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A Novel LC-MS/MS Method for the Determination of Celecoxib in Human Plasma

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Abstract : This paper describes a simple and rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the quantification of celecoxib in human plasma using celecoxib-d7 as an internal standard (IS). A C18 column with isocratic mobile phase of 5 mM ammonium acetate – acetonitrile (20:80, v/v) used for the separation of extracted analyte. The flow rate was 0.75 mL/min. The proposed linearity for celecoxib was 5.05–2519 ng/mL. A total of five linearity curves were generated with quality control samples to calculate the precision and accuracy. Also, the stability of analyte was extensively evaluated in plasma as well in extracted samples and results were met the acceptance criteria defined in US FDA guidelines. The chromatographic run time was set at 2.5 min, which makes the proposed method is high through put.
Keywords : Celecoxib; Solid–phase extraction (SPE); LC–MS/MS; Method validation.

Introduction

Celecoxib (Figure1), is a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID). The drug is used to treat the pain and inflammation of osteoarthritis, rheumatoid arthritis, acute pain in adults[1], ankylosing spondylitis[2], painful menstruation[3], and juvenile rheumatoid arthritis[4]. After oral administration, celecoxib is rapidly absorbed and achieves peak plasma concentration in about 3 hours. It is extensively metabolized in the liver and being eliminated little unchanged (<3%). The major routes of excretion for celecoxib are feces and urine[5].

Many analytical methods based on LC-MS/MS[6-10] were reported for the determination of celecoxib in human plasma, rat plasma and in rat blood samples. However, these methods are having drawbacks like use of more plasma volume (≥0.5 mL)[6], tedious sample extraction with use of non-polar solvents involves

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evaporation, drying and reconstitution steps[6-9], longer chromatographic run time (>3 min)[6-10], and use non-isotope labelled compound as internal standard[8-10]. A good analytical method should be rapid with less analysis time, requires low volume and efficient extraction to remove the endogenous matrix components[11-12].

To overcome above disadvantages, we have developed and validated a simple, reliable and rapid LC-MS/MS method for the determination celecoxib in human plasma. The present method is having run time of 2 min and utilized very low plasma 50 µL for sample preparation. Also, the sample extraction method with cartridges was efficient in obtaining high recovery for analytes with no or minimal matrix effect. Here we used celecoxib-d7 as internal standard.

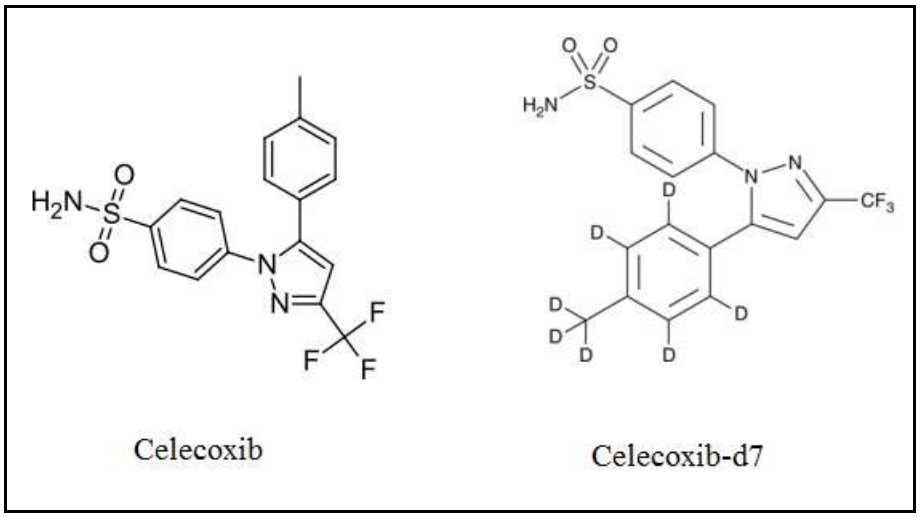

Figure 1: Chemical structure of celecoxib and celecoxib-d7 (IS)

Experimental

Standards and reagents:

The standard sample celecoxib (99.4%) and celecoxib-d7 (99.7% pure) were obtained from Clearsynth Labs Limited (Mumbai, India). Acetonitrile and methanol were of LC-MS grade were purchased from J.T. Baker (Phillipsburg, USA). Analytical grade ammonium formate was procured from Merck Ltd (Mumbai, India). Blank human plasma was obtained from Deccan's Pathological Lab's (Hyderabad, India).

