Simultaneous determination of acetaminophen and oxycodone in human plasma by LC–MS/MS and its application to a pharmacokinetic study

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A simple and rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for simultaneous determination of acetaminophen and oxycodone in human plasma. Acetaminophen-d4 and oxycodone-d3 were used as internal standards. The challenge encountered in the method development that the high plasma concentration level of acetaminophen made the MS response saturated while the desired lower limit of quantification (LLOQ) for oxycodone was hard to reach was well solved. The analytes were extracted by protein precipitation using acetonitrile. The matrix effect of the analytes was avoided by chromatographic separation using a hydrophilic C18 column coupled with gradient elution. Multiple reaction monitoring in positive ion mode was performed on tandem mass spectrometer employing electrospray ion source. The calibration curves were linear over the concentration ranges of 40.0–8000 ng/mL and 0.200–40.0 ng/mL for acetaminophen and oxycodone, respectively. This method, which could contribute to high throughput analysis and better clinical drug monitoring, was successfully applied to a pharmacokinetic study in healthy Chinese volunteers.

1. Introduction

Acetaminophen is a frequently used analgesic and antipyretic drug worldwide. Oxycodone hydrochloride, a semi-synthetic opiate derivative of thebaine, is widely used in analgesic agents due to remarkable analgesia effect and good tolerance [1]. The FDA-approved Percocet [2], combining acetaminophen with oxycodone, can be effective in pain control and help in reducing side effects and drug dependence. The commonly used dosage regimen is 325 mg and 5 mg for acetaminophen and oxycodone, respectively. The combination of acetaminophen and oxycodone provides a synergistic and opioid-sparing effect. Additionally, this combination has a safe pharmacokinetic profile, without increasing the incidence of drug addiction and acetaminophen-associated hepatotoxicity [3–7]. As the abuse of prescription opioids continues to rise, measurement of acetaminophen and oxycodone in human plasma will help researchers with better drug monitoring.

Several methods have been developed for the determination of acetaminophen in biological matrix, including immunoassay [8], gas chromatography (GC) [9,10], capillary electrophoresis [11], high performance liquid chromatography (HPLC) with UV detector [12–16] or with tandem mass spectrometer [17–19]. The analysis of oxycodone alone or with its major metabolites in human plasma with electrochemical detection [20,21], GC [22,23], liquid chromatography–tandem mass spectrometry (LC–MS) [24,25] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [26–37] has also been reported. Literature survey revealed that the majority of published methods determined acetaminophen and oxycodone in biological fluids individually, or in combination with other drugs [38–42]. Devarakonda et al. [43] quantified acetaminophen and oxycodone in plasma with liquid-liquid extraction, but the sample preparation and method validation were not clearly described.

Simultaneous determination of acetaminophen and oxycodone in human plasma by LC–MS/MS is challenged by two problems, one is the weak retention of the analytes on traditional C18 columns, and the other is that the plasma concentration of acetaminophen is much higher than that of oxycodone. The present article focuses on troubleshooting in method development, including screening of columns for better retention of the analytes, and overcoming the MS response saturation to acetaminophen...
while ensuring the detection sensitivity for oxycodone. Finally, the application of the validated method to a clinical pharmacokinetic study in healthy Chinese volunteers following oral administration of Percocet tablet is described.

2. Experimental

2.1. Chemicals and reagents

The reference standards of acetylmorphine and acetaminophen-d4 were purchased from Toronto Research Chemicals (Toronto, Canada). The reference standards of oxycodone hydrochloride and oxycodone-d3 solution were purchased from Sigma-Aldrich Company, USA. HPLC grade methanol and acetonitrile were purchased from Merck KGaA (Darmstadt, Germany). Acetic acid and ammonium acetate were obtained from Sigma-Aldrich Company, USA. Ultrapure water was generated in house with a Milli-Q system (Millipore, Bedford, MA, USA) and was used throughout the study. Blank human plasma was obtained from healthy Chinese volunteers. All the volunteers were given informed consent.

