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# Determination of betamethasone and betamethasone 17-monopropionate in human plasma by liquid chromatography–positive/negative electrospray ionization tandem mass spectrometry

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ABSTRACT

This study presents a high-performance liquid chromatography–positive/negative electrospray ionization tandem mass spectrometric (LC–ESI(+/−)–MS–MS) method for the determination of betamethasone (BOH) and betamethasone 17-monopropionate (B17P) in human plasma using beclomethasone dipropionate as the internal standard (I.S.). Both compounds were extracted from human plasma with ether–cyclohexane (4:1, v/v) and were separated by HPLC on a Hanbon Lichrospher $C_{18}$ column with a mobile phase of methanol–water (85:15, v/v) at a flow rate of 0.7 ml/min. Calibration curves were linear over the range of 0.10–50 ng/ml for BOH and 0.050–50 ng/ml for B17P. The inter-run relative standard deviations were less than 14.4% for BOH and 12.3% for B17P. The intra-run relative standard deviations were less than 9.3% for BOH and 7.9% for B17P. The mean plasma extraction recovery for BOH and B17P were in the ranges of 82.7–85.9% and 83.6–85.3%, respectively. The method was successfully applied to study the pharmacokinetics of a new formulation of betamethasone phosphate/betamethasone dipropionate injection in healthy Chinese volunteers.



## 1. Introduction

Compound betamethasone injection is made up of betamethasone phosphate (BSP, 5 mg/ml) and betamethasone dipropionate (BDP, 2 mg/ml), which is a synthetic long-acting glucocorticoid that depresses formation, release and activity of endogenous mediators of inflammation including prostaglandins, kinins, histamine, liposomal enzymes, and complement system. Also modifies body's immune response. The indications of compound betamethasone injection is systemic treatment of primary or secondary adrenal cortex insufficiency, rheumatic disorders, collagen diseases, dermatologic diseases, allergic states, allergic and inflammatory ophthalmic processes, respiratory diseases, hematologic disorders, neoplastic diseases, edematous states (resulting from nephrotic syndrome), GI diseases, multiple sclerosis, tuberculous meningitis and trichinosis with neurologic or myocardial involvement. [1].

The BSP prodrug, being highly ionized, is injected as a solution and exhibits rapid absorption after intramuscular administration. On the contrary, the BDP prodrug is highly hydrophobic, which is administered intramuscularly as a suspension. BDP has to first dissolve in the fluids of the intercellular space of muscle fibers before it can diffuse into the vascular space. So the absorption, distribution, metabolism, and excretion processes of BDP after intramuscular injection are very slow. The rate-limiting solubilization process is reflected in the terminal $t_{1/2}$ of the B17P, the active metabolite of BDP.

The chemical structures for BDP, BSP, B17P, B21P, BOH and I.S. are shown in Fig. 1. Methods for the detection and quantification of synthetic corticosteroids by gas chromatography/mass spectrometry (GC/MS) have been reported [2–4]. Although providing good sensitivity, these methods are not easy to use. Today, liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) represents a powerful alternative combining rapidity, specificity and sensitivity. Several publications have proposed the detection of corticosteroids using LC–MS–MS [5–9].

BSP is converted to its corresponding alcohol, BOH in vivo and the conversion is thought to occur rapidly and completely

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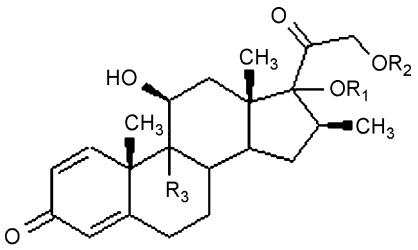


| Name | Molecular formula | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| BOH | $C_{22}H_{29}FO_5$ | H | H | F |
| B17P | $C_{25}H_{33}FO_6$ | Propionate | H | F |
| B21P | $C_{25}H_{33}FO_6$ | H | Propionate | F |
| BDP | $C_{28}H_{37}FO_7$ | Propionate | Propionate | F |
| BSP | $C_{22}H_{28}FNa_2O_8P$ | H | Phosphate | F |
| IS | $C_{28}H_{37}ClO_7$ | Propionate | Propionate | Cl |

