Journal of Chromatography B, 902 (2012) 132–136

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Metaxalone estimation in biological matrix using high-throughput LC–MS/MS bioanalytical method

Dipanjan Goswami*, Arabinda Saha, Sanjay Gurule, Arshad Khuroo, Tausif Monif, Poonam Vats

Department of Clinical Pharmacology and Pharmacokinetics, Ranbaxy Laboratories Ltd., HSIDC, GP-5, Old Delhi Gurgaon Road, Udyog Vihar Industrial Area, Gurgaon 122 015, Haryana, India

ARTICLE INFO

Article history:
Received 15 March 2012
Accepted 28 May 2012
Available online 4 June 2012

Keywords:
Metaxalone
LC–MS/MS
Method development
Matrix stability
Method validation

ABSTRACT

Metaxalone is a skeletal muscle relaxant, an approved drug for pain relief. Published bioanalytical methods lacked detailed stability evaluation in blood and plasma. An accurate, precise, high-throughput tandem mass spectroscopic method has been developed and validated. Following solid phase extraction (SPE), metaxalone and the internal standard metaxalone-d_3 were extracted from an aliquot of 200 μL of human plasma. Chromatographic separation achieved on an Ascentis Express C18 column (50 mm × 4.6 mm i.d., 2.7 μm particle size) with mobile phase is a mixture of 10 mM ammonium acetate buffer (pH 4.5)–methanol–acetonitrile (20:50:30, v/v/v), at an isocratic flow rate of 0.7 mL/min. The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via electrospray ionization (ESI) source. The mass transitions of metaxalone and metaxalone-d_3 were m/z 222.3 → 161.2 and m/z 225.3 → 163.3, respectively. The linear calibration curves were obtained in the concentration range of 0.105–10.081 μg/mL ($r^2 \geq 0.99$) with a lower limit of quantification (LLOQ) of 0.105 μg/mL. The intra- and inter-day precisions and relative error were all within 6%. Despite achieving high mean recovery (>78%), no interference peaks or matrix effects were observed. Detailed stability exercises including drug stability in blood, hemolyzed, lipemic and normal plasma were conducted to extend the method applicability in vast majority of clinical studies using 800 mg metaxalone extended release oral dosage form.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Metaxalone (Skelaxin; 5-[(3,5-dimethyl phenoxy) methyl]-2-oxazolidinone), a centrally acting skeletal muscle relaxant, is used along with rest and physical therapy, to treat injuries and other painful muscular conditions [1,2]. Metaxalone has no direct action on the contractile mechanism of sustained muscle, the motor end plate, or the nerve fiber [3,4]. The pharmacokinetic data for metaxalone are interesting. As compared to fasted conditions, the presence of a high fat meal at the time of drug administration increased metaxalone C_{max} by 177.5% and AUC (AUC_{0-t}, $AUC_{0-\infty}$) by 123.5% and 115.4% respectively. Time-to-peak concentration (T_{max}) was also delayed (4.3 h versus 3.3 h) and terminal half-life was decreased (2.4 h versus 9.0 h) under fed conditions compared to fasted condition [4–7].

There are very limited data on bioanalytical method application to clinical studies using 800 mg metaxalone extended release tablet [8]. Savard et al. reported a tedious liquid–liquid extraction method of metaxalone using LC–MS/MS technique but complete validation data were unavailable to support method application [9]. Nirogi et al. also reported liquid–liquid extraction technique to determine metaxalone in plasma by using LC–MS/MS principle, yet matrix effect using hemolyzed and lipemic plasma and stability of metaxalone in blood remained un-addressed during validation performance [10]. Moreover, there is no reported method where metaxalone recovery was achieved higher than 75% to the best of knowledge of authors. A reliable selective high-throughput method, for estimation of metaxalone in plasma using metaxalone-d_3 as an internal standard (IS), therefore, is described in this paper. The dynamic linearity ranges, high extraction recovery, validation exercises confirming stability in blood and plasma samples are discussed. The method has been successfully applied to clinical sample analysis.

