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RESEARCH ARTICLE

# Determination of (4-(1,3-dioxo-1,3-dihydro-*2H*-isoindol- 2-yl)-*N*′-[(4-ethoxyphenyl) methylidene] benzohydrazide, a novel anti-inflammatory agent, in biological fluids by UPLC-MS/MS: Assay development, validation and *in vitro* metabolic stability study

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## Abstract

A thalidomide analog, (4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-*N*′-[(4-ethoxyphenyl) methylidene] benzohydrazide), has been identified as a promising broad-spectrum anti-inflammatory agent in previous study. In this study, a sensitive and selective UPLC-MS/MS assay was developed and validated for its determination in rat plasma samples. The chromatographic separation was performed on an Aquity BEH $C_{18}$ column using mobile phase comprising of acetonitrile and 10 mM ammonium acetate in the ratio of 85: 15, at flow rate of 0.3 mL/min. The detection and quantification were performed in positive multiple reaction monitoring mode by parent to daughter ion transition of 414.06 > 148.05 for analyte and 411.18 > 191.07 for internal standard (risperidone), respectively using electrospray ionization source. The sample extraction process consisted of liquid–liquid extraction method using diethyl ether as the extracting solvent. The assay was validated by following FDA guidelines and all parameters were found to be within acceptable limits. The linearity was between 10.1 and 2500 ng/mL and the lower limit of quantification was 10.1 ng/mL. The reported results indicate that the assay could meet the requirement for analysis of this compound in amounts expected to the present in actual samples. Further, *in vitro* metabolic stability study was performed in rat liver microsomes by using the validated assay.

KEYWORDS

metabolic stability, plasma, thalidomide analog, UPLC-MS/MS

## 1 | INTRODUCTION

Thalidomide and its analogs have emerged as the potential immunomodulatory drug for the treatment of diverse chronic inflammatory diseases, including cancer (Hashimoto, 2008; Teo, 2005). Earlier in 1954, thalidomide was approved as sedative and anti-emetic in pregnancy, but was shortly withdrawn from the market due to its teratogenic effects (Teo, 2005; Mellin *et al*., 1962). Nevertheless, it possesses broad pharmacological properties, which again prompt it to be introduced in to market for the treatment of various inflammatory and autoimmune diseases, including erythema nodosum leprosum and multiple myeloma (Melchert & List, 2007; Sampaio, Sarno, Galilly, Cohn, & Kaplan, 1991). In addition, various experimental and clinical studies also confirmed that thalidomide or its analogs may produce beneficial effects in the treatment of rheumatoid arthritis, Crohn's disease, multiple myeloma, Behçet's disease, lupus erythematosus and chronic host-versus-graft (Chen, Doherty, & Hsu, 2010; Teo, Stirling & Zeldis, 2005). Moreover, it was also confirmed that the anti-inflammatory effects produced by thalidomide were due to suppressing the production of proinflammatory mediators, including tumor necrosis factor-α, interleukin (IL)-1β, IL-6 and nitric oxide (Amirshahrokhi, 2013; Mazzon *et al*., 2005). In spite of this, the teratogenicity, peripheral neuropathy and other adverse effects of thalidomide limit its use, and so the development of new analogs that

**Abbreviations:** % CV, percentage coefficient of variation; LLOQ, lower limit of quantification

  

can produce low toxicity and enhanced potency in blocking cytokine production are needed (Dinarello, 2010).

