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Research Article

An LC-MS/MS Method for Simultaneous Quantification of Paracetamol and Pamabrom in Human Plasma

Kashyap N. Thummar[1*], Falgun Patel[2], Trupesh Pethani[2], Bhumika Dudhatra[3]

[1]*Graduate School of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India.*
[2]*Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India.*
[3]*School of Applied Sciences, Gujarat Technological University, Ahmedabad, Gujarat, India*

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ABSTRACT

Back pain and menstruation pain are generally treated with a combination of paracetamol (PCM) and pamabrom (PAMA). In this study, a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of PCM and PAMA from human plasma is developed and validated for the first time. The analytes and the internal standard (IS), diclofenac, were eluted on a kromasil C_{18} (100 x 4.6 mm, 5 µm) column using a simple optimized mobile phase after being treated by liquid-liquid extraction. Using the electrospray ionization technique, quantification was carried out using a hyphenated tandem mass spectrometer with a flow rate of 0.4 mL/min and completing the entire run time within 4 minutes. For PCM and PAMA, the calibration curve was linear ($r > 0.995$) for the range of 40–8000 ng/mL and 20–4000 ng/mL, respectively. This method produced reliable findings since it was verified for specificity, accuracy, recovery, linearity, precision, matrix effect, and stability investigations, and the results were determined to be within acceptable parameters. The current study's findings demonstrated that this technology's simplicity, rapidity, and sensitivity allow for routine usage in exploratory or clinical monitoring of both medications in human plasma.

Introduction

Paracetamol (PCM), N- 4-hydroxyphenyl acetamide, is a para-aminophenol derivative of nonsteroidal anti-inflammatory medications that is extensively used as an over-the-counter analgesic-antipyretic drug with low anti-inflammatory effect (NSAIDs). PCM is an essential medicine used to treat mild to moderate pain and to reduce fever when an anti-inflammatory action is not required.[1] Pamabrom (PAMA) is a chemical compound composed of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate in a 1:1 ratio. PAMA, a xanthine diuretic, improves glomerular filtration rate and renal tubule permeability while decreasing salt and reabsorption of water in the proximal tubule.[2] This combination is recommended as an over-the-counter remedy to alleviate menstrual fluid retention.

The logic behind mixing medications for pain management is that various drugs interact with different receptors, resulting in distinct pain processes that may improve pain relief. PAMA tablet formulation is available in conjunction with PCM for treating a variety of problems such as back pain, transient water gain reduction, and menstrual pain treatment.[3]

Several analytical approaches have been described to quantitatively identify PCM in biological matrices, together with high-pressure liquid chromatography (HPLC) when combined with cetirizine,[4] and it can also be analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) when used in combination with other

***Corresponding Author:** Dr. Kashyap N. Thummar

Address: Graduate School of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India

Email ✉**:** ap_kashyap@gtu.edu.in

Tel.: +91 9427979959

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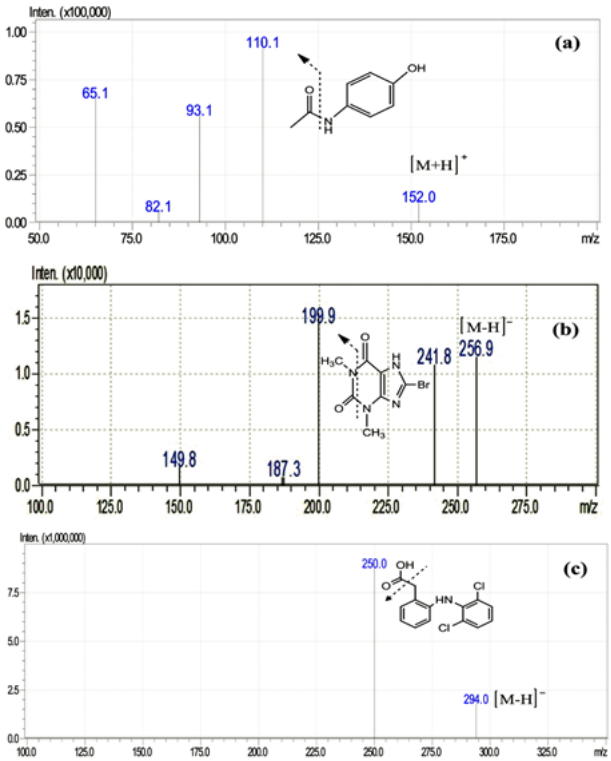

Fig. 1: The representative mass spectral scan with possible fragmentation product ion of a) Paracetamol b) Pamabrom and c) Internal Standard (Diclofenac).