2. Experimental

2.1. Chemical and materials

Working standards of metaxalone (99.89% purity) and its internal standard metaxalone-d_3 (deuterium labeled metaxalone; 99% purity) had been procured from Clearsynth India and CDN

* Corresponding author. Tel.: +91 124 4231001; fax: +91 124 4231002.
E-mail addresses: dipanjan.goswami@ranbaxy.com (D. Goswami), tausif.monif@ranbaxy.com (T. Monif).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.05.034

Isotopes, Canada, respectively. Ammonium acetate, acetonitrile and methanol were purchased from Qualigens Fine Chemicals (a division of GSK Ltd, Mumbai, India). Oasis HLB (30 mg/1 cm^3) solid phase extraction cartridge was purchased from Waters Corporation (Milford, MA, USA). Water was purified using a Milli-Q device (Millipore, Mosheim Cedex, France). Different individual lots of human plasma containing tri-potassium ethylene diamine tetra acetic acid (K_3EDTA) were collected from Yash Laboratories, Delhi, India.

2.2. LC–MS/MS instrumentation and analytical conditions

Liquid chromatographic separation was performed using Shimadzu scientific instruments (Shimadzu Corporation, Kyoto, Japan) consisting of two LC-20AD delivery pumps, an on-line DGU-20A3 prominence solvent degasser, a SIL-HTc Shimadzu auto sampler and a CBM-20A prominence column oven. Chromatographic separations were achieved on an Ascentis Express C18 column (50 mm × 4.6 mm i.d., 2.7 μm particle size; Supleco) using a mobile phase mixture of 10 mM ammonium acetate buffer (pH 4.5), methanol and acetonitrile (20:50:30, v/v/v), at isocratic flow rate 0.7 mL/min. The column oven and autosampler temperatures were maintained at 35 ± 1 °C and 10 ± 1 °C, respectively.

Samples were analyzed with API-3200 triple quadrupole mass spectrometer (MDS, Sciex®, Foster City, CA, USA) equipped with an electrospray ionization source operating in positive polarity. Unit resolution was applied to both Q1 and Q3. Ultra high purity nitrogen was used as the nebulizer, auxiliary, collision and curtain gases. Analytes were detected by tandem mass spectrometry using MRM of precursor-product ion transitions with 200 ms dwell time, at m/z 222.3/161.2 for metaxalone and 225.3/163.3 for metaxalone-d_3.

The parameters, optimized by infusing solution of metaxalone and metaxalone-d_3 into the mass spectrometer, were as follows: collision activated dissociation gas (CAD), curtain gas (CUR), nebulizer gas (GS1) and heater gas (GS2), 5, 20, 45 and 50 psi, respectively; ion spray voltage, 5500 V; source temperature, 350 °C; declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) were optimized and set at 31, 10, 16 and 14 V, respectively, for both metaxalone and metaxalone-d_3.

Calibration curves were constructed by calculating the analyte to IS peak area ratio (y) against analyte concentrations (x). Data acquisition and processing were performed using Analyst version 1.4.1 software (MDS Sciex, Toronto, Canada).

2.3. Preparation of stock solution, calibration standards and quality control samples

Stock solutions (1 mg/mL) of metaxalone and metaxalone-d_3 were prepared in methanol and were stored in refrigerator (1–10 °C). The working solution of IS (10.0 μg/mL) for routine use was prepared by diluting metaxalone-d_3 stock solution in methanol–water (50:50, v/v) and stored at room temperature. Working aqueous solutions of analyte (ranged 5.250–504.050 μg/mL), prepared in methanol–water (50:50; v/v), was used ensuring 2% spiking to yield spiked calibration standards at eight different concentrations (0.105–10.081 μg/mL) and four concentrations (0.105, 0.247, 3.856 and 7.711 μg/mL) for quality controls. Plasma samples were stored at −50 °C till analysis.