In previous study, synthesis and anti-inflammatory effects of a series of compounds of *N*-arylphthalimide derivatives (1–10P) have been reported (Bhat *et al*., 2015). The rationale behind the synthesis of new derivatives was to achieve more lipophilic phthalimide derivatives with potent anti-inflammatory activities. To achieve this, *N*-arylphthalimide derivatives, which derived from the thalidomide structural analog, were synthesized by modulating the thiomorpholine moiety with hydrophobic groups. During pharmacological evaluation, the compound (4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-*N*′-[(4-ethoxyphenyl) methylidene] benzohydrazide (6P) possesses considerable activity against important proinflammatory mediators and cytokines and is represented as a lead compound for the development of a newer, potent thalidomide analog as an anti-inflammatory agent. Compound 6P was found to considerably decrease in the cell populations of proinflammatory mediators ($CD4^+$, tumor necrosis factor-$\alpha^+$, IL-$6^+$, IL-$17^+$) and increase in the populations of anti-inflammatory mediators ($Foxp3^+$, $I\kappa B\alpha^+$, $CD4^+Foxp3^+$) in comparison to the control group in a flow cytometry experiment in mice. Further, it also decreased the expression of mRNA and proteins associated with inflammation (COX-2, STAT-3, inducible nitric oxide synthase) and increased the anti-inflammatory mediators, e.g. IL-10, IL-4 (Bhat *et al*., 2015). Considering compound 6P as a potential anti-inflammatory agent, the development of a sensitive bioanalytical assay was necessary to perform its pharmacokinetic and toxicokinetic studies. Previously, we have reported the quantification of the thiosemicarbazide derivative of isoniazid and a carvone Schiff base of isoniazid in rat plasma using UPLC-MS/MS (Iqbal, Bhat, & Shakeel, 2015; Iqbal, Ezzeldin, Bhat, Raish, & Al-Rashood, 2016). In this study, we developed a sensitive and selective UPLC-MS/MS assay for determination of the compound 6P concentration in rat plasma samples. For the reliability of the assay, it was fully validated following regulated guidelines for bioanalytical method validation. *In vitro* metabolic stability study was performed to evaluate the metabolic characteristics of this compound and the assay is being used in preclinical pharmacokinetic characterization in rats.

## 2 | EXPERIMENTAL

### 2.1 | Chemical and reagents

Compound 6P was synthesized, purified (purity, >98%) and characterized by spectroscopy in the Pharmaceutical Chemistry Laboratory, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). The internal standard (IS), risperidone (purity, 98%), was purchased from Sigma-Aldrich (St Louis, MO, USA). Figure 1 represents the molecular structure of compound 6P (molecular formula, $C_{24}H_{19}N_3O_4$; molecular mass, 413.42) and risperidone, respectively. HPLC grade acetonitrile (ACN), methanol and diethyl ether were obtained from VWR International Ltd. (Poole, UK). Ammonium acetate was obtained from Qualikems Fine Chem. Pvt. Ltd. (Vadodara, India). Dimethyl sulfoxide (purity, 99%) was purchased from Panreac Quimica (Barcelona, Spain). NADPH (purity, ≥97%) was obtained from Sigma-Aldrich, GmbH (Steinheim, Germany). Deionized water from Milli-QR Gradient A10R, pore size of 0.22 mm (Millipore, Moscheim Cedex, France) was used in this study. Blank rat plasma and liver microsomes were prepared from drug-free healthy rats that were obtained from the Animal Care and Use Centre, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia).

### 2.2 | Stock solution, calibration standards and quality control sample preparation

Stock solution of compound 6P (500 µg/mL) and IS (400 µg/mL) were prepared in dimethyl sulfoxide and methanol, respectively by accurately weighing of their standards. Separate stock solution of compound 6P was used for calibration standards (CS) and quality control (QC) samples preparation. A series of working solutions of compound 6P were also prepared by serial dilution of the stock solution in ACN/water (50: 50, *v*/v) mixture to achieve a concentration range of 100.8–25,000 ng/mL for CS and 337–22,500 for QC, respectively. The plasma CS was prepared by spiking an appropriate amount of working solutions into blank plasma to obtain eight different concentrations of 10.1, 33.6, 84.0, 210, 525, 1050, 1750 and 2500 ng/mL, respectively. The same procedure was followed to prepare three plasma QC samples: 33.7, 225 and 2250 ng/mL, and was treated as low QC, middle QC and high QC, respectively. A working solution for IS was also prepared in ACN/water (50: 50, *v*/v) mixture to obtain 40 ng/mL concentration. All spiked plasma QC and CS samples were stored in a deep refrigerator maintained at −80°C, whereas working solutions were stored at 4°C in the refrigerator.