medicines such as ketorolac enantiomers,[5] guaifenesin,[6] chlorpheniramine[7] and their tertiary combinations.[8-10] Compared to PCM, very limited methods such as stability-indicating HPLC were reported to determine PAMA in an aqueous medium,[11] and in combination with ibuprofen.[12] The PAMA was estimated from human plasma at once by HPLC-UV detection.[13] Few researchers have described the simultaneous quantification of both analytes in an aqueous medium by spectrophotometry,[14-16] HPLC,[17] HPTLC[18] and stability-indicating HPLC in presence of its potential impurities.[19,20] Until far, no LC-MS/MS approach for simultaneous analysis of PCM and PAMA from biological fluids has been published. For this reason, developing a quick and accurate LC-MS/MS approach is critical. Consequently, this work was the first to provide a simple, quick, and sensitive LC-MS/MS method for quantifying PCM and PAMA in human plasma.

Materials And Methods

Chemicals and Reagents

The PCM, PAMA and IS were provided by Rajat Pharma (Mumbai, India). Rajat Pharma supplied the PCM, PAMA, and IS (Mumbai, India). Rajkot volunteer blood bank at Gujarat, India, provided human plasma control with K3EDTA anticoagulant. Analytical grade ammonium formate and HPLC-quality acetonitrile, methanol, and tert-butyl methyl ether were provided by Spectrochem Pvt. Ltd. in India. Throughout the investigation, ultrapure

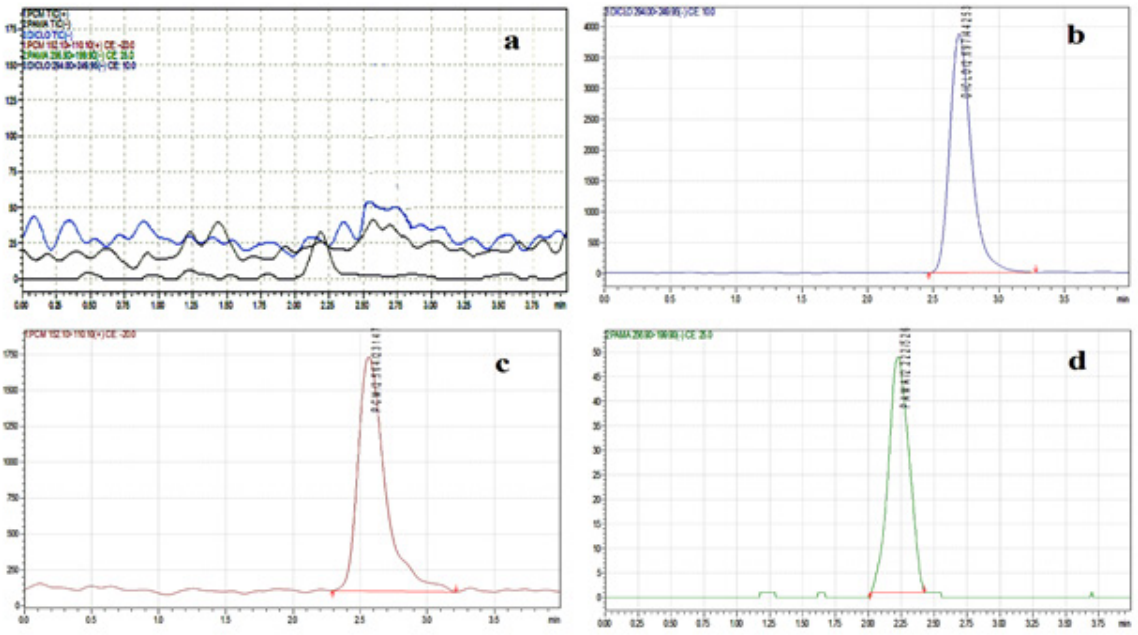

Fig. 2: Representative LC-MS/MS mass chromatogram for extracted samples of a) overlay of blank plasma for Paracetamol, Pamabrom and Diclofenac (IS), (b) Internal standard (Diclofenac) in absence of analytes (zero standard), (c) Paracetamol at LLOQ (40 ng/mL) and (d) Pamabrom at LLOQ (20 ng/mL).

