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Original Article

Development and Validation of Analytical Method for Simultaneous Estimation of Bupivacaine and Meloxicam in Human Plasma Using UPLC-MS/MS

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ABSTRACT

Bupivacaine HCl (BP) and Meloxicam (ME) are used as intra-operative local anesthesia and non-steroidal anti-inflammatory drugs respectively. A simple, rapid, selective and sensitive ultra pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been developed and validated for simultaneous quantification of BP and ME in human plasma. By following a simple protein precipitation with solvent mixture containing Water:Acetonitrile:Formic Acid (76:24:0.1,v/v/v), the analytes and internal standards were separated on Acquity HSS T3 column (2.1 x 50 mm, 1.8 µm). Chromatographic Mobile phase A consist of 10 mM Ammonium Formate and Mobile phase B consist of a mixture of Acetonitrile:water:Formic acid (96:5:0.2, v/v/v). Bupivacaine-d_9 (BUd_9) used as internal standard for BP and $^{13}C,d_3$ Meloxicam ($ME^{13}cd_3$) used for ME. The concentration of BP and ME were determined by gradient UPLC-MS/MS in Multiple reaction mode (MRM). The mass transition ion-pair for BP was m/z 289.3→140.0 and BUd_9 was 298.3→ 149.0, for ME was m/z 352.1→115.0 and $ME^{13}cd_3$ was 356.1→115.0, respectively. The proposed method was linear in the range 10 - 4500 ng/mL. The intra and inter-run precision values are within 6.8 % at a lower limit of quantization level. The overall recovery for both analytes found 98%. The total run time of the method is 2.5 min.

Key words: Human plasma, UPLC-MS/MS, Bupivacaine, Meloxicam, Liquid Chromatography.

Key Message: For the first time a new, rapid method is developed for simultaneous estimation of Bupivicaine and Meloxicam in human Plasma using UPLC-MS/MS as separation technique.

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INTRODUCTION

Bupivacaine HCl[1] is named as 1-butyl-N-(2, 6-dimethylphenyl) piperidine-2-carboxamide; hydrochloride Figure 1 is a long acting amide local anesthetic. Bupivacaine (BP) is utilized for intraoperative local anesthesia, post operative analgesia and in the treatment of chronic pain. BP is widely used in obstetrics. In lumbar epidural anesthesia, the drug appears innocuous to mother and fetus. Systemic toxic reactions, especially cardiac arrhythmias and cerebral convulsions, may occur during BP epidural analgesia because of fast absorption leading to high plasma peak levels. After injection of Bupivacaine Hydrochloride for caudal, epidural, or peripheral nerve block in man, peak levels of Bupivacaine in the blood are reached in 30 to 45 min, followed by a decline to insignificant levels during the next three to 6h.[2] The most commonly reported bupivacaine metabolites have been desbutylbupivacaine, 3'- hydroxybupivacaine, and 4'-hydroxybupivacaine.[3] The pKa[4] value for BP is 8.09. The chemical structure of BP is shown in Figure 1.

Meloxicam (ME) is named 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, belongs to the class of nonsteroidal anti-inflammatory drug category (NSAID). ME has low ulcerogenic potency and gastric irritation when compared with NSAID.[5] ME binds strongly to serum albumin (>99%) and reaches a maximum concentration at 4.5h after oral administration. ME is metabolized extensively in the liver into four pharmacologically inactive metabolites that are excreted through urine and feces[6] with absolute bioavailability of 89%.[7] The pKa[4] value for ME is 4.08. The chemical structure of ME is shown in Figure 1.

Combination of Anesthetic and Anti-inflammatory drug therapy in post operative pain medication is the most interested topic in Generic pharmaceutical industry, hence author believes that the simulations estimation method of these drugs in plasma samples would contribute greatly to pharmaceutical industry. In literature survey for estimation of Bupivacaine in plasma, several methods were proposed, such as HPLC,[8-12] by GC-MS[13] Capillary electrophoresis[14] and by LC-MS.[15] For estimation of Meloxicam in plasma several methods were proposed, such as HPLC.[16-20] and by LC-MS.[21-23] As far as we are aware there is no simultaneous UPLC-MS/MS method for estimation of Bupivacaine and Meloxicam in human plasma samples. For measuring Bupivacaine plasma concentra-