**Fig. 1.** Molecular structures of BOH, B17P, B21P, BDP, BSP and I.S.

[10–12]. Several articles reported the pharmacokinetic profiles of beclomethasone dipropionate [13–17], but no articles described the determination methods and pharmacokinetic properties of betamethasone dipropionate and betamethasone-17-monopropionate in human plasma by now. It is believed that this paper is the first report of a simple and sensitive method to determine two main active metabolites, BOH and B17P in human plasma by LC–ESI(+/−)–MS–MS and study pharmacokinetics following administration of betamethasone phosphate/betamethasone dipropionate after intramuscular injection in healthy Chinese volunteers. The alternate acquisitions of positive and negative ions in selected reaction monitoring mode (SRM) also offered lower background and higher response.

## 2. Experimental

### 2.1. Chemicals and reagents

BOH (99.6% purity), B17P (99.7% purity), B21P (99.4% purity), BSP (99.6%, purity) and BDP (99.3% purity) were supplied by Zhejiang Xianhe Pharmaceutical Technology Co., Ltd. (Zhejiang, China). The test formulation was betamethasone phosphate/betamethasone dipropionate injection (each injection containing 5 mg BDP and 2 mg BSP) provided by Zhejiang Xianhe Pharmaceutical technology Co., Ltd. Methanol was of HPLC grade (TEDIA, USA). All other reagents were of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA) and was used to prepare all aqueous solutions.

### 2.2. Instrumentation and conditions

Analysis of sample standards was performed using an LC–MS–MS system (Thermo-Finnigan, USA) consisting of a Surveyor HPLC pump with a Surveyor autosampler and a TSQ Quantum triple-quadrupole mass spectrometer equipped with an ESI probe.

LC separation was performed on a Hanbon Lichrospher $C_{18}$ column (5 μm, 150 × 4.6 mm i.d.) with a mobile phase of methanol–water (85:15, v/v) at a flow rate of 0.7 ml/min. The LC eluent was split using a zero dead-volume T-type PEEK connector, and only one seventh (100 μl/min) of the LC eluent was allowed to enter the mass spectrometer via an ESI interface. The splitting lowered the detection limits of the analytes.

The TSQ Quantum mass spectrometer was equipped with an ESI source and operated in either positive or negative ion mode. The ESI source parameters tuned for maximum abundance ions of each analyte in a mobile phase of methanol–water (85:15, v/v) at 0.2 ml/min are shown in Table 1. For quantification, the mass spectrometer was set to the data acquisition mode of SRM and the acquisition parameters are presented in Table 1.

### 2.3. Preparation of stock and working solutions

Standard solutions of B17P and BOH were prepared at the concentration of 1 mg/ml in methanol. Working solutions were prepared and obtained by tenfold successive dilution at concentrations from 1 μg/ml to 1 ng/ml. The stock (1 mg/ml) and working solution (25 ng/ml) of the I.S. were prepared in the same way. All these solutions were stored at 8 °C.

### 2.4. Sample preparation

Aliquots (0.5 ml) of plasma sample were extracted with 3 ml ether–cyclohexane (4:1, v/v) after addition of 50 μl I.S. solution. After vortex mixing for 3 min, the mixture was centrifuged for 8 min at 4000 rpm. The organic phase was separated and evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in 150 μl mobile phase, and 8-μl aliquot of the reconstituted solution was injected into the LC–MS–MS system for analysis.

### 2.5. Specificity/selectivity and matrix effect [18]

The selectivity of the assay was checked by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Each blank sample should be tested for interference.