### 2.3 | Sample preparation

The spiked CS, QC and unknown plasma samples were initially thawed and vortex-mixed before sample preparation. An aliquot of 150 µL of plasma sample was transferred into a 5 mL volume of propylene tube. Then 15 µL of IS (40 ng/mL) was transferred into each tube except the blank. Vortex-mixed and 1.5 mL of diethyl ether was transferred into each tube. Vortex-mixed again after capping of each tube for 1 min, followed by cold centrifugation at 4500 × *g* for 5 min. After centrifugation, the upper organic layer was separated into another clean tube and dried in a sample concentrator maintained at medium temperature (Thermo Scientific, Asheville, NC, USA). The dried sample was

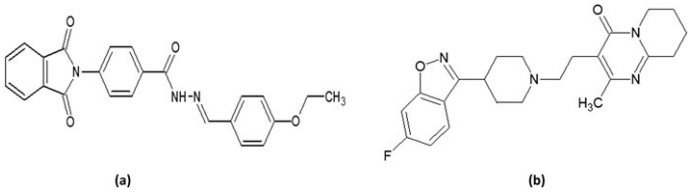


**FIGURE 1** Chemical structure of (a) compound 6P and (b) risperidone

reconstituted in 150 µL of mobile phase, vortex-mixed and 5 µL was injected for analysis in UPLC-MS/MS.

## 2.4 | Chromatographic conditions

The compound 6P and IS were separated on Aquity BEH™ $C_{18}$ column (100 × 2.1 mm; i.d., 1.7 µm) maintained at 40°C oven temperature. The mobile phase comprising of ACN and 10 mM ammonium acetate in the ratio of 85: 15, flowing at 0.3 mL/min was used in this study in the isocratic elution mode. The compound 6P and IS were retained at 0.82 and 0.92 min, respectively having 2 min run time. The autosampler temperature was maintained at 12°C and volume of injection was 5 µL.

## 2.5 | Mass spectrometry

A UPLC-MS/MS instrument, comprising of an Aquity H-class UPLC connected with a tandem quadruple detector (Waters Corp., Milford, MA, USA) was used in this study. The electrospray ionization source in positive mode was used for compound 6P and IS ionization. The detection was performed in multiple reaction monitoring (MRM) mode using parent to daughter ion transition of 414.06 > 148.05 for compound 6P and 411.18 > 191.07 for IS, respectively. The optimized MS/MS parameters were: capillary voltage (kV), 3.70; source temperature, 150°C; desolvating temperature, 350°C; dwell time, 0.106 s; desolvating gas (nitrogen) flow rate, 650 L/h; collision gas (argon) flow rate, 0.15 mL/min. The cone voltage for compound 6P and IS were optimized to 42 and 50 V, respectively whereas collision energy 40 and 24 eV, respectively. The UPLC-MS/MS system was operated by MassLynx software (Version 4.1) and data processing was performed by Target Lynx™ program.

## 2.6 | Method validation

The developed assay was fully validated to establish the selectivity, linearity, precision, accuracy, recovery, matrix effect and stability at different storage conditions according to the United States Food and Drug Administration bioanalytical method validation guidance (US FDA, 2001).

The assay selectivity was determined to ensure the absence of co-eluting peaks at the retention time of compound 6P and IS. Six blank rat plasma samples were randomly selected from different sources and were processed and extracted along with lower limit of quantification (LLOQ) samples. The resultant chromatogram of blank plasma sample was compared with the LLOQ sample.

The calibration curves of compound 6P in rat plasma at eight different points ranging from 10.1 to 2500 ng/mL were constructed between the peak area ratio (analyte/IS) and concentrations. The three calibration cures were used to determine the assay linearity by least squares regression method, which have correlation coefficient ($r^2$) values of ≥0.99. The lowest concentration of the calibration curve whose response is five times greater than the blank sample and can be analyzed with acceptable precision (percentage coefficient of variation, % CV, ≤20%) and accuracy (±20%) was considered the LLOQ of this assay.

The assay precision and accuracy were determined in plasma samples spiked at LLOQ (10.1 ng/mL) and all three QC level (33.7, 225 and 2250 ng/mL) concentrations, in five replicates for three consecutive days. Intra- and inter-day precision and accuracy were calculated by using independent calibration curve prepared for each batch. The precision value was limited to ≤15% of % CV except for LLOQ of ≤20%. The accuracy should also be limited to within ±15% of the nominal value, except at LLOQ of ±20%.