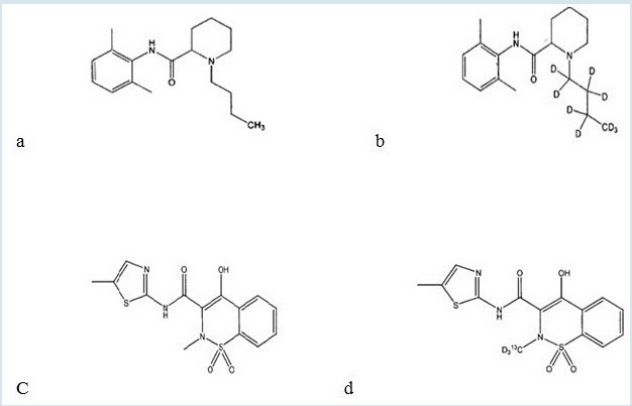

Figure 1: Chemical structures of compounds; a) Bupivacaine b) Bupivacaine-d9 c) Meloxicam d)^{13} c,d_3 Meloxicam.

tions, especially in the later phase of elimination, a highly sensitive assay is required that is affected by neither plasma components nor other drugs given simultaneously.

Our present work describes a simple, novel, rapid, sensitive and selective method for simultaneous determination of Bupivacaine and Meloxicam by advanced UPLC-MS/MS equipment. The current method offers many advantages i.e. shorter runtime, less sample volume and can estimate both compounds in a single run. In the current method Bupivacaine-d9 (BPd_9) and[16] c, d_3 Meloxicam ($ME^{13}cd_3$) were used as internal standards, the chemical structure of BP is shown in Figure 1. This method can be applied to pharmacokinetic study of plasma BP and ME concentration after oral administration.

EXPERIMENTAL

Chemicals and reagents

High purity water was obtained from Elga purified water system (Germany). Analytical grade Acetonitrile, Formic acid and Dimethyl sufoxide (DMSO) were used and obtained from E. Merck. Lithium-Heparin Plasma was obtained from a local blood blank. The reference standards Bupivacaine, Bupivacaine-d9 (Internal standard) and Meloxicam were procured from TRC Standards (Canada), the[16] c, d_3 Meloxicam (Internal standard) was procured from Chemtos.

Preparation of Standards and Quality control (QC) solutions

Preparation of standard stock solutions

Individual standard stock solutions of BP and ME were prepared by dissolving an accurately weighed quantity of BP and ME in DMSO to get final concentration of 2500 µg/mL. The solutions were further diluted to achieve standard working solutions of desired concentrations.

Preparation of Internal standard stock solutions

Individual standard stock solutions of BPd_9 and $ME^{13}cd_3$ were prepared by dissolving an accurately weighed quantity of BPd_9 and $ME^{13}cd_3$ in DMSO to get final concentration of 100 and 500 µg/mL.

Note 1: The internal standards weighing was carried out under yellow light due to light sensitivity of Bupivacaine and Meloxicam. All standards were stored at -20°C and brought to room temperature before analysis.

Preparations of working solutions, calibration standards (CC) and QC samples

The standard solutions were used to spike in blank plasma samples either for CC or QC standards of BP and ME during the validation. Calibration standards were prepared in plasma with the concentrations of 10, 20, 50, 150, 500, 1500, 4500 and 5000 ng/mL. QC samples were prepared with blank plasma at LLOQ, low, medium and high concentrations of 10, 30, 250, 2500 and 4000 ng/mL.

Preparation of Plasma Samples

Standards, QC solutions, Blank matrix and system suitability test (SST) solutions were thawed and vortex-mixed for approximately one minute before pipetting. The calibration standards were prepared in preparation tubes by adding 50 µL working standard solutions at each concentration. 50 µL of pooled human plasma was added into the Blank, Blank+IS and carry over Blank tubes. 50 µL of QC samples and SST samples were added to respective tubes. 50 µL of IS was added into the Blank+IS, Calibration standard and QC tubes. 50 µL of IS solvent added to the blank and carryover blank. Then samples were treated with 50 µL of solvent mixture contains Acetonitrile: Water: Formic Acid (50:50:0.1, v/v/v) and were added to the blank and carryover blank tubes. The tubes were capped and centrifuged for about one minute at 1000 rpm. The tubes were vortex-mixed for approximately one minute at high speed.

Protein precipitation Extraction procedure

500µL of Acetonitrile was added to all the tubes, capped and vortex-mixed for approximately five minutes. The tubes were centrifuged for approximately five minutes at 3000 rpm, and then the tubes were placed in Methanol and Dry ice mixture. A 50 µL of supernatant was transferred into each tube, 350 µL of solvent mixture Water: Acetonitrile: Formic Acid (76: 24:0.1, v/v/v) was added into all the tubes, vortex-mixed for approximately two minutes, the tubes were centrifuged for approximately 2 minutes at 3000 rpm.