Table 1: Effect of pH and polarity on paracetamol and pamabrom recovery

S. no.	*pH modifier*	*Condition*	*Solvent*	*Observation*	
				Peak intensity/area[a]	*Peak shape*[b]
1	0.1 N NaOH	Alkaline	Tert butyl methyl ether	+	+
2	0.1 N NaOH	Alkaline	Ethyl acetate	+	+
3	0.1 N NaOH	Alkaline	Di-ethyl ether : Di-chloromethane (70:30, v/v)	+	+
4	Water	Neutral	Tert butyl methyl ether	++	+
5	Water	Neutral	Ethyl acetate	+	+
6	Water	Neutral	Di-ethyl ether : Di-chloromethane (70:30, v/v)	+	+
7	0.1 N HCl	Acidic	Tert butyl methyl ether	++	++
8	0.1 N HCl	Acidic	Ethyl acetate	++	+
9	0.1 N HCl	Acidic	Di-ethyl ether : Di-chloromethane (70:30, v/v)	+	+

[a]High= ++, Low: +
[b]Symmetrical: ++, Asymmetrical: +

Table 2: Accuracy and precision of PCM and PAMA in human plasma.

Analytes	*Nominal conc. (ng/mL)*	*Intra – assay (n=6)*		*Inter – assay (n=18)*	
		Precision (% RSD)	*Accuracy (%)*	*Precision (% RSD)*	*Accuracy (%)*
PCM	40	8.5	87.6	7.9	89.3
	120	4.4	94.6	4.3	93.5
	600	2.1	97.3	1.9	97.0
	7200	9.5	97.8	6.7	104.6
PAMA	20	7.3	87.4	6.0	90.4
	60	6.7	90.3	6.3	91.4
	300	3.0	90.8	3.3	90.5
	3600	8.3	96.1	10.2	96.1

PCM: paracetamol; PAMA: pamabrom

Table 3: Extraction recovery and matrix effect of PCM, PAMA (at three QC level) and IS in the human plasma

Analytes	*Nominal conc. (ng/mL)*	*Recovery*		*Matrix effect*	
		%recovery[a] *± SD*	*%RSD*	*Matrix factor*[b] *± SD*	*%RSD*
PCM	120	92.3 ± 5.6	6.0	0.9 ± 0.1	8.1
	600	91.8 ± 1.7	1.8	0.9 ± 0.1	6.1
	7200	89.9 ± 1.9	2.1	0.9 ± 0.1	5.7
PAMA	60	105.7 ± 9.4	8.9	1.1 ± 0.1	6.8
	300	92.3 ± 2.0	2.2	0.9 ± 0.1	5.0
	3600	91.0 ± 1.5	1.6	1.0 ± 0.1	8.1
IS	500	92.7 ± 2.7	3.1	0.9 ± 0.1	8.3

PCM: paracetamol; PAMA: pamabrom; IS: internal standard (diclofenac)
a The percentage of the mean peak area of the analytes obtained from pre (C) and post (B) spiked plasma processed samples at equivalent concentrations [].
b The ratio of the mean peak area of post spiked samples (B) to those of pure standard solutions in the mobile phase (A) at the same concentrations [].

water was used from MilliQ-Elix system, (Millipore Pvt. Ltd., Gujarat, India). Without additional purification, all reagents were utilized.

Instrumentation

The LC-MS/MS system included a column oven with forced air circulation (CTO-20AC), an auto-sampler with an integrated dehumidifier, a pulse-free technique for supplying solvent (LC-20AD), and an efficient online degasser (DGU-20A5R). The HPLC was connected to an LC-MS8030 triple quadrupole mass spectrometer (Shimadzu, Japan) using an electrospray ionization (ESI) source. Lab Solution software, Shimadzu, Japan, version 5.53, was used to regulate all LC and MS parameters.

Table 4: Stability results for paracetamol and pamabrom under different conditions (n = 6).

Stability conditions	*QC samples*[a]	*PCM*	*PAMA*
		%Mean stability changed[b]	*%Mean stability changed*[b]
Benchtop stability for 6 h (at ambient temperature)	LQC	1.3	1.5
	HQC	0.1	-2.2
Freeze and thaw stability at -20°C (5 cycle)	LQC	-3.4	-7.1
	HQC	-4.2	-5.8
Dry extract stability at -20°C for 24 hours	LQC	-3.5	4.2
	HQC	1.7	2.8
Auto sampler stability at 4°C for 24 hours	LQC	-0.3	-1.0
	HQC	-0.4	0.0
Long-term stability at -20°C for 28 days	LQC	-3.0	-6.7
	HQC	-3.8	-5.9

QC: quality control; PCM: paracetamol; PAMA: pamabrom; HQC:high quality control ; LQC: low quality control

[a]The concentration of quality control sample for paracetamol is 120 ng/mL (LQC), 7200 ng/mL (HQC); and for pamabrom is 60 ng/mL (LQC), 3600 ng/mL (HQC)

[b] $$\% \text{ Mean stability changed} = \frac{\text{Mean stability samples} - \text{Mean comparison samples}}{\text{Mean Comparing samples}} * 100$$

Instrumental Parameters for Chromatography and Mass Spectrometry

The chromatographic separation was performed using a Kromasil analytical C_{18} (100 x 4.6 mm, 5 µm) column, and the column oven's temperature was kept at 40°C. The acetonitrile:methanol (50:50) solution with 2 mM ammonium formate in water (90:10, v/v) was the mobile phase, with a flow rate of 0.4 mL/min. A sample volume of 2 µL was introduced into the system, and the autosampler's temperature was maintained at 4°C and the analytical run duration was 4 minutes.