2.2. Liquid chromatography and mass spectrometric conditions

An Exion LC system (Applied Biosystems/Sciex, USA) consisted of a binary AD pump, a vacuum degasser, an autosampler (AD multiplate sampler) and a temperature-controlled compartment for column (AD column oven). Separation of analytes was performed on a Venusil ASB C18 Column (2.1 mm × 50 mm, 3 μm, 150 A; Bonna-Agela Technologies, Tianjin, China) with a Security 6500™ for curtain gas; 8 psi for collision activation dissociation; 650 °C for turbo heater temperature; 3500 V for ionspray voltage; 50 psi for turbo heater temperature; 3500 V for ionspray voltage; 50 psi for turbomass ion source in positive mode. Quantitation was performed using the multiple reaction monitoring (MRM) with a dwell time of 0.5 mL/min. The injection volume was 5 μL. The total run time was 4.5 min and the initial condition at 3.0 min and held for another 1.5 min. The composition of mobile phase B was maintained at 80% from 2.1 min to 2.8 min. The system returned to the initial condition at 3.0 min and held for another 1.5 min. The gradient elution program started at a composition of 6% B for 0.4 min, and then was ramped to 15% B at 0.5 min and held for another 1.5 min. The composition of mobile phase B was maintained at 80% from 2.1 min to 2.8 min. The system returned to the initial condition at 3.0 min and held for another 1.5 min. The total run time was 4.5 min and the flow rate was constantly 0.5 mL/min. The injection volume was 5 μL.

Detection of analytes and internal standards was operated on a triple quadrupole mass spectrometer, AB SCIEX Triple Quad™ 6500+ (Applied Biosystems/Sciex, USA), equipped with an electrospray ion source in positive mode. Quantitation was performed using the multiple reaction monitoring (MRM) with a dwell time of 100 ms per transition. The MRM parameters of acetylmorphine, oxycodone, acetaminophen-d4 and oxycodone-d3 are listed in Table 1. The optimized source parameters were as follows: 30 psi for curtain gas; 8 psi for collision activation dissociation; 650 °C for turbo heater temperature; 3500 V for ionspray voltage; 50 psi for Gas 1; 55 psi for Gas 2. Quadrupole 1 and quadrupole 3 were set at 8° and 65° for collision activation dissociation, respectively. The security curtain gas was maintained at 40 psi for curtain gas; 8 psi for collision activation dissociation; 650 °C for turbo heater temperature; 3500 V for ionspray voltage; 50 psi for turbomass ion source in positive mode. Quantitation was performed using the multiple reaction monitoring (MRM) with a dwell time of 0.5 mL/min. The injection volume was 5 μL. The total run time was 4.5 min and the initial condition at 3.0 min and held for another 1.5 min. The composition of mobile phase B was maintained at 80% from 2.1 min to 2.8 min. The system returned to the initial condition at 3.0 min and held for another 1.5 min. The total run time was 4.5 min and the flow rate was constantly 0.5 mL/min. The injection volume was 5 μL.

Table 1

| Analytes               | Precursor (Da) | Product (Da) | DP (V) | EP (V) | CE (V) | CXP (V) |
|------------------------|----------------|--------------|--------|--------|--------|--------|
| Acetylmorphine         | 152.0          | 110.1        | 38     | 8      | 42     | 12     |
| Oxycodone             | 315.1          | 241.1        | 56     | 12     | 39     | 13     |
| Acetaminophen-d4      | 155.8          | 114.1        | 90     | 14     | 30     | 11     |
| Oxycodone-d3           | 319.0          | 244.1        | 84     | 8      | 39     | 10     |

2.3. Preparation of stock and working solutions

Primary stock solutions for preparation of calibration standards and quality control (QC) samples were prepared from separate weighing. The standard stock solutions of acetylmorphine, oxycodone and acetaminophen-d4 were prepared by dissolving accurately weighed compounds in acetonitrile-water (50:50, v/v) to give a concentration of 1.0 mg/mL. The primary standard solution of oxycodone-d3 in methanol was provided at a certified concentration of 1.0 mg/mL. The combined working solutions of analytes over the desired concentration range were prepared by further dilution of stock solutions with acetonitrile-water (50:50, v/v). A combined internal standard working solution was prepared in acetonitrile-water (50:50, v/v) at 10.0 ng/mL for oxycodone-d3 and 80.0 ng/mL for acetaminophen-d4. All stock and working solutions were stored at −20 °C and brought to room temperature before use.

2.4. Preparation of calibration standards and QC samples

The calibration standards and QC samples were prepared by spiking plasma with appropriate volume of respective working solutions. Calibration standards were prepared at concentrations of 40.0, 80.0, 200, 600, 2000, 5000, and 8000 ng/mL for acetaminophen; 0.200, 0.400, 1.00, 3.00, 10.0, 25.0, and 40.0 ng/mL for oxycodone. QC samples were prepared at 40.0 ng/mL (lower limit of quantification, LLOQ), 100 ng/mL (low QC, LQC), 1200 ng/mL (middle quality control, MQC), and 6400 ng/mL (high quality control, HQC) for acetaminophen; 0.200 ng/mL (LLOQ), 0.500 ng/mL (LQC), 6.00 ng/mL (MQC), and 32.0 ng/mL (HQC) for oxycodone.