Note 2: In each quantitative analysis run a "Blank+IS" sample is included to monitor any contribution from the IS to the analyte or Vice Versa.

Ultra Pressure Liquid Chromatography (UPLC) Equipment and Conditions

Chromatographic separation was carried out by using an Acquity UPLC with HSS T3 column (Dimensions: 50 mm x 2.1 mm i.d., 1.8 µm). The sample injection volume was used as 5 µL. Mobile phase-A consists of Ammonium Formate (pH 2.4; 10 mM) and Mobile phase-B consists a mixture of Acetonitrile: water: Formic acid (96:5:0.2, v/v/v), and the mobile phase delivered at 0.5 mL/min flow rate. The total run time for each sample analysis is 2.5 min. Chromatographic Gradient program used is shown in Table 1.

Table 1: Gradient program

Time (min)	% Mobile Phase-A	% Mobile Phase-B
0	70	30
0.2	70	30
0.75	5	95
1.90	5	95
2.00	70	30
2.50	70	30

Mass Spectrometric Conditions

The mass spectrometry was performed using as API 4000 triple quadrupole mass spectrophotometer equipped with an electrospray ion (ESI) source. The mass spectrometer was operated in multiple reaction monitoring (MRM) in positive mode. The detection parameters are listed in Table 2. Method of least squares with weighting $1/X^2$ was used to calculate the peak area ratios of the target ions of the drugs to those of the IS and the calibration curve was constructed by plotting peak area ratios of BP and ME against its concentrations.

VALIDATION

The method validation for simultaneous estimation of BP and ME in plasma samples was performed in accordance with food and drug administration (FDA), European Medicines Agency and other guidelines for bioanalytical method validation.[24-26] The method was validated to check selectivity, linearity, precision, accuracy, recovery, and stability. Selectivity was performed by control concentration for BP and MEs and at one concentration for IS. The % recovery was evaluated by comparing the peak area ratios of extracted analytes to the peak areas of non-extracted standards.

The assessment of the matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the analyte ionization) constitutes

an important and integral part of validation for quantitative UPLC-MS/MS method for supporting pharmacokinetic studies. It was performed by processing six lots of different, normal controlled plasma samples in six replicates (n=6). Lower quality control (LQC) and higher quality control (HQC) working solutions were spiked with the post extraction solution. The co-efficient of variation (% C.V.) for six values at each level was calculated to test for interference at the retention times of BP, ME and IS.

The intra-and Inter-run accuracy were determined by replicate analysis of the three quality control levels along with the LLOQ and HQC levels. In each precision and accuracy batches, six replicates at each quality control level inclusive of the LLOQ and HQC levels were analyzed.

Assay precision was calculated by using the formula % C.V. = (SD / M) x 100, where M is the mean of the experimentally determined concentrations and SD is the standard deviation of M. Accuracy was defined as the percent relative error (% RE) and was calculated using the formula % RE = (E-T) x 100 / T, where E is the experimentally determined concentration and T is the theoretical concentration.

The extraction efficiencies of BP, ME and IS were determined from the analysis of six replicates at low, medium and high quality value obtained by injecting the post extracted samples prepared in duplicate from each plasma lot. The % C.V. of the area ratios of the post spiked recovery samples at LQC and HQC levels were within the acceptable limit (±15%) at each level tested for the analyte and within ±20% of the IS. Moreover, the minor enhancement of analyte signal due to endogenous matrix interferences does not affect the quantification of analyte and IS peak, which was confirmed by the post column infusion techniques.

As a part of method validation, stability was evaluated. Analytes were considered stable if the recovery of the mean test responses were within 15% of appropriate controls. Analytes were tested using the QC samples temperature of about 25°C (bench top stability) was evaluated for 6 h. The processed sample solution stability was studied by comparing the extracted plasma samples that were injected immediately (time 0h) and re-injected after 24 h by keeping in the auto sampler at 4°C. The freeze thaw stability was conducted by comparing the stability of the samples which was been frozen and thawed three times with freshly spiked QC samples. Six aliquots of each low and high concentration were used for the freeze thaw stability evaluation. For long term stability six replicates of LQC and HQC samples were spiked in plasma, kept at -20°c for 120 days, thawed to room temperature, and analyzed. Stock solution stability was performed in DMSO by comparing the areas obtained from aqueous working solutions which were kept for 6 h and up to 30 days at -20°C with freshly prepared working solutions prepared at the middle quality control (MQC) level.