The extraction recovery of compound 6P and matrix effects (ion suppression/enhancement) of this assay were determined at all three QC (33.7, 225 and 2250 ng/mL) levels in five replicates. Separate sources of blank plasma were used for each replicate sample. The recovery was calculated by comparing the peak area of blank plasma spiked with compound 6P before extraction to the peak area of blank plasma spiked with compound 6P after extraction. The matrix effects were determined by comparing the peak area of plasma spiked with compound 6P after extraction with the peak area of compound 6P spiked in mobile phase solution. The same procedure was followed to determine recovery and the matrix effects of IS in rat plasma.

The stability of compound 6P at different anticipated storage conditions was determined by using five replicates of low and high (33.7 and 2250 ng/mL) QC concentrations. The QC samples were analyzed after specific conditions as follows: (1) short-term stability (room temperature for 8 h); (2) autosampler stability (12°C for 36 h); (3) long-term stability (−80°C for 60 days); and (4) freeze–thaw stability (three freeze–thaw cycles). All samples were tested against freshly prepared calibration curves and the average deviation of the concentrations was limited to be 15% of the nominal value.

## 2.7 | Liver microsomal preparation for metabolic study

Rat hepatic microsomes were prepared by a stepwise centrifugation method as reported previously (Iba, Soyka, & Schulman, 1977). Briefly, liver tissues (weighed 4–6 g) were chopped and finely crushed tissues were homogenized in 2 mL of buffer (0.05 M Tris HCl, 1.5% KCl and 1 mM EDTA, pH 7.2). The homogenate was centrifuged at 9000 ***g*** for 20 min at 4°C. The upper supernatant layer was transferred to ultracentrifuge tubes and again centrifuged at 100,000 ***g*** for 1 h at 4°C. The pellet was washed in 2 mL of KCl 150 mM and centrifuged again at 100,000 ***g*** for 1 h at 4°C. The pellet was finally suspended in 1 mL buffer (0.05 M Tris–HCl and 1 mM EDTA, pH 7.2) containing 20% glycerol, by manual homogenization. Microsomal suspensions were stored at −80°C until use. Pierce BCA Protein Assay Kit (Thermo Scientific) was used for the estimation of protein. The estimated protein concentration of pooled microsomes was 53.23 mg/mL.

## 2.8 | *In vitro* metabolic stability of compound 6P

A metabolic stability study is one of the most important preliminary steps in the discovery and development process of a lead candidate. *In vitro* metabolic study by using animal liver microsomes, is the most common approach for early estimation and prediction of *in vivo* metabolism (Baranczewski *et al*., 2006). Therefore, in this study, the metabolic study of compound 6P was determined using rat liver

microsomes by following a previously described method (Hill, 2003). Microsomal incubation was performed in triplicate for each time point. To a 1.5 mL microcentrifuge tube, 10 µL of the compound 6P (50 µg/mL concentration) was transferred. To this, 450 mL of prewarmed (37°C) 0.1 M phosphate buffer was added. Then 25 µL of freshly prepared 20 mM NADPH was added. All samples were incubated for 5 min at 37°C in a shaking water bath and then the reaction was initiated by adding 10 µL of microsomes (final concentration 0.5 mg/mL) into each tube. The incubation continued at 37°C in a shaking water bath and was terminated by adding IS containing 250 µL of cold ACN at 0, 5, 10, 20, 30, 60, 90 and 120 min. Samples were vortex-mixed followed by cold centrifugation at 10,500 *g*, for 5 min. The supernatant was transferred to a UPLC vial for analysis. The calibration curve was prepared in mobile phase with a similar concentration (10.1–2500 ng/mL) range to those spiked in plasma. The extent of metabolism was calculated by measuring the percentage of the compound 6P turnover.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Method development

The compound 6P (400 ng/mL) displayed sensitivity in the positive mode during tuning in electrospray ionization. It produced single and stable protonated molecular ions $[M + H]^+$ at *m/z* 414.06, which produced four daughter ions at *m/z* of 120.26, 148.05, 250.10 and 267.06, respectively after fragmentation. The daughter ion of *m/z* 148.05 shown maximum sensitivity and was selected for the MS/MS transition. Similarly, the IS (risperidone), whose molecular weight is close to the analyte (410.48) produced molecular ion $[M + H]^+$ at *m/z* 411.08 and a daughter ion at *m/z* 191.07 with the highest sensitivity. Therefore, the MRM transition of 414.06 > 148.05 and 411.18 > 191.07 were used for the detection and quantification of compound 6P for IS, respectively. Figures 2 and 3 represent the MRM transition spectra of compounds 6P and IS, respectively.