Mass parameters for PCM, PAMA, and IS were tuned by the infusion of a 500 ng/mL solution of each analyte and constantly switching from positive to negative ion mode. The interface temperature and voltage of the ion spray were tuned at 300°C and 4.5 kV, respectively. Nitrogen was employed as a nebulizing (3 L/min) and drying (15 L/min) gas. MRM was used to measure analytes using argon as the collision gas and ionic transitions from m/z 152.0 →110.1 for PCM in positive mode, m/z 256.9→199.9 for PAMA, and m/z 294.0→250.0 for IS in negative mode. Collision energies for PCM, PAMA, and IS were set to be -20eV, 25eV, and 10eV, respectively. The dwell duration was limited to 100 milliseconds.

Standard and Quality Control (QC) Sample Preparation

Methanol was used to create the stock solutions of PCM and PAMA to a 5000 ng/mL as the ultimate concentration for each analyte. The working solution of the analytes was then diluted to the required concentrations using a combination of methanol and water (50:50, v/v). In order to create the calibration curves, serially diluted spike solutions were made after spiking (2%) the standard solutions into blank plasma. These calibration curves were created for PCM and PAMA, respectively, at 40, 80, 160, 400, 800, 1600, 4000, and 8000 ng/mL and 20, 40, 80, 200, 400, 800, 2000, and 4000 ng/mL, respectively.

The quality control samples (QCs) were made from the working standard solutions at four concentrations *viz.* 40, 120, 600 and 7200 ng/mL for PCM as well as for PAMA 20, 60, 300 and 3600 ng/mL respectively labelled as a LLOQ (lower limit of quantification), LQC (low quality control), MQC (middle quality control), HQC (high quality control) for both analytes, A working IS solution containing diclofenac was similarly produced to achieve an ultimate concentration of 500 ng/mL. Before being examined, all samples were maintained between 2 to 8°C temperature.

Preparation of Plasma Samples

Liquid-liquid extraction (LLE) was employed to extract PCM, PAMA, and IS from human plasma during sample preparation. The spiked samples, along with samples of standards calibration and quality control had been kept in a deep freezer at -20°C were defrosted to room temperature. Before pipetting, these defrosted samples were appropriately mixed and vortexed. These samples were then (200 µL) put into 2 mL recovery vials, added 50 µL of an IS, excluding blank samples, and vortexed for 30 s using a vortex mixer. The mixture was then vortexed with 50 µL of acidic buffer (0.1N HCl) for 2 minutes. After extraction, 1.3 mL of tert methyl butyl ether was added to the mixture as an extracting solvent and vortexed upto 5 minutes. The resultant mixture was then refrigerated centrifuged for 15 minutes around 4000 rpm at 10°C. The organic phase was then moved to a separate vial and dried at 40°C with a moderate stream of nitrogen until dry. After reconstituting the dried samples with 100 µL of reconstitution solvent (mobile phase), 2 µL of sample

was injected for chromatographic separation and analyte quantification.

Method Validation

In accordance with the USFDA's industrial guidelines,[21] the bioanalytical method validation was carried out and found to meet acceptance requirements for a several.

Selectivity

To look into endogenous interferences at the signal of analytes and IS, seven distinct lot of human plasma (blank) were screened (one heparinized plasma and six were K_3EDTA). The LLOQ QC and IS sample were made using aliquots of plasma samples. The baseline should be < 20% of the analyte responses at LLOQ QC level.[22]

Linearity, Accuracy, and Precision

Analysis of an eight-point standard curve covering the concentration ranges of 40–8000 ng/mL for PCM and 20–4000 ng/mL for PAMA (n=3), as well as the precision and accuracy batch was used to assess the method's linearity. Regression analysis was performed using the analyte to IS peak area ratio vs concentration. The concentration at each QC level is quantified using the regression equation for the calibration curve and the linear relationship between responses. Least square weighted (1/x) linear regression was used to independently assess each calibration curve.

