical method with SALLE approach is proposed for the evaluation of pharmacokinetic parameters of spherical agglomerates and marketed formulation by preclinical study.

**Experimental**

**Materials**

FEB and Timolol maleate (TIM MAL) were supplied as a gratis sample by Lupin Pharmaceutical Ltd., Mumbai, India, and Marck Biosciences Pvt. Ltd., Kheda, India, respectively. Methanol and acetonitrile of Lichrosolv grade were obtained from Mumbai, India. Potassium dihydrogen orthophosphate, triethylamine and ammonium acetate of HPLC grade were obtained from Mumbai, India. Double distilled water was prepared in a deep freezer until analysis. All other chemicals and solvents used were of analytical grade or equivalent.

**Instruments**

An HPLC system with LC solutions data handling system (Shimadzu LC-2010 CHT; Japan), with PDA detector and an auto sampler was used for the analysis. The data were recorded using LC-2010 solutions software version 1.25. Analytical balance (Shimadzu AUW220 balance, Japan), refrigerated centrifuge (TC 450 D; Eltek, Mumbai, India), Cyclo Mixer (Remi motors CM101; Mumbai, India), vacuum filtration assembly (TID 15; Mumbai, India) and AXIVA Nylon membrane filters 0.2 μm were used during the study.

**Chromatographic conditions**

Various solvents in different ratios such as methanol, acetonitrile, water along with buffer, 0.02 M potassium dihydrogen orthophosphate and 0.02 M ammonium dihydrogen orthophosphate were tried. Chromatographic separations were performed on column (250 mm × 4.60 mm) filled with octadecyl silane (ODS) chemically bonded to porous silica particles of 5 μm. The mobile phase was prepared daily, degassed by ultrasonicator and filtered through a 0.45-μm membrane filter prior to use. TIM MAL was used as an internal standard.

**Preparation of calibration standards and quality control samples**

A standard stock solution of FEB and TIM MAL (1,000 μg/mL) was prepared by separately dissolving accurately weighed 10 mg of mentioned drugs in 10 mL of methanol and was stored at −20°C in a clear glass volumetric flask and protected with aluminum foil. Working standard solution of FEB was prepared by diluting 10 mL of standard stock solution of FEB up to 100 mL with methanol to obtain a final concentration of 100 μg/mL.

In 5-μL graduated RIA vials, FEB working standard solution was spiked in appropriate volume to drug-free plasma to achieve calibration standards ranging from 0.3 to 20 μg/mL. Similarly, quality control (QC) samples were prepared by spiking and mixing the working solution to control plasma to obtain three QC levels namely, high, medium and low (20, 2 and 0.3 μg/mL), respectively. Calibration standards and QC samples were stored in a deep freezer at −20 ± 2°C until analysis.

**Optimization of conditions in SALLE**

Commonly used salts for SALLE like sodium chloride, calcium chloride, magnesium sulphate, ammonium acetate, ammonium formate and potassium carbonate at different concentrations (1, 2 and 3 M) were tried for the selection and optimization of salt concentration. Similarly, several extraction solvents like methanol, acetone, acetonitrile, methyl t-buty1 ether, ethyl acetate, 2-propanol, diethyl ether and dimethyl formamide were tried on the basis of review of literature, for the selection of the best extraction solvent. The frozen samples were thawed in water bath at room temperature. The 100 μL thawed plasma (drug spiked) were transferred into pre-labeled RIA vials and vortexed to ensure complete mixing of contents (2 min). After adding 50 μL of TIM MAL (100 μg/mL), 100 μL of above-mentioned salts with different concentrations (1, 2 and 3 M) and 1,000 μL of above-mentioned extraction solvents, the mixture was vortexed for further 5 min to ensure uniform mixing. The vials were centrifuged at 1006 ± 2°C in a refrigerated cooling centrifuge. The supernatant was collected, filtered through 0.2 μm nylon membrane filter and 20 μL of solution was further injected in RP-HPLC.

**Method validation**

Developed bioanalytical method was validated as per US FDA guideline, by determination of various parameters like linearity...
range, accuracy, precision, selectivity, sensitivity, recovery and stability (18, 19).