LC–MS/MS instrument and conditions:

An API-4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) with Turboionspray ™ (ESI) interface couple with a Shimadzu (Kyoto, Japan) HPLC system was used for the study. An isocratic mobile of 5 mM ammonium formate (pH 3.5), acetonitrile and methanol (20:20:60, v/v/v) was delivered at a flow rate of 0.9 mL/min. A 10µL aliquot of the sample was injected in to Zodiac C18 (50 x 4.6 mm, 3.0 µm) column.

The mass spectrometer was operated with ESI probe in negative mode at a source temperature of 500 °C and ion spray voltage of 4000 V. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 30, 50, 30, and 8 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were -100, -31, -10, -13 V for celecoxib and for the IS. Ions were monitored in n the multiple–reaction monitoring (MRM) mode with the transition pairs of *m/z* 380.0 precursor ion to the *m/z* 316.0 for celecoxib and *m/z* 387.2 precursor ion to the *m/z* 323.2 product ion for the IS. The chromatographic data was processed by Analyst Software™ (version 1.6.1).

Sample preparation:

Stock solutions of celecoxib and the IS were prepared in methanol at 1 mg/mL, separately. For analyte two stocks were prepared and used for the preparation of calibration standards and quality control samples.

Working solutions of analyte was prepared in water and methanol (30:70, v/v; diluent). Calibrates were prepared in plasma at concentration levels of 5.05, 10.1, 50.5, 101, 252, 505, 1009, 1511, 2015 and 2519 ng/mL. Similarly, the quality control (QC) samples were prepared at concentrations of 5.15 (lower limit of quantitation, LLOQ), 15.1 (low quality control, LQC), 379 (medium quality control, MQC1), 1262 (MQC2) and 1918 ng/mL (high quality control, HQC). The entire sample were prepared in a single batch and stored at –70±10 °C in a freezer.

To a 200 µL of thawed human plasma sample, 20 µL of the IS dilution (10 ng/mL of celecoxib-d7) was added. To each sample 500 µL of water was added after vortex mixing for 10 s. The sample mixture was loaded onto a Celerity Deluxe, (Orochem) DVB-LP (30 mg/1mL) that was pre–conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The extraction cartridge was washed with 1.0 mL of 2.0 mL of water (1 mL each time) and eluted with 1.0 mL of mobile phase and injected into the LC-MS/MS system.

Method validation parameters:

The developed method was validated as per US FDA guidelines. The parameters validated were carryover test, selectivity, matrix effect, sensitivity, linearity, precision and accuracy, recovery, dilution integrity, and stability[13].

Results and Discussion

Mass spectrometry:

A 5µL/min sample of 100 ng/mL of celecoxib was injected into ESI source to optimize the mass spectrometric conditions. The greater intensity was obtained in negative mode than the positive mode for the analyte due to its acidic nature of celecoxib. We optimized source and compound dependent mass parameters to obtain the high and reproducible response. De-protonated form of analyte and IS, $[M–H]^-$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 380.0 to 316.0 for celecoxib and from m/z 387.2 to 323.2 for the IS. The MRM technique was used to monitor the ion transitions as it provides intrinsic selectivity and sensitivity[14-18].