The matrix effect (ME) was defined as the direct or indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the sample. It was examined by comparing the peak areas of the analytes and I.S. between two different sets of samples. In set 1, analytes was

**Table 1**
Parameters for ESI source in positive and negative ion modes and SRM acquisition of BOH, B17P, B21P and I.S.

| | BOH | B17P | B21P | IS |
|---|---|---|---|---|
| Ionization mode | – | + | + | + |
| Spray voltage (V) | 3500 | 4000 | 4000 | 4000 |
| Sheath gas pressure | 10 | 10 | 10 | 10 |
| Auxiliary valve flow | 1 | 1 | 1 | 1 |
| Ion sweep gas pressure | 0.5 | 0.5 | 0.5 | 0.5 |
| Capillary temperature (°C) | 350 | 350 | 350 | 350 |
| Source CID (eV) | 15 | 18 | 18 | 16 |
| Ar gas | 1.0 | 1.0 | 1.0 | 1.0 |
| Collision energy (V) | 10 | 8 | 6 | 15 |
| Ion transition ($m/z$) for quantification | 361.0 → 307.2 | 471.1 → 397.1 | 471.1 → 451.1 | 543.1 → 433.2 |

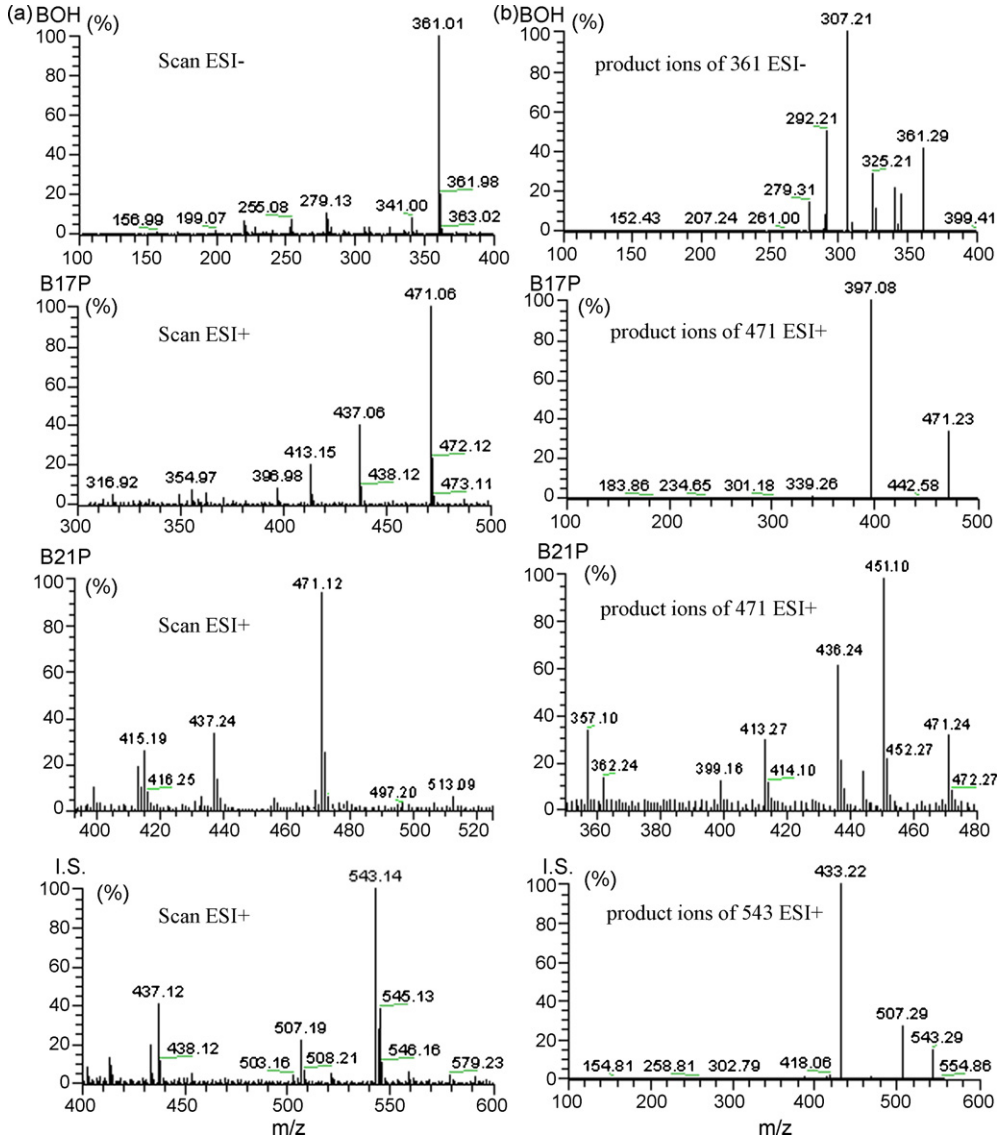


**Fig. 2.** (a) Precursor ions scan and (b) product ions scan spectra of BOH, B17P, B21P and I.S.

resolved in the blank plasma sample's reconstituted solution, and the obtained peak areas of analytes were defined as A. In set 2, analytes was resolved in mobile phase, and the obtained peak areas of analytes were defined as B. ME was calculated by using the formula: ME (%) = A/B × 100. The matrix effect of the assay was evaluated at three concentration levels of analytes, 0.20, 2.0 and 30 ng/ml for BOH and 0.080, 0.40 and 4.0 ng/ml for B17P. Five samples at each level of the analytes were analyzed. The blank plasma samples used in this study were five different batches of human blank plasma. If the ME values exceed the range of 85–115%, an endogenous matrix effect is implied.