By examining QC samples (n = 6) at each level of PCM and PAMA on the same batch and in three distinct batches, it was feasible to assess the precision and accuracy within and between assays. The batch was composed of a calibration standard, six QC sample measurements at each level, and a calculation of the % RSD (Relative standard deviation) at each level to assess accuracy. The standard deviation from the theoritcal concentration should not exceed 15.0%, except for LLOQ QC, when it should be permitted to be within 20%. For all calibration curves, a correlation coefficient (*r>99*) value was preferred.

Recovery and Matrix Factor

Six duplicates of each QC samples (120, 600, and 7200 ng/mL for PCM and 60, 300, and 3600 ng/mL for PAMA) were processed by LLE and evaluated for extraction recovery and matrix effect. Peak areas from the plasma processing samples before (C) and after (B) the addition of a spiked drug were compared. The recovery (%) was calculated using . To assess the matrix effect on plasma, the peak area after spiked samples (B) was compared to that of pure standard solutions in the mobile phase (A) at the same concentrations. By applying, the matrix factor was computed. IS extraction recovery and matrix factor were assessed similarly at working concentration (500 ng/mL). A method's matrix factor must be within the range of 0.85 to 1.15 at each QC level to ensure it is free from the matrix effect. The acceptable limits were set at a 15% deviation from the mean recovery.

Dilution Integrity

Dilution consistency was tested to ensure that samples may be diluted with a blank matrix without changing the final concentration if the genuine samples exceeded the method's certified calibration range. To do this, predetermined reference samples were created at concentrations 1.5 and 4 times greater than the maximum limit of the calibration range (above ULOQ). To create diluted quality control samples (DQCs), aliquots were diluted (1:2 and 1:10) with human plasma to reach concentrations of DQC (1/2) 6000 ng/mL and DQC (1/10) 3200 ng/mL for PCM and DQC (1/2) 3000 ng/mL and DQC (1/10) 1600 ng/mL for PAMA, respectively. These diluted samples were processed and evaluated along with calibration samples.

Stability

Six duplicates of QC samples (120, 7200 ng/mL for PCM and 60, 3600 ng/mL for PAMA) were examined under various settings to assess the sample stability in human plasma. The bench-top (6 h at room temperature), freeze-thaw (-20 ± 5°C), auto-sampler (at 4°C for 24 hours), dry extract (2–8°C for 24 hours), and long-term stability (-20 ± 5°C for 28 days) were all calculated. Concentrations of both stability samples and newly generated samples were determined to determine stability. Stability was represented as the % mean change in computed concentrations, and sample solutions were deemed stable if the percent mean stability change was found to be within 15% of the starting concentration.

Result And Discussion

Method Development

A fast and accurate assay for the concurrent measurement of PCM and PAMA has been developed and validated in human plasma, as well as the MS parameter, extraction method, and chromatographic conditions.

Mass Spectrometric Condition Optimization

The analytes were initially examined by system injection of separate standard solutions without column. It was decided to use the PCM in positive, PAMA, and IS in negative ionization modes since they provided more effective ionization. To get the highest sensitivity feasible, the MS/MS conditions were tuned. The MRM was used to keep track of precursor and product ions that might improve selectivity and lessen interference. Similarly, the molecular ion $[M+H]^+$ of PCM was detected at 152.0 m/z, the collision-induced (-20 eV) dissociation due to loss of neutral molecule, namely ketene (CH_2=C=O) with proton

transfer to produce a primary ammonium ion at 110.1 m/z. The corresponding mass spectrum is shown in Fig. 1a.

The PAMA showed quasi-molecular ion $[M-H]^-$ at 256.90 m/z of 8-bromotheophyllinate and at collision energy of 25 eV, a significant fragment ion formed at 199.9 m/z. Its m/z of 256.9 to 199.9 transition, i.e., 57 unit losses was due to CH_3NCO loss from di-methyl uracil ring (*retro-Diels-Alder fragmentation* reaction). The corresponding mass spectrum was shown in Fig. 1b.

Diclofenac contains two chlorine atoms and can be detected in the negative ionization mode. The usual isotope pattern for the molecular ion [M-H]- was discovered at 294.0 m/z. when the collision energy was set at 10 eV, a significant fragment ion was identified at 250.0 m/z. This product ion was formed due to loss of CO_2 $[M-CO_2-H]^-$ (Fig. 1c).