**Calibration and linearity range**

The linearity was established by construction of calibration curve of response in form of FEB/TIM MAL peak area ratio plotted against concentration of FEB (in micrograms per milliliter). The response of six standard solutions in the range of 0.3–20 µg/mL was subjected to regression analysis to establish the calibration equation, and correlation coefficient was computed by least square regression analysis. Moreover, Bartlett’s test was applied to evaluate the homoscedasticity of data in terms of variance (20, 21).

**Sensitivity**

Sensitivity in terms of lower limit of quantification (LLOQ) is the lowest concentration of analyte on the calibration curve, which should be quantified reliably with an acceptable accuracy and percent coefficient of variance (%CV) should be ≤20%. Limit of detection (LOD) was defined as signal/noise ratio of three.

**Selectivity**

The selectivity of the method was assessed at LLOQ by extracting FEB and TIM MAL from two different blank plasma samples. The absence of interfering peaks at the retention time of analyte or internal standard and %CV <20% confirmed selectivity of the method.

**Accuracy and precision**

The accuracy was measured as the percentage of measured concentration to theoretical concentration by injecting five replicates of three QC samples (0.3, 2 and 20 µg/mL) and expressed in terms of %CV. Intraday precision was analyzed on three QC samples (0.3, 2 and 20 µg/mL) as five replicates on 1 day, whereas interday precision was assessed by analyzing three QC samples (0.3, 2 and 20 µg/mL) on 3 subsequent days. The precision of the method was expressed as %CV. The mean value should be within 15% CV of the actual value except at LLOQ, where it should not deviate by >20%.

**Extraction recovery**

Extraction recoveries from human plasma were determined by comparing the response ratio of extracted plasma samples spiked with known amount of FEB at high (20 µg/mL), middle (2 µg/mL) and low (0.3 µg/mL) QC levels (n = 5) before and after extraction followed by calculation of percent extraction recovery using equation,

\[
\%\text{Extraction recovery} = \left( \frac{\text{Mean peak area ratio of extracted sample}}{\text{Mean peak area ratio of non-extracted sample}} \right) \times 100
\]

**Stability studies**

Stability of FEB under various storage conditions was investigated.

Freeze/thaw stability study was determined at two concentration levels, i.e. low quality control (LQC) (0.3 µg/mL) and high quality control (HQC) (20 µg/mL) stored at −22°C for 24 h and thawed unassisted at room temperature. The freeze/thaw cycle was repeated three times and compared with freshly prepared QC samples. The short-term temperature stability study was also determined at −22°C for 12 h and compared with freshly prepared QC samples.

addition, the long-term stability study for a period of about 30 days was evaluated at LQC (0.3 µg/mL) and HQC (20 µg/mL) stored at −22°C by comparing against the freshly prepared QC samples.

**Method applicability for pharmacokinetic study**

The study was conducted in accordance with the ethical guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) and was approved by the Institutional Animal Ethics Committee (IAEC) (Registration no. 277/PO/ReBi/2000/CPCSEA) of Anand Pharmacy College, Anand, Gujarat, India (Protocol no. 1213 dated 30 November 2012). Rats were housed in plastic cages on corn-cob bedding in a temperature controlled room (20°C ± 2°C) with a 12 h light/dark cycle. Female Wistar albino rats, 250 ± 10 g, fasted overnight with free access to water for at least 12 h, were dosed orally by gavages with 7.085 mg/kg of FEB. Rats were divided into three groups (n = 4) based on the time of blood sampling having four animals each. The first group received pure FEB, second group received prepared spherical agglomerates of FEB and third group received marketed formulation. The blood samples (~500 µL) were collected from the retro-orbital vein in sodium citrate tubes at 0, 15, 30, 60, 90, 120 and 150 min after the administration. Samples were immediately centrifuged at 4000 rpm for 10 min, and the plasma was frozen at −20°C and stored until analysis. The estimation of FEB in all the samples was undertaken within 6–7 h of blood collection by the method as described earlier.

The pharmacokinetic parameters were calculated by using a non-compartmental approach. The area under the plasma concentration vs. time curve after oral administration, AUC, was calculated using the linear trapezoidal rule up to the last measured plasma concentration and extrapolated to AUC_{0→∞}. The area under the first moment curve to the last measured plasma concentration, AUMC, was also calculated using the linear trapezoidal rule. Peak plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), elimination rate constant (K_{el}), terminal elimination half-life (t_{1/2}), absorption rate constant (K_{ab}) and absorption half-life (t_{1/2}) were determined for pure FEB, marketed formulation and developed spherical agglomerates of FEB.