### 2.6. Calibration standards and quality controls

Calibration standards of seven concentrations of BOH (0.10, 0.30, 1.0, 3.0, 10, 20 and 50 ng/ml) and B17P (0.050, 0.10, 0.20, 0.50, 1.0, 2.0 and 5.0 ng/ml) were extracted and assayed. The calibration curve was prepared and assayed along with the quality control (QC) samples and each batch of the clinical plasma samples.

The quality control (QC) samples were prepared in the blank plasma at concentrations of 0.20, 2.0 and 30 ng/ml for BOH, and 0.080, 0.40 and 4.0 ng/ml for B17P. The QC samples were prepared independently of the calibration standards, and analyzed with processed test samples at intervals in each run. The results of the QC samples provided the basis of accepting or rejecting the run.

### 2.7. Precision, accuracy and extraction recovery [18]

Validation samples were prepared and analyzed on three separate runs to evaluate the accuracy, intra-run and inter-run precision of the analytical method. The accuracy, intra-run and inter-run precision of the method were determined by analyzing five spiked samples at 0.20, 2.0 and 30 ng/ml for BOH, and 0.08, 0.4 and 4 ng/ml for B17P on each of three runs. Assay precision was calculated using the relative standard deviation (R.S.D.%). Accuracy is defined as the relative deviation in the calculated value ($E$) of a standard from that of its true value ($T$) expressed as a percentage (RE%). It was calculated by using the formula: RE% = $(E - T)/T \times 100$. The extraction recoveries of BOH and B17P were evaluated by analyzing five replicates at concentrations of 0.20, 2.0 and 30 ng/ml for BOH, and 0.080, 0.40 and 4.0 ng/ml for