2.5. Sample preparation

50 μL aliquot of plasma sample was transferred to a clean 96-well plate and mixed with 25 μL of internal standard working solution. The mixture was deproteinized with 200 μL acetonitrile, vortex-mixed for 10 min, and then centrifuged at 4000 rpm for 10 min. 30 μL of the supernatant was transferred to another clean 96-well plate, to which 330 μL of acetonitrile-water (10:90, v/v) was added. After vortex mix for 3 min, the mixture was injected into the LC-MS/MS system for analysis.

2.6. Method validation

The validation of this method was carried out following the USFDA guidelines [44]. The validation included specificity, linearity, precision and accuracy, recovery, matrix effect, dilution integrity, carryover effect and stability.

The specificity was tested for interference in the MRM channels using the proposed extraction procedure and LC-MS/MS conditions. Six batches of blank plasma obtained from six individual volunteers were analyzed and the results were compared to those obtained from samples at LLOQ. Best-fit calibration curves of peak area ratio versus analyte concentrations were drawn for acetylmorphine and oxycodone. The calibration curve was fitted to a 1/x² weighed regression analysis where x is the concentration of the analyte. Precision and accuracy were evaluated at four concentration levels (LLOQ, LQC, MQC, and HQC) in six replicates. Intra-run precision and accuracy were assessments of precision and accuracy during a single analytical run. Inter-run precision and
accuracy were evaluated over two days by quantification of three validation runs. Recovery experiments were performed by comparing peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (QC working solutions spiked in extracted plasma) at LQC, MQC and HQC levels. The matrix effect was assessed by comparing the peak area obtained from an amount of the analyte added in extracted blank plasma to the peak area obtained from equal concentration of the analyte in solvent. Dilution integrity was performed with six replicates each for samples spiked at concentrations of HQC and 2 times of the upper limit of quantification (ULOQ) diluted 5-fold with blank plasma. In this study, stability tests evaluated the stability of acetaminophen and oxycodone in stock solutions and plasma samples under different conditions. For stock solutions, the stability was evaluated by comparing the area response of analytes in stability samples to that of freshly prepared stock solutions. For plasma samples, bench top stability, autosampler stability, freeze-thaw stability and long-term stability were evaluated with three replicates each at LQC and HQC levels.

2.7. Pharmacokinetic study

Ten healthy Chinese volunteers were enrolled in the clinical pharmacokinetic study. Preliminary screening involved a medical history, physical examination, medical and laboratory evaluations. The protocol approval was obtained from a local ethics committee and informed consent was obtained from each subject. After an overnight fast of at least 10 h, all subjects were administered with a single dose of one Percocet tablet (acetaminophen/oxycodone, 325 mg/5 mg, Endo Pharmaceuticals Inc.). Blood samples were collected before drug administration (0 h) and at 0.17, 0.33, 0.5, 0.75, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 h post-dosing. Whole blood was collected into heparinized tubes and centrifuged at 3000 rpm for 5 min. The plasma samples were stored at −20 °C until analysis. The pharmacokinetic parameters were calculated with non-compartment model using WinNonlin software version 6.4.