2.4. Sample preparation

Plasma samples retrieved from freezer were thawed at room temperature and 200 μL were aliquoted to polypropylene tubes (13 mm × 50 mm) containing 50 μL of IS dilution (10.0 μg/mL). The samples were vortex mixed and diluted with 200 μL of HPLC-grade water. Diluted samples were then extracted employing Oasis HLB extraction cartridges (30 mg/1 cm^3) using centrifuge system and evaporated to dryness at 20 psi and 50 °C under a stream of dry nitrogen using a Zymark TurboVap LV evaporator (Caliper, Hopkinton, MA, USA). Finally the residue was reconstituted with 500 μL of mobile phase and 10 μL of each sample were injected into the column for analysis.

2.5. Method validation

The method was validated for selectivity, sensitivity, linearity of response, accuracy, precision, recovery, matrix effect and stability of analyte during both short-term sample processing and long-term storage conditions according to FDA guidelines [11].

Normal K_3EDTA human plasma samples from six different individuals, two different lots of hemolyzed K_3EDTA plasma and two different lots of lipemic K_3EDTA were used to evaluate selectivity of the method. Calibration curves based on peak area ratio of analyte to IS were prepared in triplicate and linearity was assessed by least-squares regression with a weighting factor of $1/x^2$. The correlation coefficient $r > 0.98$ was mandatory for all the calibration curves to be accepted. Intra- and inter-day precision and accuracy were determined on the basis of six replicates of QC samples included in each run. The lowest standard concentration on the calibration curve was to be accepted as LLOQ, if the analyte response was at least five times more than that of drug-free (blank) extracted plasma. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with accuracy within ±20% and a precision ≤ 20%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ±15%. Recovery (process efficiency) was determined by measuring the mean peak area response of six replicates of extracted quality control samples against the mean peak area response of neat aqueous solutions. Matrix factor (MF) was evaluated by comparing the analyte peak area in post-extracted blank matrix samples against the analyte peak area in neat aqueous sample (reference solution), whereas IS normalized matrix factor was determined by comparing the peak area ratio of analyte/IS in post-extracted blank samples against the mean peak area ratio of analyte/IS in reference solution. Matrix effect was assayed at two concentration levels (low and high quality control concentrations). The matrix effect is nullified if the accuracy is within ±15% and precision is ≤15% at the low and high QC concentrations.

Stability of metaxalone in matrix was examined at low and high QC levels by analyzing four replicates of QC samples against freshly spiked calibration standards. The stability data from various exercises, e.g., autosampler stability (ASS), bench-top stability (BTS) in plasma, freeze/thaw stability (FTS) and long-term stability (LTS) were evaluated as per regulatory guidelines. For sample collection and handling stability, fresh human K_3EDTA whole blood was spiked with the metaxalone at the low and high QC levels (0.247 and 7.711 μg/mL). This spiked whole blood sample was split into two aliquots (A and B). Aliquot A was placed for 10 min at room temperature, centrifuged at 4 °C and the resulting plasma was used as comparison sample. Aliquot B was kept at room temperature for 120 min, centrifuged at 4 °C and the resulting plasma (stability sample) was analyzed with the comparison sample in the same batch to access the percentage of change during the sample collection process.

3. Result and discussions

3.1. Optimization of LC–MS parameters

In ESI source, analyte and internal standard formed protonated molecules $[M+H]^+$ under acidic condition due to the addition

Table 1
Intra-day and inter-day relative error (RE) and precision for metaxalone.