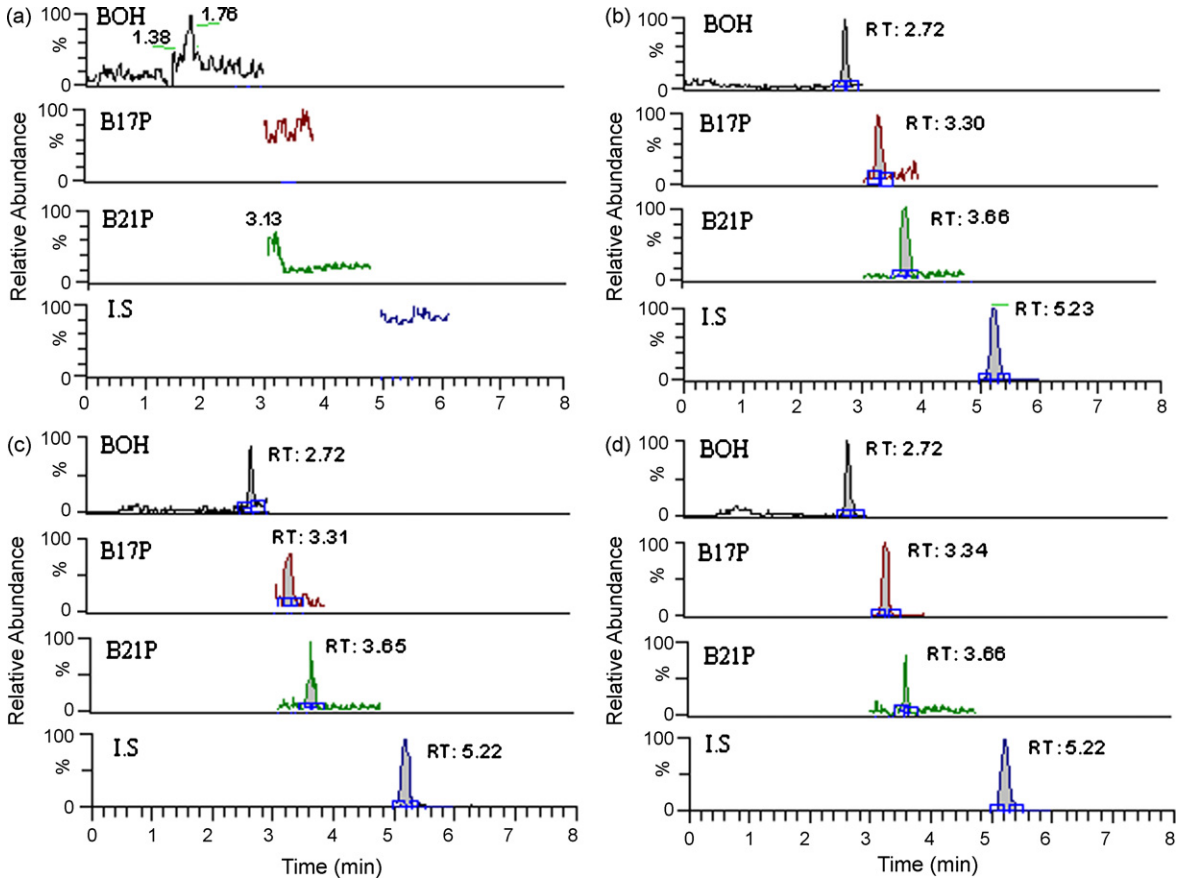


**Fig. 3.** (a) Typical SRM chromatograms of blank plasma, (b) plasma spiked with 0.2 ng/ml for BOH, 0.10 ng/ml for B17P, 0.10 ng/ml for B21P and the I.S., (c) LLOQ for BOH (0.10 ng/ml), for B17P (0.05 ng/ml), for B21 (0.05 ng/ml) in plasma and I.S. and (d) plasma obtained from a volunteer at 0.5 h administrated a single dose of BSP (2 mg)/BDP (5 mg) by intramuscular injection.

B17P. The recovery was calculated by comparison of the peak areas of analytes extracted from plasma samples with those of injected standards.

### 2.8. Clinical study design and pharmacokinetic analysis [19]

The clinical pharmacokinetic study was approved by the Ethic Committee of Nanjing First Affiliated Hospital of Nanjing Medical University. The volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki [20]. Ten healthy young male Chinese volunteers participated in this study. Each volunteer administrated a single dose of BSP (2 mg)/BDP (5 mg) by intramuscular injection. Blood was sampled pre-dose and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72, 120, 216 and 336 h post-dose. Model-independent pharmacokinetic parameters were calculated for both BOH and B17P. To overcome the problem of sample stability, blood samples was stabilized using a 2 M concentration of an esterase inhibitor, sodium arsenate [11], mixed immediately and placed on the ice. The blood sample was then centrifuged at 2500 rpm for 10 min (4 °C) to obtain plasma sample, then plasma samples were stabilized with 50% (w/v) sodium fluoride, which inhibits plasma esterase [21]. Plasma samples were stored at −70 °C prior to determination of BOH, B17P and I.S.