RESULTS AND DISCUSSION

Method development and optimization

During literature Survey there was no UPLC-MS/MS method to estimate BP and ME simultaneously. The present work was aimed to develop and validate a simple, novel, rapid and sensitive method for extraction and quantitation of BP and ME simultaneously for its pharmacokinetics studies. To achieve this, during development, different trails were carried out to optimize sample extraction, detection parameters and chromatography. Based on solubility properties both the anlytes and its internal standards were found soluble in DMSO, hence DMSO was selected as a solvent to dissolve the standards and internal standards. After trying different individual solvents and mixtures, the extraction solvent mixture "Water: Acetonitrile: Formic Acid (76: 24:0.1, v/v/v)" was found to be most reproducible and gave less variation between batch analysis. To prevent from any ionization during chromatographic separation, based on the pKa values of analytes, the mobile pH was selected as 2.4. The gradient mobile phases with mixture of Ammonium Formate, Milli-Q-water, Acetonitrile and Formic acid resulted in improved response. The matrix effect was determined by post column infusion technique. There was no ion suppression or enhancement observed at the RT of analytes and internal standards. Different UPLC columns were tried to reduce the run time and flow rates, and Acquity UPLC HSS T3 (50 mm x 2.1mm, 1.8µm) column found suitable. The RT of BP, BPd_9, ME and $ME^{13}cd_3$ found 0.96, 0.95, 1.45 and 1.44 min respectively (Figure 4 and 5) Selectivity.

No interference peaks of endogenous compounds were observed at RT of anlaytes and IS in blank Lithium-Heparin human plasma from six different lots. The typical chromatogram obtained for Blank Plasma Figure 2 and 3, and plasma spiked with LLOQ for BP and BPd_9 and ME and $ME^{13}cd_3$ are represented in Figure 4 and 5. Similarly a typical chromatogram obtained for blank plasma and its nominal concentration for 'IS' i.e. BPd_9 and $ME^{13}cd_3$ represented in Figure 6 and 7.

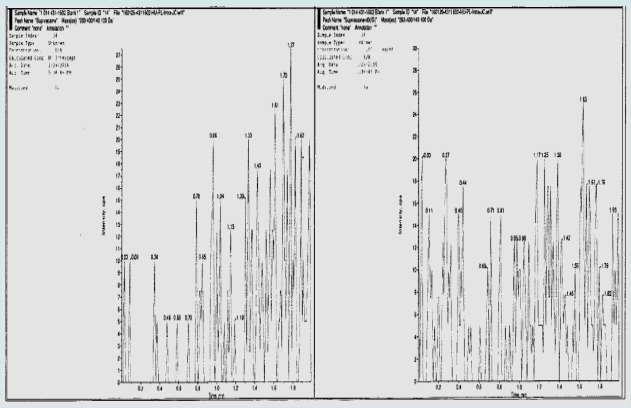

Figure 2: Typical chromatogram for Blank plasma for BP and BPd_9.

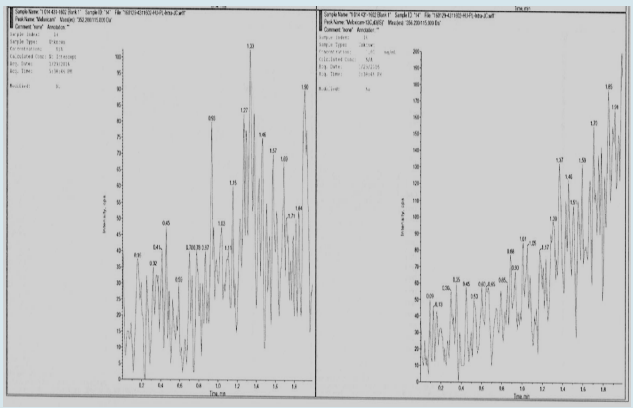

Figure 3: Typical chromatogram for Blank plasma for ME and $ME^{13}cd3$.