Optimization of Sample Preparation

The sample preparation process should be rapid, simple, and need the least quantity of chemicals while maximizing analyte recovery. In this context, a study of the literature found that the LLE approach was used to extract PCM and PAMA.[23] However, the recovery of analytes using this extraction method was only about 60%. Another approach that has been published likewise used a larger amount of plasma for sample preparation and a more volume of injection for getting results.[24] Liquid-liquid extraction (LLE) and protein precipitation technique (PPT) procedures were examined in sample preparation for improved recovery, extraction efficiency, and observation of signal-to-noise ratio at lower concentrations. It was observed that recovery efficiency was less in PPT compared to LLE, which might be owing to less protein binding capacity of PCM and PAMA. In LLE method, to assess the impact of extraction solvent and pH on analyte recovery, various organic solvents which were covered within expedient polarity range such as diethylether, dichloromethane, tert butyl methyl ether, ethyl acetate, were used at different concentrations and amount of pH modifier like HCl, NaOH and also without pH modifier.[25,26] The results expressed that optimal extraction of selected analytes can be obtained using less polar organic solvents. As compared to acidic conditions, recovery of PCM was better in unchanged and alkaline condition, whereas better recovery value for PAMA and IS was observed in acidic conditions. Therefore, subsequent experiments were performed in acidic condition using methyl tert-butyl ether as solvent. Sample preparation was required to reduce processing time and achieve the specified analyte recoveries. There was no endogenous or exogenous plasma matrix interference. Hence the analyte recovery, sensitivity, and/or ion suppression remained unaltered. The summary of results obtained from the different extraction process is provided in Table 1.

Optimization of Chromatographic Condition

The technique was made cost-effective by choosing the mobile phase considering the gaussian peak shape and early completion of run time, which makes less use of organic solvent. The mobile phases containing different proportion of ammonium acetate (2 mM, 5 mM, 10 mM), ammonium formate (0.1%), formic acid in water (0.5%) as a buffer, and acetonitrile and methanol as organic solvents were screened to obtain symmetric peak shape and better sensitivity. Finally, the desired findings were seen in the Kromasil C_{18} (100 x 4.6 mm, 5 µm) analytical column using a variety of combinations, demonstrating that mixture of acetonitrile and methanol (50:50) 2 mM ammonium formate in water (90:10, v/v) meets the necessary purpose with maximum efficacy. A small injection volume of 2 µL minimized analyte overloading of the column, allowing for numerous sample analyses on the same column. The suggested chromatographic settings were created with the goal of increasing sensitivity while decreasing chromatographic run time (4 minutes).

Method Validation

Specificity

No substantial interference from endogenous compounds was found in drug-free human plasma. PCM, PAMA, and IS had retention durations of 2.57, 2.20, and 2.86 minutes, respectively (Fig. 2). At this concentration level, the baseline noise was found to be < 20% of the PCM, PAMA, and IS responses.

Linearity, Precision and Accuracy

For PCM and PAMA, the chromatographic responses were linear across the concentration ranges of 40–8000 ng/mL and 20–4000 ng/mL, respectively, in human plasma. The average regressions of the area ratio of peak analytes to IS at various concentrations were fitted to generate a typical equation for PCM and PAMA. Average linear equation obtained for PCM and PAMA were $y = (0.006 \pm 0.0002)\ x + (0.583 \pm 0.0329)$ and $y = (0.002 \pm 0.0001)\ x + (0.020 \pm 0.0047)$, respectively where y represents the ratio of peak of analytes to that of IS, and x represents plasma concentration. Throughout validation, the correlation coefficient, r, was continuously equal to or better than 0.995 for both analytes. Samples from calibration curves with back calculated concentrations were within 15% of the theoretical value. The proposed method has a 200 times wide dynamic linearity range for both analytes that can cover the therapeutic range of PCM and PAMA.

PCM and PAMA intra- and inter-assay precision and accuracy were determined by evaluating QCs samples at four concentrations (7200, 600, 120, and 40 ng/mL for PCM and 3600, 300, 60, and 20 ng/mL for PAMA) in six repetitions on a within day and between days, respectively, and the concentration was back-calculated using the calibration curve. The technique was rapid and consistent,

with intra and inter-assay accuracy was ranged from 87.6–97.8% for PCM and 87.4–96.1% for PAMA, and for precision %RSD values were less than 7.9% for PCM and 10.3% for PAMA. With the exception of LLOQ QC, where a value up to 20% over the theoretical value was permitted, all accuracy and precision levels felt within the allowable limitations of 15%. The values in Table 2 indicate better precision and accuracy.