During the optimization of chromatographic separation parameters, both methanol and ACN were tried with different volatile buffers (formic acid, ammonium acetate and ammonium formate). The organic and aqueous mixtures were tried with different compositions and flow rates. The mobile phase containing ACN with 10 mM ammonium acetate in the ratio of 85: 15 produced the best separation of both compounds 6P and IS at flow rates of 0.3 mL/min. Both analyte and IS peaks were well resolved and eluted within 1 min.

For sample preparation, protein precipitation was initially tried by using methanol, ACN and 1-butanol as the precipitating agent. However, the result was not satisfactory due to poor recovery and ion suppression effects. Then liquid–liquid extraction was tried using ethyl acetate, diethyl ether, dichloromethane and n-hexane. Although, both ethyl acetate and diethyl ether produced satisfactory results, but

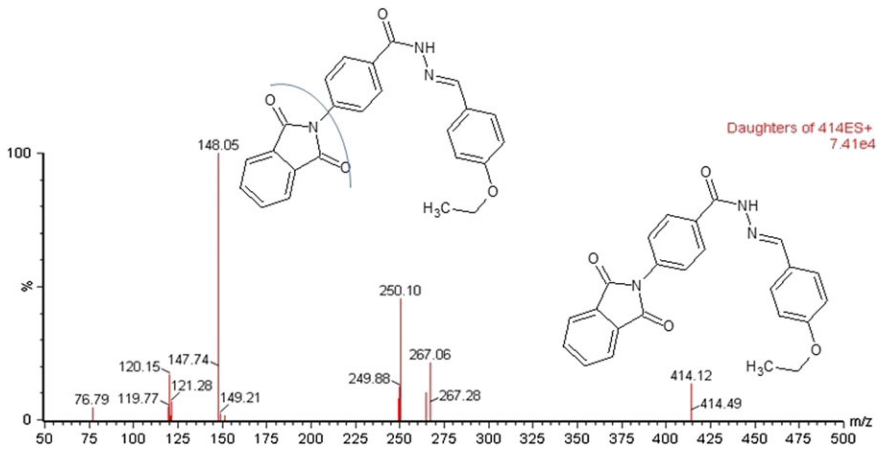


**FIGURE 2** Parent to daughter ion transition (MS/MS) spectra of compound 6P

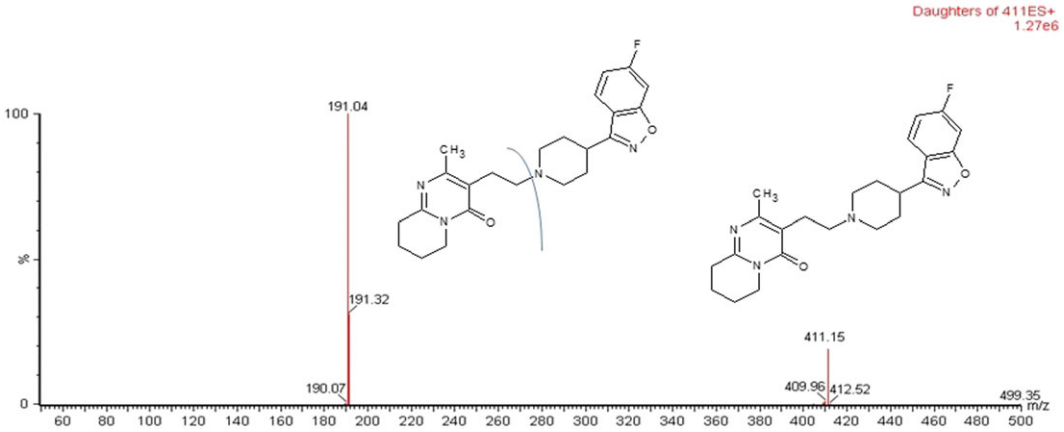


**FIGURE 3** Parent to daughter ion transition (MS/MS) spectra of internal standard

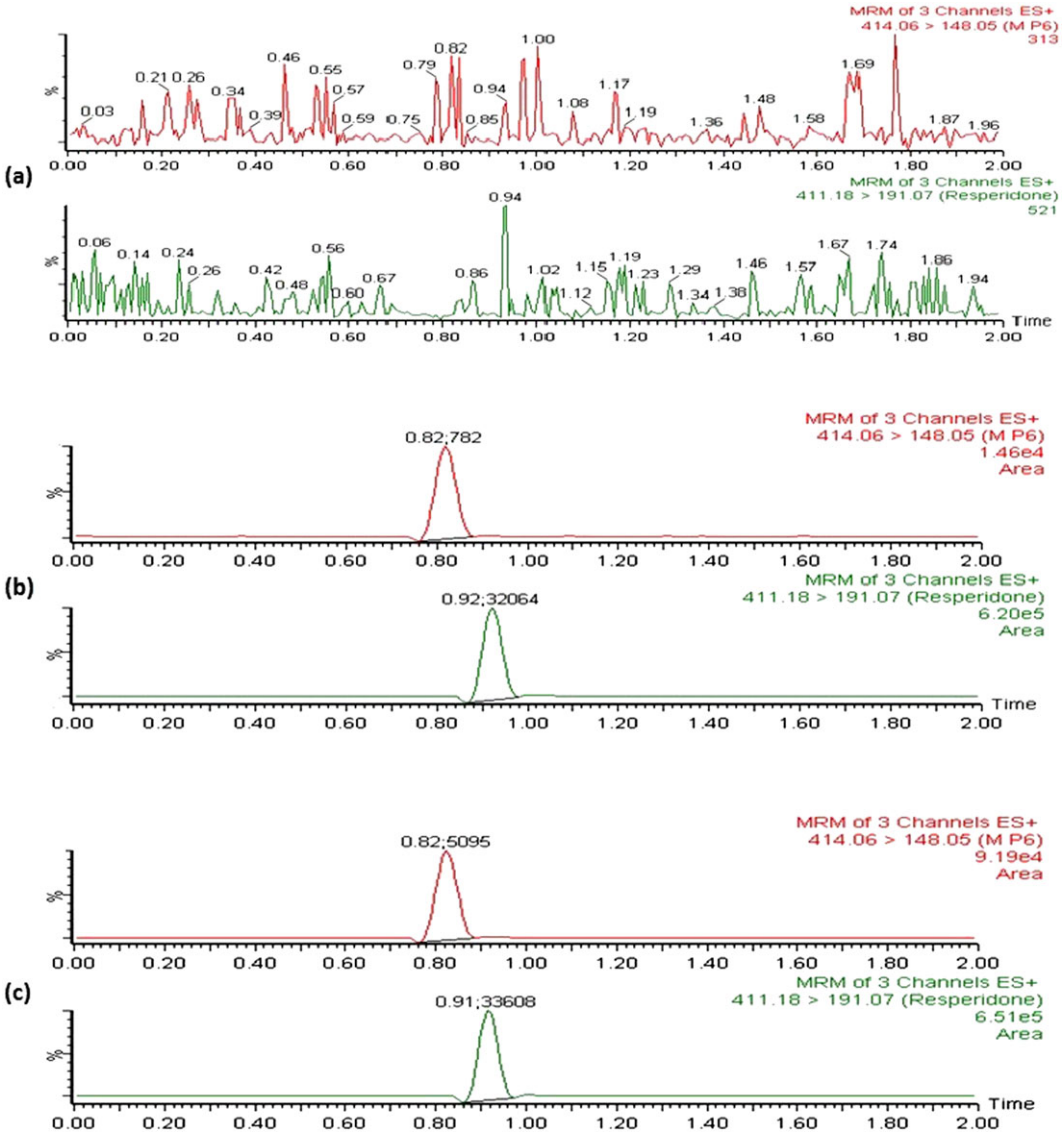


**FIGURE 4** Representative chromatograms of compound 6P and internal standard in (a) blank plasma (b) plasma spiked at lower limit of quantification level and (c) 1 h after oral administration of compound 6P (10 mg/kg) in rat. MRM, multiple reaction monitoring

diethyl ether produced maximum recovery and lower interference and was considered for sample extraction procedures.