Table 2: Ion source and MRM parameters

Ion source				
Ion mode	Electrospray +			
Source temperature	500 °C			
Curtain gas(CUR)	35 psi (Nitrogen)			
Collision gas(CAD)	6 psi (Nitrogen)			
Ion spray voltage(IS)	5500 V			
Entrance potential (EP)	10 V			
Scan duration	2 min			
Ion source gas 1	50 psi (Nitrogen)			
Ion source gas 2	50 psi (Nitrogen)			
MRM parameters	BP	BPd9	ME	$ME^{13}cd_3$
Precursor ion (m/z)	289.3	298.3	352.1	356.1
Product ion (m/z)	140.0	149.0	115.0	115.0
Collision energy(eV)	65.0	65.0	33.0	33.0
Declustering Potential (V)	94.0	94.0	90.0	90.0
Collision exit potential (V)	10.0	10.0	8.0	8.0

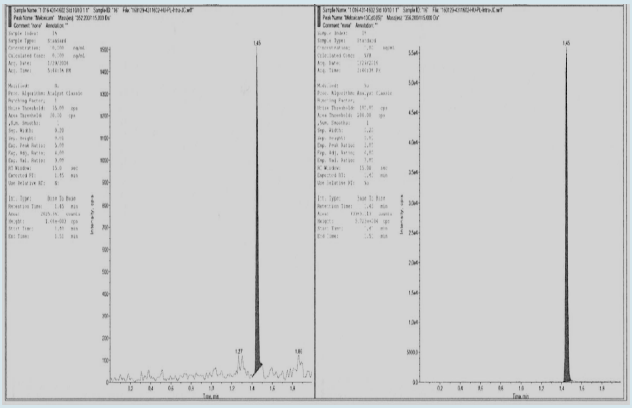

Figure 5: Typical chromatogram for ME and $ME^{13}cd_3$ at LLOQ.

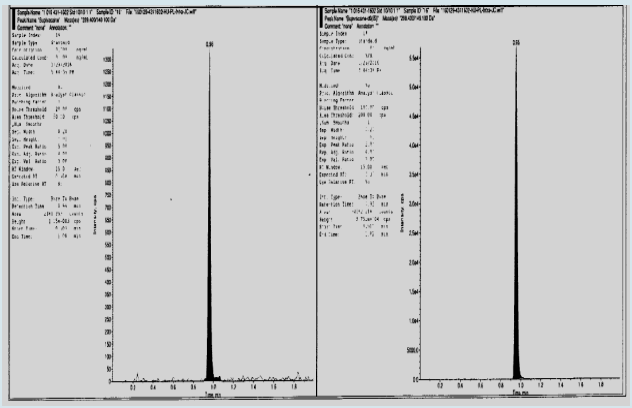

Figure 4: Typical chromatogram for BP with BPd_9 at LLOQ.

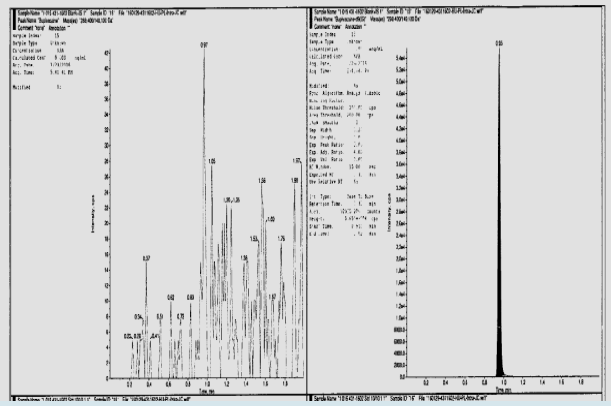

Figure 6: Typical chromatogram for Blank with IS (BPd_9)

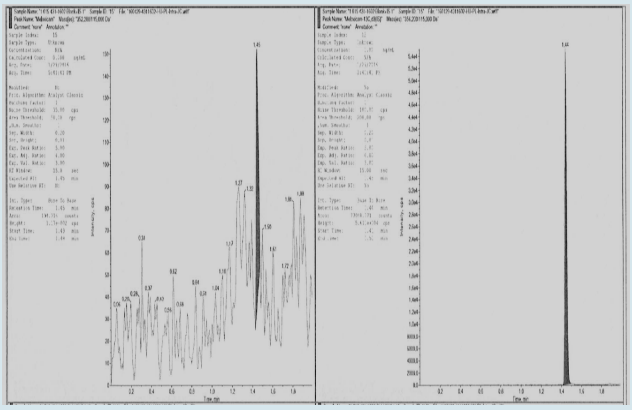

Figure 7: Typical chromatogram for Blank plasma with IS ($ME^{13}cd_3$).

Linearity

The peak area ratios for CC standards were proportional to the concentration of analyte in each assay over the nominal concentration range of 10-5000 ng/mL for both BP and ME. The calibration curves showed linear and were well explained by least squares lines, a weighting factor on $1/X^2$ was chosen to achieve homogeneity of variance. The correlation coefficients were greater than 0.99 for both BP and ME. Across the eight points taken as calibration standards the % C.V. obtained over five batches are less than 3.6 for BP Table 3. And 4.6 for ME Table 4.