Method development:

Ionization of analyte and the IS is mainly affected by the pH and the composition of the mobile phase. To opt a suitable mobile phase, a series of buffers (acidic buffers like ammonium acetate and ammonium formate and volatile acids like formic acid and acetic acid) were tested in combination with methanol and acetonitrile as organic modifiers. Chromatography of analyte and the IS were not good and the response was not reproducible with ammonium acetate, ammonium formate in combination with methanol and acetonitrile. Similar results were obtained with volatile buffers. Hence the ammonium formate pH was adjusted to 3.5 with formic acid. A mobile phase composed of 5 mM ammonium formate (pH 3.5, adjusted with formic acid), acetonitrile and methanol (20:20:60, v/v/v) achieved symmetric peak shape and reproducible response even with short retention time. Among the different C_{18} columns tested, Zodiac C18 (50 x 4.6 mm, 3.0 µm) column gave good peak shape and response even at LLOQ level for the analyte. The chromatographic run time was 2.5 min, having retention time of 1.2 min for analyte and the IS. The mobile phase flow rate was set at 0.9 mL/min.

The earlier publications have employed LLE and PP for sample preparation. However, SPE gives superior sample cleanup with minimal or no matrix effect. Hence, during the method development we tried to extract plasma samples with Celerity Deluxe DVB-LP, Oasis HLB, Strata X polymeric sorbent and Orpheus C_{18} cartridges. Among all the above, high recovery for analyte and the IS was obtained with Celerity Deluxe- DVB-LP cartridges. A deuterated internal standard will have the same extraction recovery, ionization response in ESI mass spectrometry and the same chromatographic retention time. Also, these compounds will co-elute with the compound to be quantified. Hence, in the present work celecoxib stable labeled isotope celecoxib-d7 was used as internal standard and found to be best for the present purpose.

Selectivity and specificity:

Method selectivity was achieved through analyzing 6 K_2 EDTA plasma lots obtained from individuals. These include one lipemic and one haemolyzed plasma. Figure 2 shows that, no significant interference in the

blank plasma at the retention time of analyte and the IS. Also, no interference was observed from the IS channel at the retention time of analyte (Figure 3). A representative chromatogram LLOQ samples was displayed in Figure 4.