## 3.2 | Method validation

### 3.2.1 | Selectivity and specificity

No significant interferences from the endogenous peak and/or MS response were observed in the blank chromatograms of compounds 6P and IS. Figure 4(a) represents the blank chromatograms of compounds 6P and IS. These results confirmed that the developed assay is selective and specific for compound 6P determination.

### 3.2.2 | Linearity of plasma calibration curve and lower limit of quantification

The resultant three calibration curves were generated in rat plasma and showed good linearity in the concentration range of 10.1–2500 ng/mL with a coefficient of determination ($r^2$) value of ≥0.998. The back concentration calculation for all points was done by using weighing

**TABLE 1** Intra-day and inter-day precision and accuracy of compound 6P in rat plasma

| Nominal concentration (ng/mL) | Measure concentration, mean ± SD | Precision (% CV) | Accuracy (%) |
|---|---|---|---|
| Intra-day variation ($n$ = 5) | | | |
| 10.1 | 11.6 ± 0.86 | 7.42 | 115.2 |
| 33.7 | 29.8 ± 1.88 | 6.31 | 88.4 |
| 225 | 239 ± 9.25 | 10.2 | 97.3 |
| 2250 | 2023 ± 80.5 | 3.98 | 89.9 |
| Inter-day variation ($n$ = 15) | | | |
| 10.9 | 11.4 ± 0.70 | 6.16 | 113.2 |
| 33.7 | 30.9 ± 2.43 | 7.87 | 91.7 |
| 225 | 221 ± 18.41 | 8.32 | 98.3 |
| 2250 | 2025 ± 117.9 | 5.82 | 90.0 |

CV, coefficient of variation.

**TABLE 2** Recovery and matrix effect of compound 6P (three quality control samples) and IS in rat plasma ($n = 5$)

| Compound | Nominal concentration (ng/mL) | Extraction recovery | | Relative matrix effects | | IS normalized matrix effects | |
|---|---|---|---|---|---|---|---|
| | | % Mean ± % SD | % cv | % Mean ± % SD | % cv | % Mean ± % SD | % cv |
| Compound 6 | 33.7 | 83.1 ± 5.13 | 6.17 | 96.8 ± 3.93 | 4.06 | 1.02 ± 0.07 | 7.30 |
| | 225 | 80.7 ± 9.03 | 11.19 | 95.0 ± 3.78 | 3.97 | 1.00 ± 0.04 | 4.26 |
| | 2250 | 84.7 ± 2.70 | 3.19 | 91.0 ± 4.35 | 4.78 | 0.96 ± 0.09 | 9.09 |
| | Mean | 82.9 ± 2.02 | 2.43 | 94.30 ± 2.97 | 3.15 | 0.99 ± 0.03 | 3.09 |

CV, coefficient of variation; IS, internal standard.

factor of $1/x^2$ least square linear regression. With the stated calibration curve, its lower point of 10.1 ng/mL concentration could be reliably distinguished from baseline with a signal to noise ratio of ≥5. Therefore, it was considered as the LLOQ of the developed method. Moreover, it was analyzed with an acceptable limit of precision (≤20%, CV) and accuracy (±20%). Figure 4(b) and (c) represent the chromatograms of compounds 6P and IS at the LLOQ level and 1 h after oral administration of compound 6P (10 mg/kg) in rats, respectively. Both compounds 6P and IS were eluted at 0.82 and 0.92 min, respectively with a total run time of 2 min only.

### 3.2.3 | Precision and accuracy

The intra- and inter-day accuracy and precision data are presented in Table 1. The intra-day and inter-day precision were found to be ≤10.2 and ≤8.32%, respectively whereas the intra-day and inter-day accuracy were within 88.4–115.2% and 90.0–113.2%, respectively compared to nominal concentrations. These findings confirmed that the method is reproducible and precise, and suitable for accurate determination of compound 6P in real plasma samples.