3. Results and discussion

3.1. Method development

The method development included selection of mobile phase, column types, MS detector, MS parameters and sample extraction. Acetaminophen and oxycodone are compounds with low lipophilicity and retain weakly on C18 columns; thus the initial organic phase ratio should be low to achieve appropriate chromatographic separation. In the column screening stage, appropriate retention for both acetaminophen and oxycodone could not achieve on numerous C18 columns, such as a Zorbax Eclipse Plus C18 column (2.1 mm × 50 mm, 3.5 μm; Agilent Technologies), a Zorbax Extend-C18 column (2.1 mm × 50 mm, 3.5 μm; Agilent Technologies) and a Poroshell 120 SB-C18 column (2.1 mm × 50 mm, 2.7 μm; Agilent Technologies), except on an Ultimate XB-C18 column (2.1 mm × 100 mm, 3 μm; Welch Materials, Inc., Shanghai, China). It was observed that pure water and acetonitrile as mobile phase gave the best sensitivity and peak shape on the Ultimate XB-C18 column. Tailing peak was observed when methanol was used as mobile phase. Addition of acid in mobile phase A resulted in oxycodone eluted at dead time and addition of ammonium acetate led to unacceptable peak shapes of oxycodone. However, endogenous interference and matrix effect existed while pure water
was employed as mobile phase A. A Venusil ASB C18 column (2.1 mm × 50 mm, 3 μm, 150 Å) was then tested. The optimized mobile phase was composed of 2 mM ammonium acetate containing 0.1% (v/v) acetic acid and acetonitrile to achieve symmetrical peak shapes and better MS response. Higher concentration of ammonium acetate at 5 mM and 10 mM would inhibit MS response for all analytes. More addition of acid like 0.2% (v/v) acetic acid or 0.1% (v/v) formic acid in aqueous mobile phase would weaken the chromatographic retention of the analytes. The difference between the above two columns lies in that the Ultimate XB-C18 column is a traditional reversed-phase column with end-capping while the Venusil ASB C18 column is an uncapped hydrophilic column. The end-capping process might increase the lipophilicity of stationary phase, resulting in weak absorption of polar compounds. The hydrophilic column could provide better chromatographic retention and separation for strong polar compounds within a pH range of 1–5. The proportion of organic phase started at 6% to elute endogenous substance, and then increased to 15% to separate acetaminophen and oxycodone. Optimized flow rate of 0.5 mL/min helped in separation and elution of all compounds in 2 min. Whereafter, the composition of mobile phase B was set at 80% for 0.8 min to wash column and inhibit matrix effect. The elution program changed to the initial condition in order to back toward equilibrium state.

The MS-MS detection was initially performed on an API 4000 mass spectrometer (Applied Biosystems/Sciex, USA). The plasma concentration of acetaminophen was much higher than that of oxycodone after oral administration of Percocet tablet. If the analytes in plasma were not diluted, the plasma concentration of acetaminophen would be so high that the saturation of MS response to acetaminophen made it challenging that both analytes in processed samples could give suitable signal intensities simultaneously via the same sample preparation. In order to avoid the MS response saturation to acetaminophen as well as guarantee the LLOQ of oxycodone detection, the analytes in plasma were diluted to a limited extent. Despite massive dilution of analytes in plasma, the MS response saturation to acetaminophen could not be avoided completely. Optimization of collision energy was also conducted to make fewer product ions of acetaminophen generated in Q3 to avoid the MS response saturation to acetaminophen. The MS condition optimization test showed that to obtain the highest intensity for acetaminophen, the collision energy of 20 V should be adapted. Actually, a collision energy we wanted should inhibit the MS response saturation to acetaminophen. This meant that the collision energy should be increased appropriately to decrease the generation of the product ions of acetaminophen to an acceptable extent. Finally, the collision energy for acetaminophen was adjusted to 42 V to obtain adaptive MS response. Other optimized parameters are listed in Table 1.

Previous pharmacokinetic research of drug formulations combining acetaminophen and oxycodone reported extraction of acetaminophen, oxycodone simultaneously from human plasma with liquid-liquid extraction [43]. As the purpose was to develop a simple and rapid method, protein precipitation was tested. Precipitants including methanol, acetonitrile and methanol-acetonitrile (1:1, v/v) were compared. Single precipitant of acetaminophen showed higher extraction recovery and negligible matrix effect. Due to the saturation of MS response to acetaminophen at high concentration level, the volume of acetonitrile-water (10:90, v/v) used to dilute the supernatant of deproteinized samples was tested. The results of a 4-fold dilution, an 8-fold dilution and a 12-fold dilution are given in Fig. 2. A 12-fold dilution, 30 μL of the supernatant diluted with 330 μL of acetonitrile-water (10:90, v/v), showed the best linearity. The major advantage was its efficiency in simultaneous extraction of both analytes and internal standards with single step of protein precipitation.

3.2. Method validation

3.2.1. Specificity

The typical chromatograms of blank plasma, drug-free plasma spiked with both analytes at LLOQ and a clinical sample from a volunteer at 4 h after single oral administration of one Percocet tablet are shown in Fig. 3. No significant endogenous interference was observed in blank plasma at the retention time of acetaminophen, oxycodone and internal standards. Potential interference of stable isotope-labeling internal standards was also evaluated in this study. The internal standards had no interference in the channel of acetaminophen and oxycodone.

3.2.2. Linearity

The calibration curves showed good linearity over the concentration of 40.0–8000 ng/mL for acetaminophen and 0.200–40.0 ng/mL for oxycodone. The correlation coefficient ($r$) of the
weighed calibration curves for both analytes during the validation was ≥ 0.995. Precision and accuracy of the back-calculated concentrations of calibration standards well met the acceptance criteria.