Matrix Factor and Recovery

LLE was employed to extract PCM, PAMA, and IS from spiked human plasma. The %mean recoveries ranged from 89.9 to 92.3% for PCM and from 91.0 to 105.7% for PAMA, with the matrix factor having a mean value of 0.9 ± 0.1 for PCM and 1.0 ± 0.1 for PAMA. The mean recovery and matrix factor of IS were achieved 92.7% and 0.9, respectively. The recovery of PCM, PAMA and IS was high and were noted to be consistently precise and reproducible (Table 3). Thus, we could conclude that the proposed method efficiently recovered PCM, PAMA and IS from human plasma.

Dilution Integrity

The precision (%RSD) values for consistency for $1/2^{nd}$ and $1/10^{th}$ dilutions for PCM were 9.5 and 10.8%, respectively, and 8.9 and 8.2% for PAMA. While PCM accuracy was 97.35 and 99.52%, respectively, PAMA accuracy was 92.86 and 95.03%. The precision (%RSD) and accuracy (85–115%) results were within the acceptable limits. Therefore, the real subject plasma concentrations above the ULOQ can also be assessed by this method.

Stability

QC samples matched to low and high matrix levels were used for the stability testing. Since there were no significant variations from the predicted concentration, PCM and PAMA were stable throughout all test conditions. Results fell under the 15% mean stability changed acceptability threshold. Therefore, it has been demonstrated that this method may be used for regular analysis (Table 4).

Conclusion

A rapid and easy LC-MS/MS technique for measuring PCM and PAMA levels in human plasma was created and successfully validated as per the guidance document of USFDA. This method has considerable benefits over previously described methods in terms of very effective liquid-liquid extraction, shorter chromatographic run time (4 min), only 2% spiking with biological matrix, and lower sample injection volume (2 µL), making it helpful in high-throughput bioanalysis. Furthermore, analytes were stable under typical handling and processing conditions and provided effective analyte and IS recoveries in the absence of matrix effect, which contributed to the development of a reliable method for simultaneously measuring PCM and PAMA in human plasma. This method may be used in bioavailability and bioequivalence research, as well as regular dose-dependence profiles with the desired precision and accuracy.

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Competing Declaration

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data And Material Availability

The datasets of the study are provided as supplementary material.

References

1. Rang HP, Ritter JM, Flower RJ, Henderson G. Rang & Dale's Pharmacology. 5th edition (India) ed. New Delhi: Elsevier; 2005. 244-52 p.
2. Mudge GH, Weiner IM. The mechanism of action of mercurial and xanthine diuretics. Annals of the New York Academy of Sciences. 1958;71(4):344-54. 10.1111/j.1749-6632.1958.tb46761.x.
3. Turley SM. Understanding Pharmacology for Health Professionals: Pearson Education; 2014.
4. Nagaralli BS, Seetharamappa J, Gowda BG, Melwanki MB. Liquid chromatographic determination of ceterizine hydrochloride and paracetamol in human plasma and pharmaceutical formulations. Journal of Chromatography B. 2003;798(1):49-54. http://dx.doi.org/10.1016/j.jchromb.2003.08.048.
5. Ing-Lorenzini KR, Desmeules JA, Besson M, Veuthey J-L, Dayer P, Daali Y. Two-dimensional liquid chromatography–ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomers and paracetamol in human plasma: Application to a pharmacokinetic study. Journal of Chromatography A. 2009;1216(18):3851-6. http://dx.doi.org/10.1016/j.chroma.2009.02.071.
6. Chen X, Huang J, Kong Z, Zhong D. Sensitive liquid chromatography–tandem mass spectrometry method for the simultaneous determination of paracetamol and guaifenesin in human plasma. Journal of Chromatography B. 2005;817(2):263-9. http://dx.doi.org/10.1016/j.jchromb.2004.12.011.
7. Celma C, Allue JA, Prunonosa J, Peraire C, Obach R. Simultaneous determination of paracetamol and chlorpheniramine in human plasma by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A. 2000;870(1-2):77-86. 10.1016/s0021-9673(99)01252-2.
8. Lou HG, Yuan H, Ruan ZR, Jiang B. Simultaneous determination of paracetamol, pseudoephedrine, dextrophan and chlorpheniramine in human plasma by liquid chromatography-tandem mass spectrometry. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2010;878(7-8):682-8. 10.1016/j.jchromb.2010.01.005.
9. Mohamed D, Hegazy MA, Elshahed MS, Toubar SS, Helmy MI. Liquid chromatography–tandem MS/MS method for simultaneous quantification of paracetamol, chlorzoxazone and aceclofenac in human plasma: An application to a clinical pharmacokinetic study. Biomedical Chromatography. 2018;32(7):e4232. 10.1002/bmc.4232.