### 3.2.4 | Recovery and matrix effects

The extraction recovery and matrix effects, which were evaluated in the rat plasma matrix are presented in Table 2. The average value of recovery between three QC concentrations was 82.9% with % CV of 2.43 by the proposed extraction method. It confirms that the recovery of compound 6P is consistent, precise and concentration independent. The matrix effects were determined by the post-extraction method. No significant suppression effects of ions were observed due to matrix components in all three QC samples. The mean ion suppression effects measured between three QC concentrations were 94.30% with % CV of 3.15. The mean calculated value of IS normalized matrix effects was equivalent to 1 with a % CV of 3.09. Hence, it ruled out any possible interference from matrix effects in compound 6P quantification.

### 3.2.5 | Stability

The stability of spiked QC samples of compound 6P at different anticipated storage conditions were presented in Table 3. No significant loss of compound 6P was observed in different conditions as described in Section 2.5. The calculated percentage accuracies were within ±15% with precision of ≤15% for both low and high QC concentrations. These results rule out any possibility of degradation or loss of compound 6P during different anticipated storage conditions.

## 3.3 | *In-vitro* metabolic stability study

The concentrations of compound 6P at different time intervals were calculated by using the prepared calibration curve in mobile phase between the area ratio of compounds 6P and IS versus nominal concentrations of compound 6P. The percentage residual concentrations of parent compound 6P that remained in the incubation mixtures at different time intervals were measured. Figure 5 represents the percentage of compound 6P remained versus the incubation time point of the metabolic study. These results indicate that the compound 6P is slowly and partially metabolized, as 50% of the parent compound was still present after 2 h incubation in reaction mixtures.

**TABLE 3** Stability of compound 6P in different storage condition ($n = 5$)

| Stability | Nominal concentration (ng/mL) ($n = 5$) | Mean ± SD | Precision (% CV) | Accuracy (%) |
|---|---|---|---|---|
| Bench top (8 h) | 33.7 | 36.9 ± 2.90 | 7.87 | 109.5 |
| | 2250 | 2142 ± 161 | 7.51 | 95.2 |
| Freeze–thaw (3 cycle) | 33.7 | 33.1 ± 1.62 | 4.88 | 98.3 |
| | 2250 | 2037 ± 194 | 9.55 | 90.6 |
| In injector (36 h) | 33.7 | 35.9 ± 2.02 | 5.61 | 106.6 |
| | 2250 | 2134 ± 98.1 | 4.60 | 94.8 |
| 60 days at −80°C | 33.7 | 33.7 ± 3.36 | 9.95 | 100.1 |
| | 2250 | 2019 ± 87.0 | 4.31 | 89.7 |

CV, coefficient of variation.

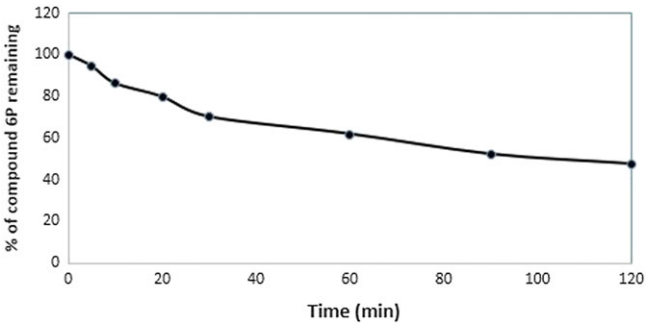


**FIGURE 5** Metabolic stability profile of compound 6P in rat liver microsomes

## 4 | CONCLUSION

In this study, the UPLC-MS/MS assay was developed and validated for the determination of compound 6P, a potential anti-inflammatory agent in biological fluids. The assay was sensitive, selective and had a large calibration range (10.1–2500 ng/mL), and was suitable for preclinical pharmacokinetic and toxicokinetic studies. The reported results indicate that the assay could meet the requirement for analysis of this compound in amounts expected to be present in actual samples. The developed assay was applied in the metabolic stability study of the compound 6P in rat liver microsomes and is being used for preclinical pharmacokinetic studies. The results of a metabolic study indicate that the compound 6P is slowly and partially metabolized in rat hepatic microsomes.

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