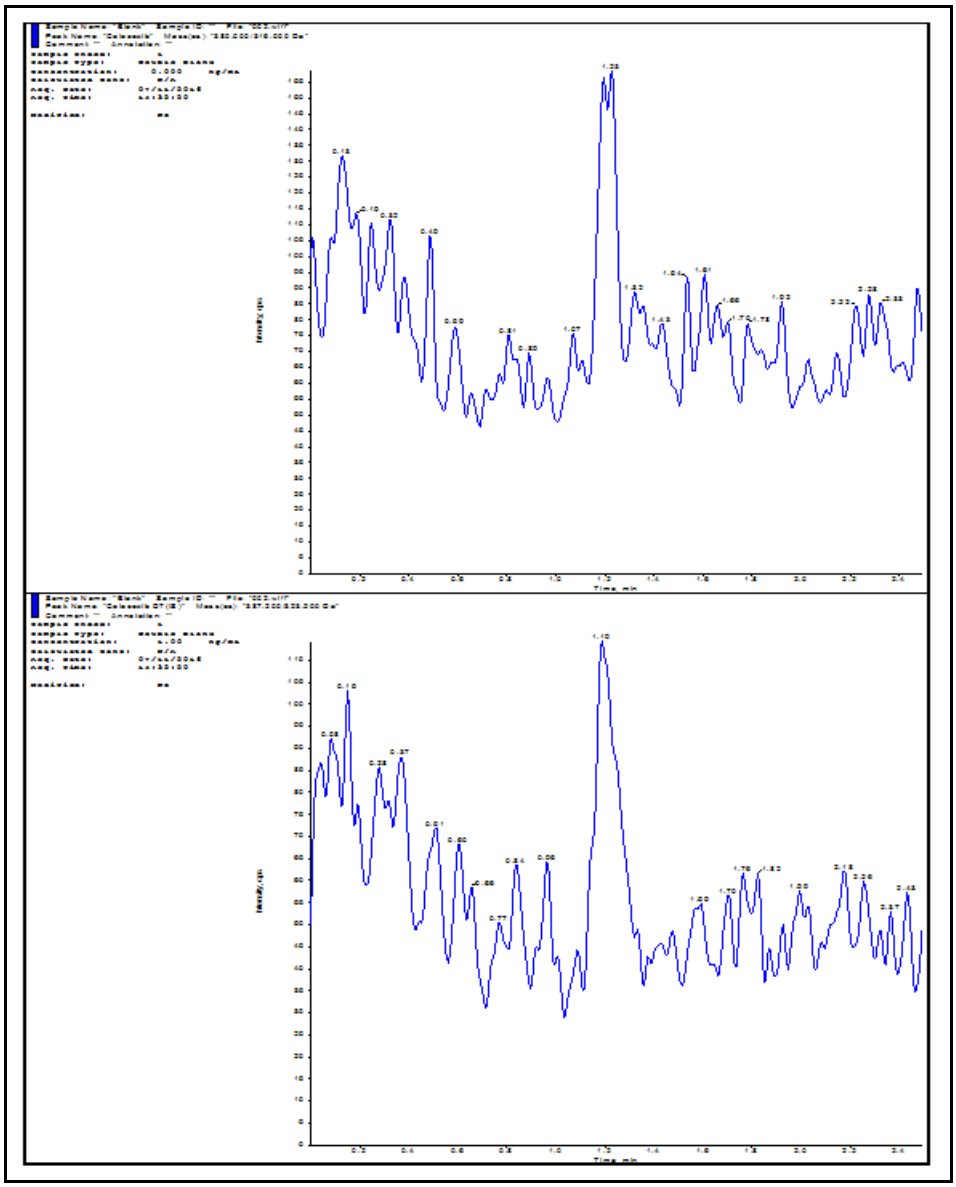

Figure 2. Typical MRM chromatograms of celecoxib (upper panel) and IS (lower panel) in human blank plasma