Lowest limit of quantitation (LLOQ)

The LLOQ is defined as the lowest concentration that could be analyzed with acceptable accuracy and precision of 20%. The LLOQ of BP and ME was found to be 10.024 and 10.016 ng/mL respectively.

Precision and Accuracy

Across all the four levels tested, the intra-run precision at LQC level was less than 4.2% for BP and 4.5% for ME where as at LLOQ level it was 5.9% for BP and 5.8% for ME and the accuracy was within the range of 95.95% -103.36% for BP and 97.07% -104.08% % for ME Table 5. The

Table 3: Results of Linearity (BP)

Parameters	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8
Actual concentration (ng/mL)	10	20	50	150	500	1500	4500	5000
Calculated concentration (ng/mL)								
1	9.78	20.05	47.45	154	514	1500	4440	4870
2	9.98	19.10	49.55	152	530	1470	4470	4815
3	9.56	20.80	48.10	155	531	1490	4345	4675
4	9.78	19.85	49.30	156	502	1525	4095	4940
5	9.81	19.65	49.75	150	512	1510	4340	4945
Mean	9.78	19.89	48.83	153	517	1499	4338	4849
SD	0.15	0.62	1.00	2.41	12.59	20.74	147.42	111.10
% C.V.	1.5	3.1	2.1	1.6	2.4	1.4	3.4	2.3
% RE(±)	2.2	0.5	2.3	2.3	3.5	0.1	3.6	3.0
% Nominal	97.8	99.5	97.7	102.3	103.5	99.9	96.4	97.0

Table 4: Results of Linearity (ME)

Parameters	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8
Actual concentration (ng/mL)	10	20	50	150	500	1500	4500	5000
Calculated concentration (ng/mL)								
1	9.67	21.25	53.65	152	504	1485	4300	4795
2	10.16	20.80	50.25	152	516	1445	4385	5125
3	10.08	20.35	48.35	155	529	1500	4515	4665
4	9.75	20.80	53.00	154	519	1475	4415	4600
5	9.99	20.2	50.95	162	505	1495	4095	5010
Mean	9.93	20.68	51.24	155	514	1480	4342	4839
SD	0.21	0.42	2.140	3.95	10.47	21.79	158.02	224.81
% C.V.	2.1	2.0	4.2	2.5	2.0	1.5	3.6	4.6
% RE(±)	0.7	3.4	2.5	3.2	2.9	1.3	3.5	3.2
% Nominal	99.3	103.4	102.5	103.2	102.9	98.7	96.5	96.8

Table 5: Accuracy and Precision (Intraday) results in human plasma (n=6)

Parameters	LLOQ		LQC		MQC		HQC	
	BP	ME	BP	ME	BP	ME	BP	ME
Nominal Conc.(ng/mL)	10	10	250	250	2500	2500	4000	4000
Mean	9.33	9.24	254	255	2496	2481	3898	3825
SD(±)	0.548	0.533	10.759	11.472	54.850	56.166	87.446	90.994
% C.V.	5.9	5.8	4.2	4.5	2.2	2.3	2.2	2.4
%RE(±)	6.7	7.6	1.7	2.0	0.2	0.8	2.6	4.4
% Nominal	95.95	103.28	100.07	104.08	103.36	101.01	100.75	97.07

Table 6: Accuracy and Precision (Interday) results in human plasma (n=18).

Parameters	LLOQ		LQC		MQC		HQC	
	BP	ME	BP	ME	BP	ME	BP	ME
Nominal Conc.(ng/mL)	10	10	250	250	2500	2500	4000	4000
Mean	9.42	9.50	252	255	2485	2487	3884	3843
SD(±)	0.553	0.642	8.117	12.019	58.169	54.059	86.201	88.310
% C.V.	5.9	6.8	3.2	4.7	2.3	2.2	2.2	2.3
%RE(±)	5.8	5.0	0.9	2.0	0.6	0.5	2.9	3.9
% Nominal	94.22	95.04	100.93	102.04	99.38	99.47	97.11	96.08

inter-run precision and accuracy were determined by pooling all individual assay results of replicate (n=18) QC samples over separate batch runs. The inter-run precision was less than 3.2% for BP and 4.7% for ME at LQC where as at the LLOQ it is 5.9% for BP and 6.8% for ME. The inter-run accuracy was within the range of 94.22% -100.93% for BP and 95.04% -102.04% for ME Table 6.