**Results**

**Optimization of chromatographic conditions**

Various mobile phases comprising different ratios of methanol, acetonitrile and 0.02 M potassium dihydrogen orthophosphate were tried. Acetonitrile when used as mobile phase produced splitted peak at 2.8 min and methanol as a mobile phase produced peak at 8.49 min but peak shape was not Gaussian. Hence, various ratios of methanol and buffer were tried that produced acceptable peak shape of FEB and TIM MAL. Finally, the optimized mobile phase, methanol: 0.025 M potassium dihydrogen orthophosphate adjusted to pH 6.8 with triethylamline (65:35 v/v) gave acceptable retention time FEB (5.56 ± 0.095 min) and TIM MAL (8.86 ± 0.02 min), theoretical plates (2474.25 ± 141.23), tailing factor (1.15 ± 0.10) with appropriate resolution (9.62 ± 0.06) for FEB and internal standard, TIM MAL at 314 nm and flow rate 1.0 mL/min. The injection volume to carry out chromatography was set at 20 µL. Under these optimized conditions, FEB and TIM MAL were eluted at retention time of 5.56 and 8.86 min, respectively.
Optimization of SALLE conditions

Selection of salts along with its concentration as well as extracting solvent is a critical step in SALLE procedure. Various salts: sodium chloride, calcium chloride, magnesium sulphate, ammonium acetate, ammonium formate and potassium carbonate at different concentration levels (1, 2 and 3 M) and various extraction solvents: methanol, acetone, acetonitrile, ethyl acetate, methyl t-butyl ether, 2-propanol, diethyl ether and dimethyl formamide were tried in different combinations. The percentage extraction recovery using magnesium sulphate, calcium chloride and potassium carbonate at different concentration was found to be very low (Figure 2). Moreover, the percentage extraction recovery using ammonium acetate was found to be higher than ammonium formate (Figure 2). Percentage recovery of FEB from human plasma using acetonitrile and methyl t-butyl ether with different salts and their specified 2 M concentration gave optimum results as evident from Figure 2. Therefore, 1000 µL acetonitrile as an extracting solvent and 2 M ammonium acetate as salt concentration gave 94% extraction recovery of FEB and was finally selected as the optimum extraction conditions. These results are also in accordance with the results of Wu et al. (10).

Method validation

Calibration and linearity range

The linearity of the method was determined by using ordinary least square regression analysis and calibration curve was linear from 0.3 to 20 µg/mL with acceptable correlation coefficient ($r^2$), slope of regression line, $y$-intercept and linear regression equation (Table I). Furthermore, homoscedasticity of variance was confirmed by Bartlett’s test and the response of peak area for FEB showed homogenous variance that was exemplified by the $\chi^2$ value less than the tabulated value (Table I) (20, 21). Thus, from the obtained results, there was no further need of weighting and transformation approach.

Sensitivity

The LOD and LLOQ were found to be 0.0659 and 0.1995 µg/mL, respectively. The percentage accuracy was found to be 85% at LOD level and 92% at LLOQ level. The %CV was found to be 9.16% and 7.69% indicating sensitivity of the proposed method. Representative chromatogram of FEB and TIM MAL at LOD and LLOQ are shown in Figure 3.

Table I. Linear Regression Parameters of FEB

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient$^a$</td>
<td>0.9997</td>
<td>0.00</td>
</tr>
<tr>
<td>Slope of regression line$^a$</td>
<td>0.593</td>
<td>1.56</td>
</tr>
<tr>
<td>$y$-intercept$^a$</td>
<td>−0.063</td>
<td>9.08</td>
</tr>
<tr>
<td>Linearity range</td>
<td>0.3–20 µg/mL</td>
<td></td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>$y = 0.593 \times −0.063$</td>
<td></td>
</tr>
<tr>
<td>Bartlett's test ($\chi^2$)</td>
<td>0.000574236</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Average of five replicates; $\chi^2$ critical value = 9.488 at $\alpha = 0.05$.