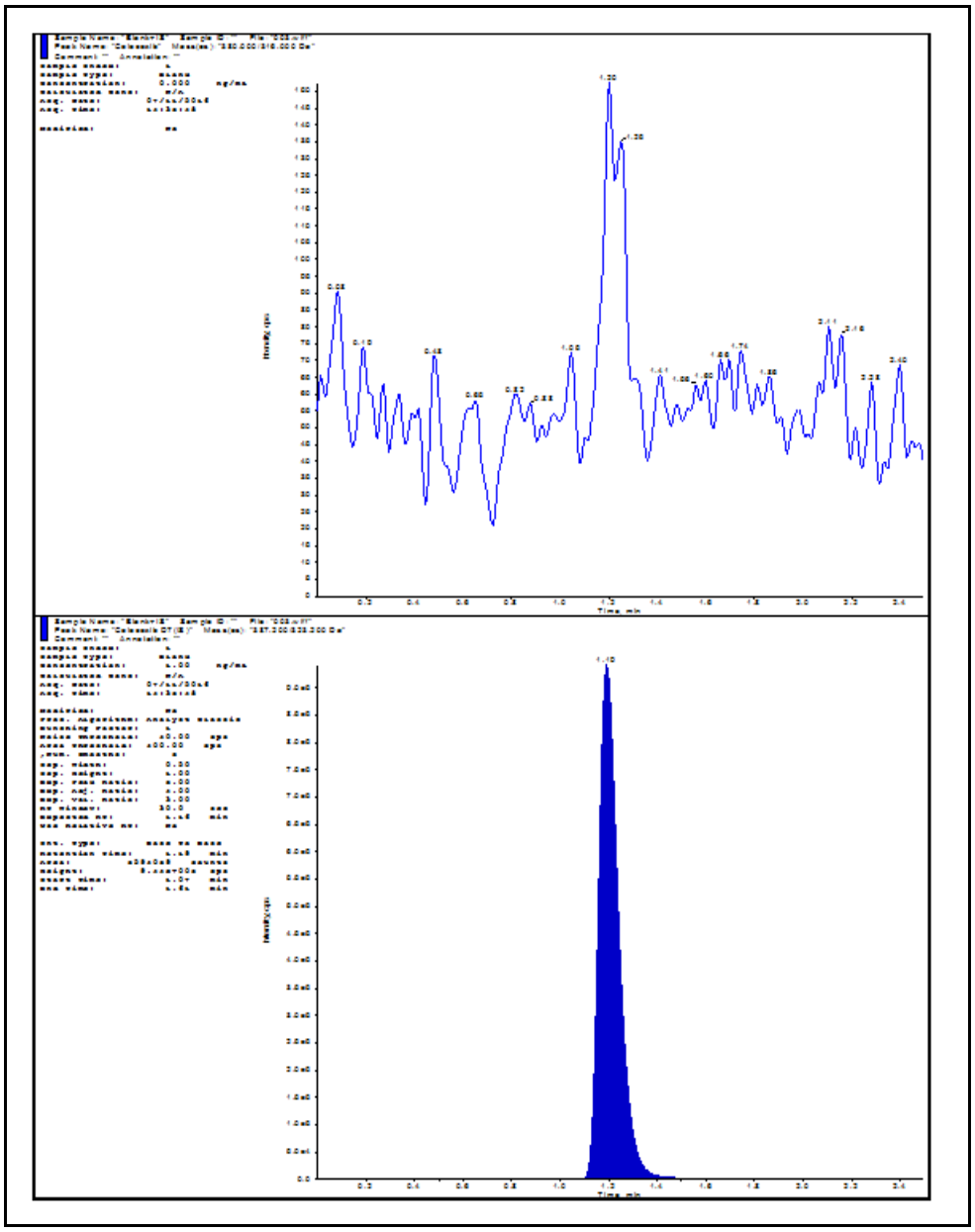

Figure 3. Typical MRM chromatograms of celecoxib (upper panel) and IS (lower panel) in human plasma spiked with IS