Nominal concentration (μg/mL)	Intra-day (n = 6)				Inter-day (n = 18)			
	Mean	S.D. (±)	%RE	%CV	Mean	S.D. (±)	%RE	%CV
0.105	0.1042	0.00223	−0.8	2.1	0.1058	0.00366	0.7	3.5
0.247	0.2497	0.01467	1.1	5.9	0.2537	0.01016	2.7	4.0
3.856	4.0010	0.04637	3.8	1.2	3.9770	0.08574	3.1	2.2
7.711	7.8750	0.07062	2.1	0.9	7.9016	0.13518	2.5	1.7

of proton to the nitrogen atom of oxazolidine ring system. Several fragment ions were observed in the product ion spectra of both analyte as well as internal standard. Fragment ion 161.2 was selected for metaxalone and 163.3 for metaxalone-d_3 as these ions are abundant, selective and produced stable response. MSMS spectra for metaxalone-d_3 showed two product ions 163.3 and 164.3, and the most stable and intense product ion is 163.3. This could be due to H/D-exchange in isotope-labeled compound (especially featured in d_3-labeled compounds). Capillary temperature and ion spray voltage significantly influenced the MS behavior of analyte as well as its deuterated internal standard and was best suited at 350 °C and 5500 V, respectively. To improve peak shape dwell time was set 200 ms and found to be critical for the analysis.

After optimizing mass parameters liquid chromatographic conditions were tuned. Ascentis Express-C18 was selected after trying other C18 columns since it provided good peak shape and high intensity with greater signal to noise ratio (S/N) when acetonitrile in combination with methanol was used as organic component in mobile phase. Analyte and IS responses in presence of ammonium acetate were optimum and stable at pH 4.5 (adjusted with glacial acetic acid). The mobile phase consisting of 10 mM ammonium acetate buffer (pH 4.5), methanol and acetonitrile (20:50:30, v/v/v) was used to complete each run within 1.8 min. Cleanest sample was