Selectivity

The selectivity was studied at LLOQ by analyzing two different plasma samples. The retention time of FEB and TIM MAL was found to be 5.56 and 8.86 min, respectively (Figure 4), with no other peak eluting at the retention times of FEB or TIM MAL in the blank samples from two different sources.

Accuracy and precision

The accuracy was estimated, for each spiked QC samples as five replicate by comparing the theoretical concentration with the assayed concentration (Table II). The %CV was found to be within 15% indicating that the proposed method provides acceptable accuracy for the determination of FEB. The results of precision study were found to be acceptable with %CV <9.49 % (Table II).

Extraction recovery

Extraction recovery study performed at three concentration levels showed 82.19–95.59% recovery of spiked drug, %CV found was <15%, which shows high efficiency of extraction procedure and sensitivity of proposed method (Table II). However, Gide et al. reports 72.13–74.34% extraction recovery of FEB using diethyl ether as extracting solvent in LLE procedure (8). Hence, proposed method is more beneficial than reported LLE procedure.

Stability studies

The %CV of freeze/thaw stability, short-term and long-term stability studies at two concentration levels was found to be <15 indicating stability of drug in plasma (Table III).
Table II. Accuracy, Precision and Extraction Recovery of FEB by Proposed Method

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (µg/mL)</th>
<th>Accuracy</th>
<th>Precision</th>
<th>Extraction recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean concentration found (µg/mL)</td>
<td>%CV</td>
<td>Mean concentration found (µg/mL)</td>
</tr>
<tr>
<td>LQC</td>
<td>0.3</td>
<td>0.26</td>
<td>7.35</td>
<td>0.26</td>
</tr>
<tr>
<td>MQC</td>
<td>2</td>
<td>1.88</td>
<td>3.56</td>
<td>1.81</td>
</tr>
<tr>
<td>HQC</td>
<td>20</td>
<td>19.06</td>
<td>2.89</td>
<td>19.87</td>
</tr>
<tr>
<td>TIM MAL</td>
<td>4</td>
<td>84.63</td>
<td>6.11</td>
<td></td>
</tr>
</tbody>
</table>

*aAverage of five determinations; MQC, middle quality control.

Table III. Stability Study by Proposed Bioanalytical Method

<table>
<thead>
<tr>
<th>Stability</th>
<th>%Mean recovery a</th>
<th>%CV</th>
<th></th>
<th>%Mean recovery a</th>
<th>%CV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LQC: 0.3 µg/mL</td>
<td>HQC: 20 µg/mL</td>
<td></td>
<td>LQC: 0.3 µg/mL</td>
<td>HQC: 20 µg/mL</td>
<td></td>
</tr>
<tr>
<td>Freeze/thaw stability</td>
<td>71.06</td>
<td>95.69</td>
<td>9.03</td>
<td>3.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-term stability</td>
<td>85.98</td>
<td>96.79</td>
<td>7.99</td>
<td>4.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term stability</td>
<td>84.23</td>
<td>86.45</td>
<td>8.58</td>
<td>5.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aAverage of three determinations; HQC, high quality control.
Table IV. Pharmacokinetic Parameters of Spherical Agglomerates and Marketed Formulation

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Spherical agglomerates(^a)</th>
<th>Marketed formulation(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-150}) (µg min/100 µL)</td>
<td>4776.93 ± 3.65</td>
<td>4204.05 ± 6.21</td>
</tr>
<tr>
<td>AUMC(_{0-∞}) (µg min/100 µL)</td>
<td>356344.12</td>
<td>316862.85</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>74.59</td>
<td>70.58</td>
</tr>
<tr>
<td>(C_{max}) (µg/100 µL)</td>
<td>58.18 ± 3.54</td>
<td>52.31 ± 2.14</td>
</tr>
<tr>
<td>(t_{max}) (min)</td>
<td>60 ± 0.65</td>
<td>50 ± 6.54</td>
</tr>
<tr>
<td>(K_{ab}) (min(^{-1}))</td>
<td>0.0276</td>
<td>0.0172</td>
</tr>
<tr>
<td>(K_{el}) (min(^{-1}))</td>
<td>0.0153</td>
<td>0.0037</td>
</tr>
<tr>
<td>(t_{1/2}) (Absorption) (min)</td>
<td>25.10</td>
<td>40.29</td>
</tr>
<tr>
<td>(t_{1/2}) (Elimination) (min)</td>
<td>45.29</td>
<td>22.57</td>
</tr>
</tbody>
</table>