10. Idris M, John C, Ghosh P, Shukla SK, Baggi TRR. Simultaneous determination of methaqaulone, saccharin, paracetamol, and phenacetin in illicit drug samples by HPLC. Journal of Analytical Science and Technology. 2013;4(1):4. 10.1186/2093-3371-4-4.
11. Shah U, Kavad M, Raval M. Development and Validation of Stability-indicating RP-HPLC Method for Estimation of Pamabrom in Tablets. Indian Journal of Pharmaceutical Sciences. 2014;76(3):198-202.
12. Jun H-S, Kang J-S, Park J-S, Cho C-W. Simultaneous analysis of ibuprofen and pamabrom by HPLC. Journal of Pharmaceutical Investigation. 2015;45(6):555-60. 10.1007/s40005-015-0203-2.
13. González-de la Parra M, Lucio-Maldonado S, Cruz-Salinas MA, López-Bojórquez E, Pérez-Becerril F, Burke-Fraga V. Bioanalytical Method for Pamabrom (Determined as 8-Bromotheophylline) and its Application to a Pharmacokinetic Study. Journal of Bioequivalence Studies. 2017;3(1):1-8. 10.15744/2575-551X.3.101.
14. Bambhrolia S, Rajput SJ. Simultaneous estimation of Paracetamol and Pamabrom in bulk drugs and in pharmaceutical formulation by spectrophotometry. International Journal of ChemTech Research. 2013;5(4):1802-7.
15. Salem H, Mohamed D. Simultaneous determination of pamabrom and paracetamol in bulk powders and womankit tablet using spectrophotometric and chemometric-assisted spectrophotometric methods. Analytical Chemistry: An Indian Journal. 2013;13(1):6-16.
16. Abdelaleem EA, Naguib IA, Hassan ES, Ali NW. Development and Validation of Three Spectrophotometric Methods for Simultaneous Determination of Paracetamol and Pamabrom in Bulk and Pharmaceutical Formulation. Analytical Chemistry Letters. 2016;6(1):13-23. 10.1080/22297928.2016.1138882.
17. Patel HH, Patel H. Development and validation of reverse phase high performance liquid chromatographic method for simultaneous estimation of paracetamol and pamabrom in combined dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(Spec.Issue4):1303-13, 11.
18. Prajapati PP, Captain AD, Patel DS. Development and validation of HPTLC method for simultaneous determination of pamabrom and paracetamol in synthetic mixture. International Research Journal of Pharmacy. 2012;3(11):167-71.
19. Abdelaleem EA, Naguib IA, Hassan ES, Ali NW. HPTLC and RP-HPLC methods for simultaneous determination of Paracetamol and Pamabrom in presence of their potential impurities. Journal of pharmaceutical and biomedical analysis. 2015;114:22-7. 10.1016/j.jpba.2015.04.043.
20. El-Houssini OM. RP-LC and TLC densitometric determination of paracetamol and pamabrom in presence of hazardous impurity of paracetamol and application to pharmaceuticals. Analytical chemistry insights. 2013;8:73-81. 10.4137/ACI.S12349.
21. USFDA. Guidance for Industry- Bioanalytical method validation. U.S. Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research (CDER), Centre for Veterinary Medicine (CVM); May 2001.
22. Patel JR, Pethani TM, Vachhani AN, Sheth NR, Dudhrejiya AV. Development and validation of bioanalytical method for simultaneous estimation of ramipril and hydrochlorothiazide in human plasma using liquid chromatography-tandem mass spectrometry. Journal of Chromatography B. 2014;970:53-9. https://doi.org/10.1016/j.jchromb.2014.08.023.
23. Zhou L-j, Gu L-h, Wang Y-j, Liang J-y. Determination of acetaminophen and pamabrom in compound tablets in human plasma by HPLC. Chinese Journal of New Drugs and Clinical Remedies 2007;3(3):187-90.
24. Wang H, Jin X, Zhao L, Chen J, Lu L, Zhang G. Determination of paracetamol and pamabrom in human plasma and urine by LC/MS method. Pharmaceutical Care and Research. 2010;10(3):198-201.
25. Thummar K, Solanki P, Mardia R, Sheth N. Quantitative determination of lacosamide in human plasma using liquid chromatography-tandem mass spectrometry and its application to pharmacokinetic study. International Journal of Pharmaceutical Sciences & Research. 2021;12(1):321-29. 10.13040/ijpsr.0975-8232.12(1).321-29.
26. Thummar K, Jethva D, Sheth N. Quantitative analysis of Topiramate in human plasma using LC-MS/MS and its application to pharmacokinetic study. World Journal of Pharmacy and Pharmaceutical Sciences. 2018;7(5):1016-32. 10.20959/wjpps20185-11511.

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