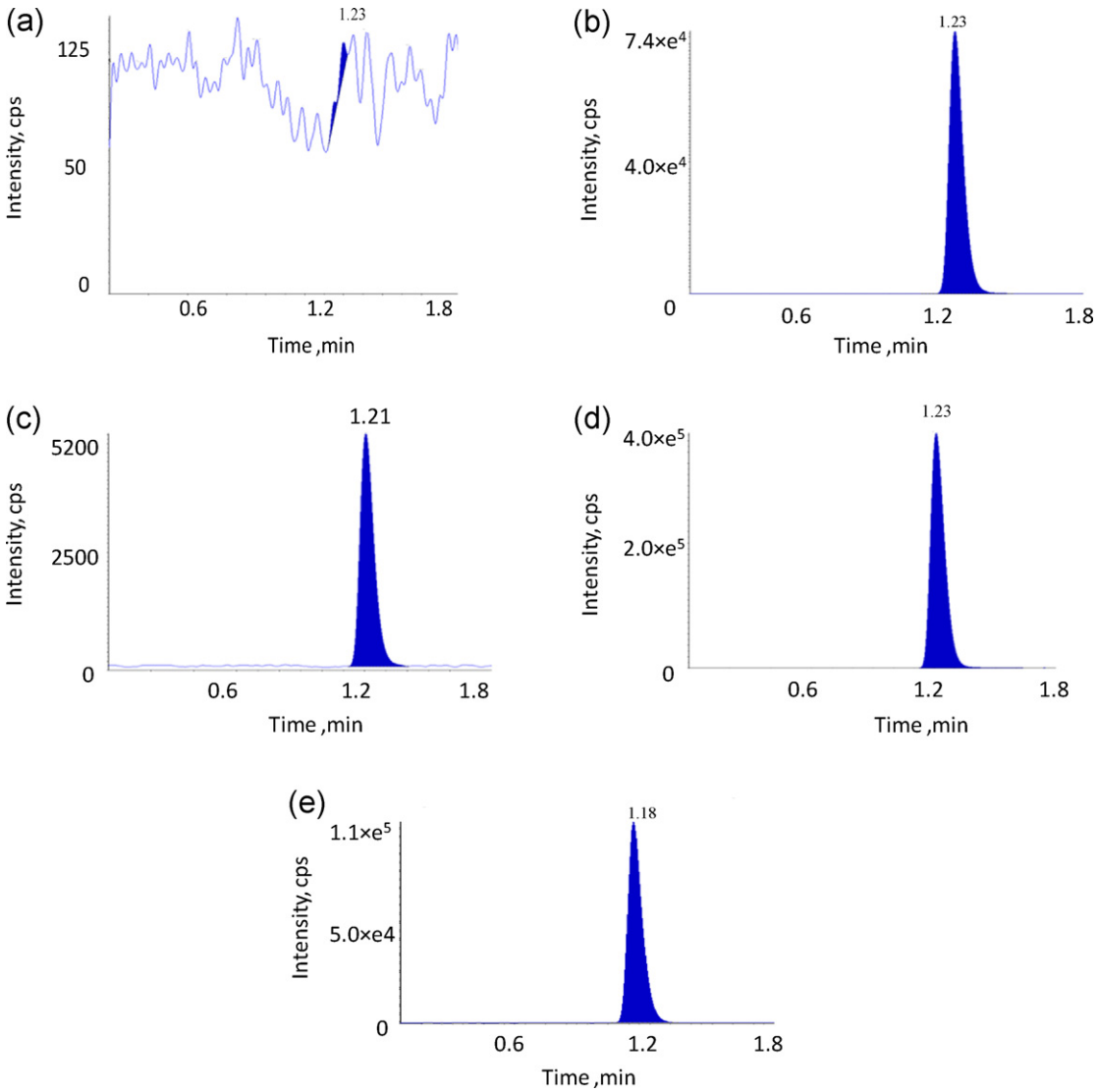

Fig. 1. Chromatograms of (a) blank plasma spiked with IS sample [at RT of metaxalone], (b) blank plasma spiked with IS sample [at RT of metaxalone-d_3], (c) LLOQ, (d) ULOQ and (e) real subject sample (2.463 μg/mL, after 4.5 h of oral administration).

Table 2
Absolute matrix effect and process efficiency for metaxalone.

QC level	A[a] (%CV)[b]	B[c] (%CV)[b]	C[d] (%CV)[b]	Absolute matrix effect (%ME)[e]	Process efficiency (%PE)[f]
LQC	72,486 (1.4)	71,103 (0.8)	55,000 (6.0)	98.1	75.9
MQC	934,242 (1.7)	925,343 (2.5)	767,425 (5.4)	99.0	82.1
HQC	1,679,026 (0.6)	1,627,332 (4.2)	1,414,439 (4.0)	96.9	84.2

[a] Mean area response of six replicate samples prepared in mobile phase (neat samples).
[b] Coefficient of variation.
[c] Mean area response of six replicate samples prepared by spiking in extracted sample blank plasma.
[d] Mean area response of six replicate samples prepared by spiking before extraction.
[e] $B/A \times 100$.
[f] $C/A \times 100$.

obtained using SPE principle as compared with solvent extraction and protein precipitation technique and recovery improved by 30% using HLB cartridges in comparison to MCX cartridges.

3.2. Validation parameters

The assay was found to be linear for metaxalone in the range 0.105–10.081 μg/mL. The typical regression lines were found: $y = 0.697x + 0.00421$ with a regression coefficient (r^2) of 0.9997 (where y represents the peak area ratio of metaxalone to that of IS and x represents the plasma concentration of metaxalone). As shown in Table 1, intra- and inter-day precision is less than 6% and relative error ranged from −0.8 to 3.8% for metaxalone. Representative chromatograms of blank plasma spiked with IS, lower limit of quantification (LLOQ), upper limit of quantification (ULOQ) and real subject sample are shown in Fig. 1.