3.2.3. Precision and accuracy

The intra-run and inter-run precision and accuracy was summarized in Table 2. The regression equations for calibration curves were used to back-calculate the measured values of QC samples at

| Analytes     | Intra-run Mean concentration (ng/mL) | Accuracy (%) | CV% | Inter-run Mean concentration (ng/mL) | Accuracy (%) | CV% |
|--------------|-------------------------------------|--------------|-----|-------------------------------------|--------------|-----|
| Acetaminophen|                                     |              |     |                                     |              |     |
| LLOQ QC (40.0 ng/mL) | 38.7 ± 0.5                        | 96.8         | 1.3 | 39.3 ± 3.3                         | 98.3        | 8.4 |
| LQC (100 ng/mL)   | 97.0 ± 4.1                        | 97.0         | 4.2 | 101 ± 9                            | 101.0       | 8.9 |
| MQC (1200 ng/mL)  | 1160 ± 17                         | 96.7         | 1.5 | 1187 ± 42                          | 98.9        | 3.5 |
| HQC (6400 ng/mL)  | 6097 ± 201                        | 95.3         | 3.3 | 6130 ± 180                         | 95.8        | 2.9 |
| Oxycodone       |                                     |              |     |                                     |              |     |
| LLOQ QC (0.200 ng/mL) | 0.196 ± 0.019                    | 98.0         | 9.7 | 0.198 ± 0.020                      | 99.0        | 10.1|
| LQC (0.500 ng/mL) | 0.513 ± 0.028                    | 102.6        | 5.5 | 0.507 ± 0.010                      | 101.4       | 2.0 |
| MQC (6.00 ng/mL)  | 5.96 ± 0.16                       | 99.3         | 2.7 | 6.05 ± 0.15                        | 100.8       | 2.5 |
| HQC (32.0 ng/mL)  | 32.0 ± 1.4                        | 100.0        | 4.4 | 32.2 ± 1.1                         | 100.6       | 3.4 |

The mean concentration was presented in the form of mean ± SD. CV, coefficient of variation.
four concentration levels. For both acetaminophen and oxycodone, the deviations of mean value from the nominal value were all within ± 15% and the coefficient of variation (CV) determined at each concentration level did not exceed 10.1%.

3.2.4. Recovery
Six replicates at LQC, MQC and HQC were prepared for recovery experiment. The recovery extent of each analyte and its corresponding internal standard was consistent and reproducible. The mean recovery data (with the precision) at LQC, MQC and HQC was 96.0% (7.6%), 94.7% (5.0%), and 95.7% (4.5%) for acetaminophen; 91.1% (5.4%), 94.0% (2.4%), and 94.7% (2.3%) for oxycodone. The recovery of acetaminophen-d4 was 95.3% with the precision of 9.3%. The recovery of oxycodone-d3 was 94.1% with the precision of 2.6%.

3.2.5. Matrix effect
No significant matrix effect was observed in six batches of human plasma for both analytes at three concentration levels (low, middle and high). The matrix effects at concentrations of LQC, MQC and HQC were (96.7 ± 6.0) %, (97.8 ± 3.2) %, and (98.5 ± 1.4) % for acetaminophen; (96.6 ± 2.7) %, (98.6 ± 1.1) %, and (97.3 ± 1.0) % for oxycodone. The matrix effect of acetaminophen-d4 and oxycodone-d3 was (92.4 ± 1.6) % and (97.8 ± 1.6) %, respectively.

3.2.6. Dilution integrity
The diluted samples were prepared by a 5-fold dilution of samples at HQC and 2 times of the ULOQ with blank plasma. The determined concentrations were corrected for dilution factor. The mean back calculated concentrations for both analytes at each concentration level did not exceed 10.1%.

3.2.7. Carryover effect
Carryover was assessed and monitored by analyzing blank plasma samples instantly following plasma samples at ULOQ. No obvious response at the retention time of analytes was observed in blank matrix samples.

3.2.8. Stability
The stock solution was found to be stable for 20 h at room temperature and 33 days at −20 °C. Table 3 lists the stability of acetaminophen and oxycodone in human plasma under different storage conditions.