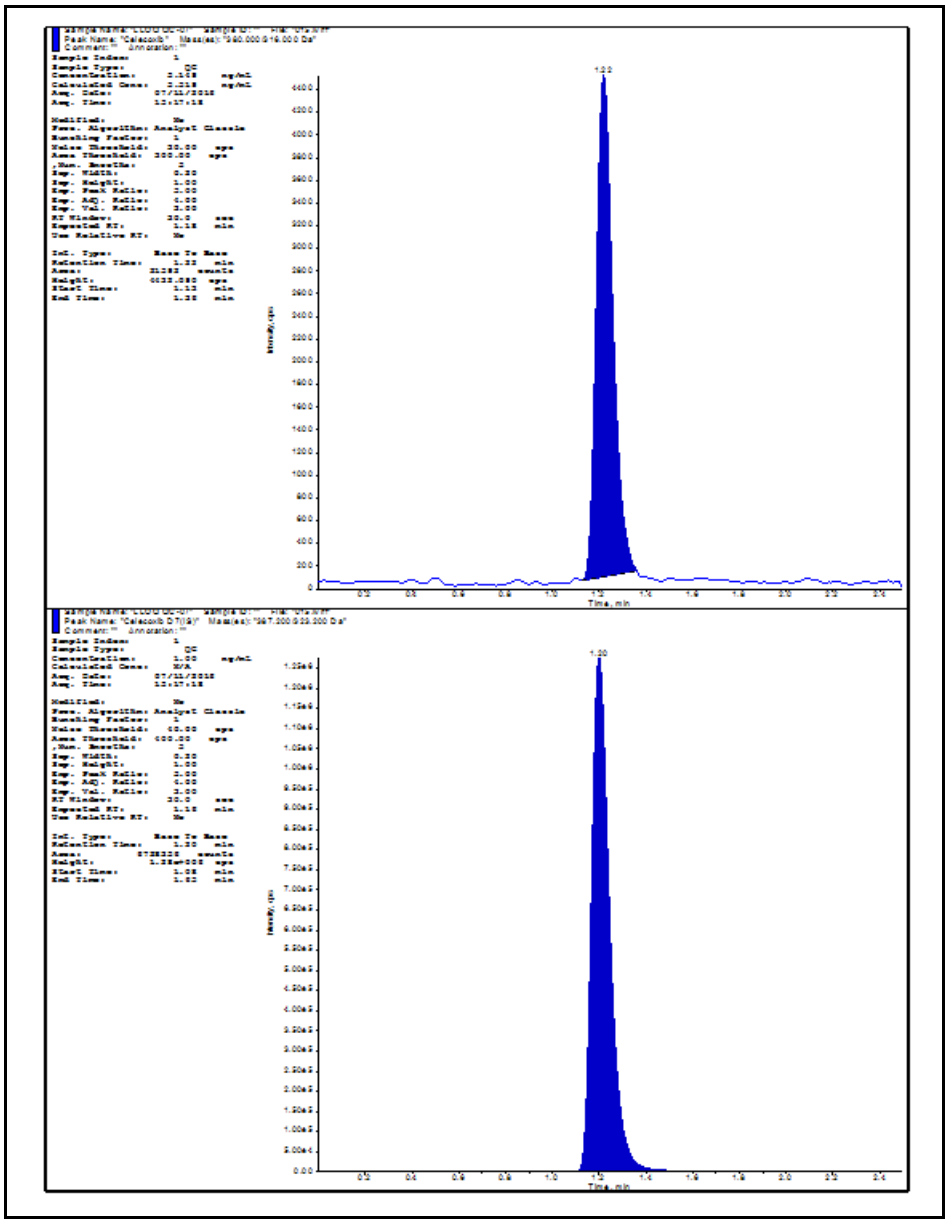

Figure 4. Typical MRM chromatogram of an LLOQ sample of Celecoxib (upper panel) along with IS (lower panel)

For specificity experiment, interference at the retention time analyte was assessed by injecting the IS alone. Similarly, interference at the retention time the IS was assessed by injecting the ULOQ concentration of analyte alone. Results show that there was no significant cross talk was observed. The carryover test was performed by injecting the highest concentration of analyte and working concentration of the IS. The results obtained for carryover test shows no significant carryover effect in the blank samples after injection of highest concentration of analyte (ULOQ).

Sensitivity and matrix effect

Sensitivity was established at 5.05 ng/mL, which is known as lowest limit of reliable quantification (LOQ). The accuracy and precision at this concentration was 2.55 and 105%, respectively. Also, the signal–to–noise ratio (S/N) measured was ≥10.

Matrix effect expressed as IS normalized matrix factor (MF) and was determined at LQC and HQC levels. The response of post–extraction spiked samples was compared with mean area of neat samples. The IS normalized matrix factor was 1.01 for LQC and 1.00 for HQC. The results indicate no significant matrix effect was found in all the plasma lots tested.

Dilution integrity

The upper linearity concentration (ULOQ) can be extended up to 8580 ng/mL by 1/2 and 1/4 dilutions with screened human blank plasma. The precision (%CV) an accuracy for two-fold dilution was 1.03% to 90.9%, respectively. Similarly, the precision (%CV) an accuracy for four-fold dilution was 1.55% to 90.3%, respectively.

Linearity, precision and accuracy

A total of five successful calibration curves run during the validation in the concentration range of 5.05–2519 ng/mL for celecoxib with a mean correlation coefficient of ≥ 0.99. After comparing the two weighting models (1/x and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration was found to produce the best fit for the concentration–detector response relationship.

The results of intra–day and inter–day analysis are summarized in Table 1. The intra–day and inter day precision deviation values were all within 15% of the relative standard deviation (RSD) at low, middle and high quality control level, whereas within 20% at LLOQ QCs level. The intra–day and inter–day accuracy deviation values were all within 100 ± 15% of the actual values at low, middle, and high quality control level, whereas within 100± 20% at LLOQ QCs level. The results revealed good precision and accuracy.