The process efficiency (PE) of metaxalone at low, medium and high QC levels was 75.9%, 82.1% and 84.2%, respectively. The mean recovery of IS was 75.5%. Matrix effects were absent as shown by the fact that concentrations of analyte as a percentage of nominal concentrations for metaxalone low and high QC samples were 101.2 ± 2.3% and 98.2 ± 2.3%, respectively. The IS normalized matrix factor at low and high concentrations from six lots of plasma samples was 0.987 and 0.980, respectively. The precisions of absolute MF and IS normalized MF from six lots of plasma samples were ≤1.9%. These results showed that ion suppression or enhancement from the plasma matrix was negligible under the current conditions. Both matrix effect and extraction process efficiency results are presented in Table 2.

The mean calculated concentration of metaxalone in normal blood/plasma as well as in hemolyzed and lipemic plasma is depicted in Fig. 2 (CV ≤ 5% in different types of matrixes at LQC and HQC level). There was no impact of matrix in all cases as observed concentrations of metaxalone were comparable at LQC and HQC level in different matrices. Stability results of metaxalone were as follows: BTS at room temperature for 6.2 h, three freeze/thaw cycles stability, LTS storage at −50 °C for 124 days and ASS for 49.6 h under auto sampler condition (maintained at 10 °C). The working solutions and stock solutions of metaxalone and the IS were also evaluated for stability at room temperature for 8.3 h and at refrigerator temperature (between 1 and 10 °C) for 11 days, respectively. Results of stability tests are represented in Fig. 3, where negligible degradation (accuracy and precision are all within 6%) in plasma could be inferred.

4. Application

The validated bioanalytical method was successfully applied to evaluate the bioavailability of metaxalone reference drug (SKELAXIN® 800 mg tablets; Core Pharma. LLC, USA) in 18 healthy male Indian volunteers (age: 26.5 ± 2.0 years; mean weight: 65.4 ± 7.2 kg) using two doses of IR innovator Skelaxin 800 mg, administered in six hourly manner. All volunteers signed informed consent after protocol was discussed and had normal examination and clinical laboratory test results. Selection was based on the fact that all participants were not on any medications for at least 2 weeks prior to and during the period of the study. The study was approved by ethics committee of Institutional Review

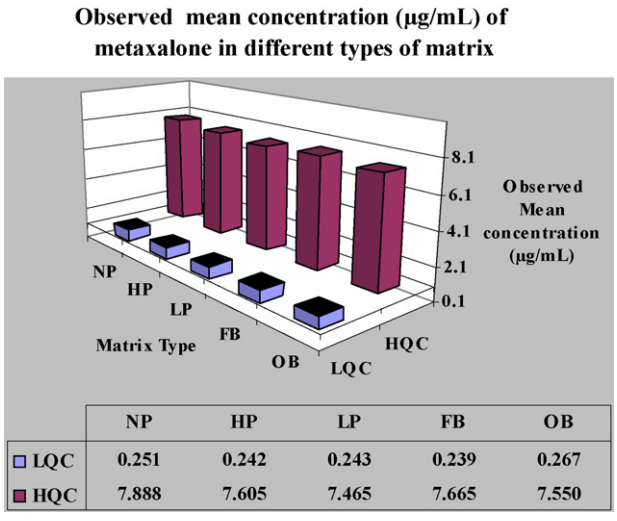

Fig. 2. A comparative analysis based on observed mean concentration of metaxalone in different type of matrices (K_3EDTA normal plasma, NP; K_3EDTA hemolyzed plasma, HP; K_3EDTA lipemic plasma, LP; plasma separated from fresh whole blood, FB; plasma separated after 2.2 h from whole blood, OB).