Matrix Effect

The matrix study was performed by analyzing LQC, HQC samples. Average matrix factor (MF) values (MF=Response of post spiked concentration / Response of neat sample concentration) obtained at LQC and HQC respectively for BP was + 1.08 and 1.01 and for ME was + 1.24 and 1.19, where as the MF for 'IS' is + 1.0.

Recovery

Accuracy of an analytical method describes the closeness of test results obtained to the true value. Accuracy was determined by replicate analysis of samples containing known amounts of anlaytes and expressed as percent. Six replicates at LQC, MQC and HQC concentrations for BP and ME were prepared for recovery determination. The mean recovery for BP and ME was 97.9 % and 98.2 %, respectively. The % CV obtained was not more than 2.5% for both compounds.

Stability

The bench top and process stabilities for BP and ME were performed at LQC and HQC levels. The obtained results revealed that BP and ME were stable in plasma for 24 h at room temperature and 120 hr at 4ºC. It was confirmed that repeated freeze thawing (three cycles) of plasma spiked with BP and ME at LQC and HQC level did not affect the stability of analytes. The long-term stability results also indicated that the analytes were stable in the matrix up to 120 days at the storage temperature of -70°C. Study on working solution stability proved that they were stable for 6hr at room temperature and stable for 30 days at -20ºC.

CONCLUSION

A novel, simple, selective, rapid, and sensitive UPLC-MS/MS method for the simultaneous determination of BP and ME in human plasma has been developed. The present method is advantageous with respect to simultaneous estimation, shorter run time (2.5 min) and less mobile phase consumption (Flow rate at 0.5 mL/Min). As developed method is cost and time effective, it is concluded that it can be applied for the routine pharmacokinetic evaluation of BP and ME in human subjects after oral administration of the same.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

ABBREVIATION USED

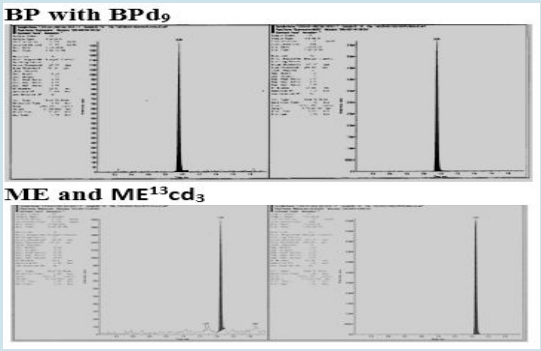
ML: Milli Liter; **µL:** Micro Liter; **Min:** Minutes; **µg:** Micro gram; **Ng:** Nano Gram; **%:** Percentage; **RSD:** Relative Standard Deviation; **n:** Number; **ICH:** International council for Harmonization; **ºC:** Degree Centigrade; **LLOQ:** Lower Limit of Quantitation; **Conc.:** Concentration; **CV:** Coefficient of Variation; **Hr:** Hour.