Table 1. Precision and accuracy data for celecoxib

Quality control	**Run**	**Concentration found Mean±SD (ng/mL)**	**Precision (%)**	**Accuracy (%)**
Intra–day (*n*=12)				
LLOQ		5.43 ± 0.12	2.28	106
LQC		16.0 ± 0.18	1.10	105
MQC1		407 ± 3.20	0.79	108
MQC2		1166 ± 7.67	0.66	92.4
HQC		1964 ± 11.5	0.59	102
Inter–day (*n*=30)				
LLOQ		5.51 ± 0.17	3.01	107
LQC		16.0 ± 0.28	1.72	106
MQC1		407 ± 5.56	1.37	108
MQC2		1168 ± 10.3	0.89	92.6
HQC		1971 ± 22.2	1.12	103
Nominal concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 5.15, 15.1, 379, 1262 and 1918 ng/mL, respectively.				

Recovery

The SPE gives highest and reproducible recoveries for the analyte and the IS. The recovery was determined at LQC, MQC2 and HQC levels and was found to be 101%, 99.1% and 98.6%, respectively with mean recovery of 99.4±1.01%. Similarly, the recovery of IS was 99.8% with the precision range of 0.74–2.02%.

Stability studies

The mean % nominal values were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels and the precision (% CV) values were within 15% (Table 2) for all the stability tests carried out during the entire course of method validation. All the above stability results were found to be within the acceptable limits during the entire validation.

Table 2. Stability data for celecoxib in plasma (*n*=6)

Stability test	QC (spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/ Stability (%)
Auto-sampler (50 h)	15.1	16.5 ± 0.43	2.64	109
	1918	1905 ± 22.3	1.17	99.4
Wet extract stability (48 h)	15.1	16.0 ± 0.52	3.26	105
	1918	1930 ± 23.3	1.21	101
Bench top (15 h)	15.1	15.4 ± 0.49	3.19	102
	1918	1916 ± 8.54	0.45	99.9
freeze and thaw (4 Cycles)	15.1	15.1 ± 0.20	1.35	99.9
	1918	1929 ± 16.5	0.85	101
Re-injection (36 h)	15.1	16.0 ± 0.34	2.14	105
	1918	1955 ± 12.9	0.66	102
Long–term (70 days)	15.1	15.4 ± 0.35	2.26	101
	1918	1918 ± 6.33	0.33	100

Conclusions

The present LC–MS/MS assay method is simple, rapid and sensitive for the determination of celecoxib in human plasma. This method was fully validated as per US FDA guidelines and is well suitable for pharmacokinetic or bioavailability/bioequivalence application. Simple and one step SPE extraction give high and reproducible recovery celecoxib and the IS. Also, the method was rapid with run time of 2.5 min and alternative for existing methods for routine drug analysis. Thus, the advantage of this method is that a relatively large number of samples can be analyzed in short time, thus increasing the output.

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References

1. Neal M.D., Andrew J.M., Ric O.D. and Kenneth M.W., Clinical pharmacokinetics and pharmacodynamics of celecoxib, a selective cyclo-oxygenase-2 inhibitor, Clin. Pharmacokinet., 2000, 38, 225-242.
2. Poddubnyy D.A., Song I.H. and Sieper J., The safety of celecoxib in ankylosing spondylitis treatment, Expert Opin. Drug Saf., 2008, 7, 401-409.
3. Daniels S., Robbins J., West C.R. and Nemeth M.A., Celecoxib in the treatment of primary dysmenorrhea: results from two randomized, double-blind, active- and placebo-controlled, crossover studies, Clin. Ther., 2009, 31, 1192-1208.
4. Krishnaswami S., Hutmacher M.M., Robbins J.L., Bello A., West C. and Bloom B.J., Dosing celecoxib in pediatric patients with juvenile rheumatoid arthritis. J. Clin. Pharmacol., 2012, 52, 1134-1149.
5. Food and Drug Administration. New drug application #20998: clinical pharmacology/biopharmaceutics review section celecoxib. Bethesda (MD): FDA, 1998.
6. Werner U., Werner D., Pahl A., Mundkowski R., Gillich M. and Brune K., Investigation of the pharmacokinetics of celecoxib by liquid chromatography-mass spectrometry. Biomed. Chromatogr., 2002, 16, 56-60.
7. Bräutigam L., Vetter G., Tegeder I., Heinkele G. and Geisslinger G., Determination of celecoxib in human plasma and rat microdialysis samples by liquid chromatography tandem mass spectrometry, J.