### Table 3

| Storage conditions          | Analytes     | Nominal (ng/mL) | Mean (ng/mL) | RE (%) | CV (%) |
|----------------------------|--------------|-----------------|--------------|--------|--------|
| Autosampler stability       | Acetaminophen| 100             | 99.7         | −0.3   | 5.5    |
| (24 h, 8 °C)                | Oxycodone    | 6400            | 6247         | −2.4   | 5.8    |
|                            |              | 6000            | 5994         | 0.3    | 5.0    |
|                            |              | 5800            | 5794         | 0.1    | 4.8    |
| Bench top stability         | Acetaminophen| 100             | 98.7         | −1.3   | 1.0    |
| (24 h, room temperature)    | Oxycodone    | 6400            | 6170         | −3.6   | 1.6    |
|                            |              | 6000            | 5970         | 0.3    | 3.2    |
|                            |              | 5800            | 5770         | 0.2    | 3.0    |
| Freeze-thaw stability       | Acetaminophen| 100             | 103          | 3.0    | 2.9    |
| (3 cycles, −20 °C)          | Oxycodone    | 6400            | 5740         | −10.3  | 4.2    |
|                            |              | 6000            | 5510         | −13.5  | 4.6    |
|                            |              | 5800            | 5380         | −16.7  | 5.0    |
| Long-term stability         | Acetaminophen| 100             | 93.3         | −6.7   | 5.0    |
| (23 days, −20 °C)           | Oxycodone    | 6400            | 5740         | −10.3  | 0.5    |
|                            |              | 6000            | 5510         | −13.5  | 1.0    |
|                            |              | 5800            | 5380         | −16.7  | 1.0    |

**RE**, relative error; **CV**, coefficient of variation.

3.3. Method application
The validated method was applied to quantify acetaminophen and oxycodone in human plasma samples collected from healthy Chinese volunteers after a single oral administration of one Percocet tablet. The mean plasma concentration-time profile of acetaminophen and oxycodone is shown in Fig. 4. The typical pharmacokinetic parameters are presented in Table 4. No literature revealed the pharmacokinetic profile of acetaminophen after administration of Percocet. Gammaitoni et al. [30] only evaluated the pharmacokinetics of oxycodone following single-dose administration of Percocet at three dose levels. For the 325 mg/5 mg (acetaminophen/oxycodone) dose level, the reported mean values of peak plasma concentration (Cmax), area under the plasma concentration-time curve from 0 h to 24 h (AUC0–24), time to peak plasma concentration (Tmax) and terminal elimination half-life (T1/2) were 9.96 ng/mL, 48.62 h ng/mL, 1.33 h and 3.28 h, respectively. AUC0–24 and T1/2 of oxycodone listed in Table 4 were consistent with those earlier reported values. Cmax of oxycodone in Table 4 was slightly higher than the value in the previous study while Tmax was somewhat lower. This may be due to differences in race, age and genetics of the study subjects.
Table 4
Pharmacokinetic parameters of acetaminophen and oxycodone in healthy Chinese volunteers following oral administration of one Percocet tablet (mean ± SD, n = 10).

| Parameters | Acetaminophen | Oxycodone |
|------------|---------------|------------|
| C_{max} (ng/mL) | 6326 ± 2574 | 12.8 ± 4.6 |
| AUC_{0-24} (ng/mL) | 16,205 ± 5871 | 46.5 ± 17.0 |
| AUC_{0-t} (ng/mL) | 16,744 ± 6026 | 51.2 ± 18.1 |
| t_{1/2} (h) | 0.48 ± 0.41 | 0.77 ± 0.36 |
| t_{1/2} (h) | 4.56 ± 2.16 | 3.74 ± 1.18 |
| Ke (1/h) | 0.185 ± 0.083 | 0.204 ± 0.072 |
| MRT_{0-24} (h) | 3.81 ± 1.04 | 4.17 ± 0.90 |
| MRT_{0-\infty} (h) | 4.54 ± 1.42 | 5.49 ± 1.38 |

C_{max}, peak concentration in plasma; AUC, area under the plasma mean concentrations-time curve; T_{max}, time to peak concentration; t_{1/2}, terminal elimination half-life; Ke, elimination rate constant; MRT, mean residence time.

4. Conclusion

A simple method has been developed and validated for determination of acetaminophen and oxycodone in human plasma. To the best of our knowledge, no published methods are available for simultaneous quantitation of acetaminophen and oxycodone in human plasma using protein precipitation so far. The validated method has advantages in terms of the usage of simple protein precipitation extraction and a short analysis time, which promotes high-throughput pharmacokinetic study and is useful for routine therapeutic drug monitoring. This method has been successfully applied to a pharmacokinetic study in healthy Chinese volunteers following oral administration of one Percocet tablet.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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