REFERENCES

1. Babst CR, Gilling BN. Bupivacaine a Review. Anesthesia progress, 1978;25(3):87-91.
2. home page of Drugs.com, (cited on 10.01.17): Available from; https://www.drugs.com/pro/bupivacaine.html
3. Schieferecke MA, McLaughlin KJ, Faibushevich AA, Lunte CE. Determination of bupivacaine and three of its metabolites in rat urine by capillary electrophoresis. Electrophoresis. 1998;19(16-17):2997-3002.
4. National center for Biotechnology information, (cited on 10.01.17): Available from; https://www.ncbi.nlm.nih.gov/pccompound,
5. Engelhardt G, Homma D, Schlegel K, Schnitzler CHR. Anti-Inflammatory, Analgesic, Antipyretic and Related properties of Meloxicam, a new Non-steroidal Anti-Inflammatory Agent, Inflamm Res. 1995;44(10):423-33.
6. Schmid J, Busch U, Heinzel G, Bozler G, Kaschke D, Kummer M. Pharmacokinetics and Metabolic Pattern after Intravenous Infusion and Oral Administration to Healthy subjects, Drug. metab. Dispos. 1995; 23(11):1206-13.
7. Davies NM, Skjodt NM. Clinical pharmacokinetics of meloxicam. Clinical pharmacokinetics. 1999;36(2):115-26.
8. Murello I, Costa J, Salva P. Determination of Bupivacaine in Human Plasma by HPLC, Journal of liquid chromatography. 2007;16:3509-14.
9. Qin WW, Jiao Z, Zhong MK, Shi XJ, Zhang J, Li ZD, *et al.* Simultaneous determination of procaine, lidocaine, ropivacaine, tetracaine and bupivacaine in human plasma by high-performance liquid chromatography. Journal of Chromatography B. 2010;878(15):1185-9.
10. Verheesen PE, Brombacher PJ, Cremers HM, De Boer R. Determination of Low Levels of Bupivacaine (Marcaine®) in Plasma During Epidural Analgesia. Clinical Chemistry and Laboratory Medicine. 1980;18(6):351-4.
11. Yu Z, Westerlund D. Direct injection of large volumes of plasma in a column-switching system for the analysis of local anaesthetics II. Determination of bupivacaine in human plasma with an alkyldiol silica precolumn. Journal of Chromatography A. 1996;725(1):149-55.
12. Michaelis HC, Geng W, Kahl GF, Foth H. Sensitive determination of bupivacaine in human plasma by high-performance liquid chromatography, Journal of Chromatography B, Biomedical Sciences and Applications. 1990;527:201-07.
13. Tahraoui A, Watson DG, Skellern GG, Hudson SA, Petrie P, Faccenda K. Comparative study of the determination of bupivacaine in human plasma by gas chromatography-mass spectrometry and high-performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 1996;15(2):251-7.
14. Schieferecke MA, McLaughlin KJ, Faibushevich AA, Lunte CE. Determination of bupivacaine and three of its metabolites in rat urine by capillary electrophoresis. Electrophoresis. 1998;19(16-17):2997-3002.
15. Daryanavard SM, Jeppsson-Dadoun A, Andersson LI, Hashemi M, Colmsjö A, Abdel-Rehim M. Molecularly imprinted polymer in microextraction by packed sorbent for the simultaneous determination of local anesthetics: lidocaine, ropivacaine, mepivacaine and bupivacaine in plasma and urine samples. Biomedical Chromatography. 2013;27(11):1481-8.
16. Enikő C, Ibolya F, Daniela-Lucia M. Development of a HPLC-UV method for determination of meloxicam in human plasma and pharmaceutical dosage forms. Acta Medica Marisiensis. 2014;60(4):142-5.
17. Cox S, Hayes J, Yarbrough J, Veiga-Parga T, Greenacre C. High-Performance Liquid Chromatography Determination of Meloxicam and Piroxicam with Ultraviolet Detection. Chromatography Research International. 2014.
18. Emara LH, Emam MF, Taha NF, Raslan HM, El-Ashmawy AA. A Simple and Sensitive HPLC/UV Method for Determination of Meloxicam in Human Plasma for Bioavailability and Bioequivalence Studies, Journal of Applied Pharmaceutical Science. 2016; doi: 10.7324/JAPS.2016.60702.
19. Jaafar JI. Al-tamimi. Development and validation of hplc/uv method for determination of meloxicam in human plasma and application in pharmacokinetic studies, International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7:370-78.
20. Elbary ABD, Foda N, Elkhateeb M. Reversed phase liquid chromatographic determination of meloxicam in human plasma and its pharmacokinetic application, Journal Analytical Letters. 2001;34:1175-87.

21. Modesto RH, Duarte MG, Carter CBN, Moreno RA. Meloxicam determination in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) in Brazilian bioequivalence studies, International Journal of Clinical Pharmacology & Therapeutics. 2006;44(10):489-98.
22. Yuan Y, Chen X, Zhong D. Determination of meloxicam in human plasma by liquid chromatography–tandem mass spectrometry following transdermal administration. Journal of Chromatography B. 2007;852(1):650-4.
23. Ganesh M, Hemalatha P, Peng MM, Sakthimanigandan K, Jang HT. Estimation of meloxicam in human plasma by liquid chromatography-tandem mass spectrometry (lc-ms/ms). Journal of Liquid Chromatography & Related Technologies. 2013;36(7):867-80.
24. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research: Rockville, MD, 2001.
25. European Medicines Agency, guideline on bio analytical method validation (2011).
26. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, *et al.* Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharmaceutical Research. 2007;24(10):1962-73.

PICTORIAL ABSTRACT



SUMMARY

- Bupivacaine HCl (BP) and Meloxicam (ME) are used as intra-operative local anesthesia and non-steroidal anti-inflammatory drugs respectively.
- A simple, rapid, selective and sensitive ultra pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been developed and validated for simultaneous quantification of BP and ME in human plasma.
- The method validation proved that the method is selective, Linear, Precise and Accurate.
- The present method is advantageous with respect to simultaneous estimation, shorter run time (2.5 min) and less mobile phase consumption (Flow rate at 0.5 mL/Min) and it can be applied for the routine pharmacokinetic evaluation of BP and ME in human subjects after oral administration of the same.

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