Chromatogr. B., 2001, 761, 203-212.
8. Park M.S., Shim W.S., Yim S.V. and Lee K.T., Development of simple and rapid LC-MS/MS method for determination of celecoxib in human plasma and its application to bioequivalence study, J. Chromatogr. B., 2012, 902, 137-141.
9. Ma Y., Gao S. and Hu M., Quantitation of celecoxib and four of its metabolites in rat blood by UPLC-MS/MS clarifies their blood distribution patterns and provides more accurate pharmacokinetics profiles, J. Chromatogr. B., 2015, 1001, 202-211.
10. Thappali S.R., Varanasi K., Veeraraghavan S., Arla R., Chennupati S., Rajamanickam M., Vakkalanka S. and Khagga M., Simultaneous determination of celecoxib, erlotinib, and its metabolite desmethyl-erlotinib(OSI-420)
in rat plasma by liquid chromatography/tandem mass spectrometry with positive/negativeion-switching electrospray ionization, Sci. Pharm., 2012, 80, 633-646.
11. Matta M.K., Pilli N.R. and Jillela V.L.N.S.R., A validated liquid chromatography and tandem mass spectrometric method for simultaneous quantitation of tenofovir, emtricitabine and efavirenz in human plasma and its pharmacokinetic application. Acta Chromatogr., 2015, 27, 27-39.
12. Putluru S.P., Matta M.K., Ahire D., Subramanian M., Sinz M. and Mandlekar S., A novel liquid chromatography tandem mass spectrometry method for the estimation of bilirubin glucuronides and its application to in vitro enzyme assays, Drug Metab. Lett., 2016, 10, 264-269.
13. US DHHS, FDA and CDER. Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, 2001. Available at: http://www/ fda.gov/cder/guidance/index.htm.
14. Matta M.K., Pilli N.R., Inamadugu J.K., Burugula L. and Jillela V.L.N.S.R., Simultaneous quantification of lamivudine, zidovudine and nevirapine in human plasma by liquid chromatography – tandem mass spectrometry and its application to a pharmacokinetic study, Acta Pharm Sin B., 2012, 2, 472-480.
15. Matta M.K., Burugula L., Pilli N.R., Inamadugu J.K. and Jillela V.L.N.S.R., A novel LCMS/MS method for simultaneous quantification of tenofovir and lamivudine in humanplas ma and its application to a pharmacokinetic study, Biomed. Chromatogr., 2012, 26, 1202-1209.
16. Adireddy V., Kovvasu S.P.R. and Ravella V.N., Simple and rapid LC-MS/MS method for the determination of rasagiline in human plasma, Eur. J. Biomed. Pharmaceu. Sci., 2017, 4, 695-699.
17. Gandla K., Repudi L., Kovvasu S.P.R. and Rao R.N., Simple and rapid determination of rosuvastatin in human plasma by LC-MS/MS, World J. Pharm. Pharmaceu. Sci., 2017, 6, 1027-1037.
18. Janjanam K.C., Bimireddy B.P.K., Kovvasu S.P.R. and Ratna J.V., Bioanalysis of darunavir in human plasma using liquid chromatography coupled with tandem mass spectrometry, Int. J. PharmTech Res., 2018, 11, 28-34.

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