\(^a\)Each value indicates mean ± SD of four wistar albino rats; AUC\(_{0-∞}\), area under the plasma concentration vs. time curve from 0 to infinity; AUMC\(_{0-∞}\), area under the plasma concentration \(\times\) time vs. time curve from 0 to infinity; MRT, mean residence time; \(C_{max}\), maximum concentration; \(t_{max}\), time of peak concentration; \(K_{ab}\), absorption constant; \(K_{el}\), elimination constant; \(t_{1/2}\), half-life.

Method applicability for pharmacokinetic study

The above validated method was applied for the determination of FEB in rat plasma samples. It clearly appears from the pharmacokinetic parameters that absorption of FEB from spherical agglomerates is increased as compared to pure FEB and marketed formulation (Table IV, Figures 5 and 6). The plasma concentration–time profile showed higher \(C_{max}\) for spherical agglomerates (58.18 ± 3.54 µg/100 µL) compared to marketed formulation (52.31 ± 2.14 µg/100 µL), respectively. \(C_{max}\) value hence reveals that the solubility of spherical agglomerates is more resulting in more bioavailability in blood, compared to marketed formulation.

Discussion

There are quite a few bioanalytical methods for FEB reported in literature (3–6). However, most of these methods utilize the conventional sample preparation approaches like PPE, LLE and SPE. This study reveals development and application of a fast high-throughput salting-out assisted liquid–liquid extraction technique. SALLE is a technique based on LLE in which an appropriate concentration of salt is added to achieve the separation of aqueous phase from the partially miscible organic phase and simultaneously the target solutes are extracted into the separated organic phase. Addition of salt increases the ionic strength of the solution and decreases the solubility of organic analyte. Moreover, no drying down step is required in this method compared with PPE, SPE and offline SPE method. Hence, SALLE is an environment-friendly sample preparation method (7–12). Generally methanol, acetonitrile, ethyl acetate and diethyl ether are suitable candidates for SALLE (11, 14–17). Inorganic salts such as NaCl, MgSO\(_4\), CaCl\(_2\), K\(_2\)CO\(_3\), (NH\(_4\))\(_2\)SO\(_4\) and ammonium acetate were evaluated at different concentrations (10–12, 14, 17). In this study, acetonitrile was selected due to its polarity and higher extraction efficiency. NH\(_4\)Ac was a better salting-out agent, which helps in deproteination. In the extraction of FEB from human plasma, the sample went through double protein precipitation by acetonitrile and ammonium acetate salt. In nutshell, 1000 µL acetonitrile as the extracting solvent and 2 M ammonium acetate as salt concentration gave 94.00% extraction recovery of FEB and was finally chosen as the optimum extraction condition. These results are also in accordance with the results of Wu et al. (10). Pharmacokinetic study by the proposed validated SALLE-HPLC bioanalytical method showed high concentration for spherical agglomerates of FEB resulting in more solubility and bioavailability compared to marketed formulation.

Conclusion

A novel sensitive RP-HPLC method for the estimation of FEB in plasma was developed using SALLE method, by optimization of various SALLE conditions, salting-out solvent, salt and its concentration. Acetonitrile and ammonium acetate salt showed high extraction recovery >90% for the FEB from plasma. Moreover, the results of pharmacokinetic study showed that the absorption profile of spherical agglomerates of FEB compared to marketed formulation and pure FEB was higher showing greater systemic absorption. Pharmacokinetic parameter, \(C_{max}\) demonstrate that spherical agglomerates of FEB showed higher concentration compared to marketed formulation and pure FEB resulting in more solubility and increased bioavailability in blood. Hence, the prepared spherical agglomerates of FEB is capable of surmounting the short-comings of pure febuxostat, BCS Class II drug, such as least solubility and bioavailability. The developed SALLE–with simple UV detector offered a number of features including good quantitative ability, wide linear range, high recovery, simple operation process and short analysis time, as well as low cost and environmental benignity.
References