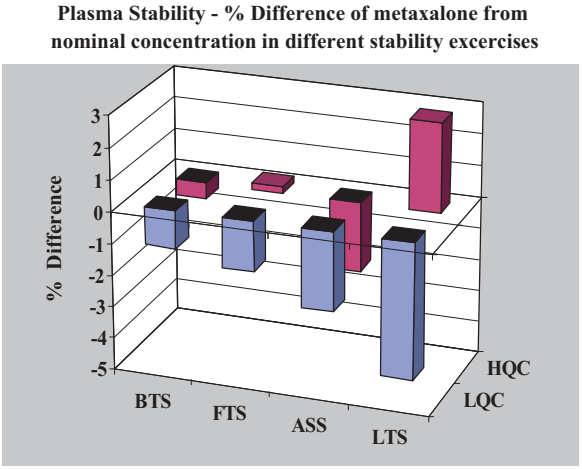

Fig. 3. Plasma stability calculation from mean QC concentrations ($n = 4$): comparative analysis from bench top stability (BTS), freeze/thaw stability (FTS), auto sampler stability (ASS) and long term stability (LTS) exercises performed during validation.

Board at Majeedia hospital (New Delhi, India). In this study after an overnight fast of at least 10.0 h, all volunteers singly orally dosed with the assigned tablets were served with high-fat high calorie breakfast (after 45 min of dosing). Blood samples (4 mL) were drawn just before and at 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0, 12.0, 14.0, 16.0, 18.0, 24.0, 30.0, 36.0, and 48.0 h after drug administration. Samples were centrifuged at 4000 rpm for 15 min and separated plasma samples were stored at −50 °C until analysis. The clinical samples from 18 volunteers were analyzed using the current method and the results showed that for innovator product C_{max} was 3.246 μg/mL, $AUC_{0-\infty}$ was 30.170 μg hr/mL and AUC_{0-12} was 16.922 μg hr/mL. These data are very helpful for conducting bioavailability and bioequivalence studies for formulation dosage of 800 mg or twice dosing in 6 hourly manner.

5. Conclusion

The developed LC–MS/MS method for metaxalone is selective, rugged and suitable for fast measurement of routine subject samples. This method has significant advantages in terms of clean and reproducible SPE extraction procedure and a short chromatographic run time of 1.8 min. The extraction method achieved consistent high recoveries for analyte and IS from human plasma, without any matrix interference or ion suppression though matrices range has been extended in current research. These advantages would make it efficient for the analysis of large number of plasma samples obtained from exploratory pharmacokinetics study with Skelaxin® 800 mg metaxalone tablets.

Acknowledgement

The authors wish to acknowledge the support and facilities received from Ranbaxy Research Laboratories, Gurgaon, India, for carrying out this work.

References

[1] J.K. Elenbass, Am. J. Hosp. Pharm. 37 (1980) 1313.
[2] R.N. Harden, C. Argoff, J. Back Musculoskelet. Rehabil. 15 (2000) 63.
[3] P.P. Toth, J. Urtis, Clin. Ther. 26 (2004) 1355.
[4] T. Bosworth, Prim. Care Spec. 6 (2002) 1.
[5] K.A. Moore, B. Levine, D. Fowler, Forensic Sci. Int. 149 (2005) 249.
[6] J.L. Poklis, J.D. Ropero-Miller, D. Garside, R.E. Winecker, J. Anal. Toxicol. 28 (2004) 537.
[7] A.C. Gruszecki, S. Kloda, G.T. Simmons, T.M. Daly, R.W. Hardy, C.A. Robinson, J. Forensic Sci. 48 (2003) 432.
[8] M. Jemal, Biomed. Chromatogr. 14 (2000) 422.
[9] C. Savard, G. Havard, M. Théberge, J. Couture, F. Vallée, AAPS, http://www.aapsj.org/abstracts/AM_2006/staged/AAPS2006-000872.PDF.
[10] R.V. Nirogi, V.N. Kandikere, M. Shukla, K. Mudigonda, W. Shrivastav, P.V. Datla, J. Anal. Toxicol. 30 (2006) 245.
[11] (a) http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf (last accessed May 2011);
(b) http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500018062 (last accessed May 2011).