The plasma concentrations of these samples were determined, and the pharmacokinetics parameters were calculated. The maximum plasma concentration ($C_{max}$) was noted directly. The elimination rate constant ($k_e$) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. The elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_e$. The area under the plasma concentration–time curve from the start of the infusion to the

**Table 2**
Matrix effect evaluation of BOH, B17P and the I.S. in human plasma ($n = 5$)

| Samples | Nominal concentration (ng/ml) | Matrix effect (%) |
|---|---|---|
| BOH | 0.020 | 110.1 |
| | 0.20 | 108.5 |
| | 30 | 107.1 |
| B17P | 0.080 | 105.5 |
| | 0.40 | 103.2 |
| | 4.0 | 111.5 |
| I.S. | 2.5 | 105.7 |

**Table 3**
Accuracy and precision for the analysis of BOH and B17P in human plasma (in prestudy validation, three runs, five replicates per run)

| Analytes | Nominal concentration (ng/ml) | Intra-run R.S.D. (%) | Inter-run R.S.D. (%) | RE (%) |
|---|---|---|---|---|
| BOH | 0.20 | 9.3 | 14.4 | −4.7 |
| | 2.0 | 2.5 | 2.0 | −3.0 |
| | 30 | 2.0 | 8.7 | 0.1 |
| B17P | 0.080 | 7.9 | 12.3 | −3.4 |
| | 0.40 | 2.3 | 9.2 | −3.9 |
| | 4.0 | 3.3 | 7.5 | −10.0 |

*Note:* R.S.D., relative standard deviation; RE, relative error.

**Table 4**
Stability (%) of BOH and B17P in human plasma samples under various conditions

| | BOH | | B17P | |
|---|---|---|---|---|
| | 0.20 ng/ml | 30 ng/ml | 0.080 ng/ml | 4.0 ng/ml |
| Room temperature (5 h) | 94.2 ± 3.0 | 105.6 ± 7.0 | 99.1 ± 6.7 | 106.5 ± 5.8 |
| Three freeze–thaw cycles | 92.5 ± 4.5 | 102.3 ± 8.7 | 93.8 ± 5.7 | 101.9 ± 7.2 |
| Keep in autosampler | 93.7 ± 5.4 | 106.5 ± 5.7 | 93.1 ± 6.2 | 107.5 ± 5.3 |
| 2 weeks at −70 °C | 96.5 ± 10.8 | 101.2 ± 8.3 | 94.8 ± 8.6 | 96.3 ± 9.1 |

Stability was expressed as the percentage ratio of measured concentration to the nominal concentration ($n = 3$).

time of the last determined concentration ($AUC_{0-336}$) was calculated using the linear trapezoidal rule. The area under the plasma concentration–time curve to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-336} + C_{336}/k_e$, in which the $C_{336}$ was the plasma concentration of B17P at 336 h post-dose.

## 3. Results and discussion

### 3.1. Conditions of chromatography

In order to achieve the maximum signal response under ESI+/− condition and the shortest analysis time, the percentage of organic phase in the mobile phase was maintained as high as possible while still avoiding the early front peak which contained most of the hydrophilic response-suppressing endogenous interferences. Methanol and acetonitrile were screened for the organic phase. Methanol was selected due to the better selectivity. The aqueous portion was also investigated and the test results showed that the mobile phase of ammonium acetate solution could not improve the peak shapes and sensitivity of B17P, BOH and I.S. Further experiment results showed that acidifying the mobile phase with formic acid decreased the MS sensitivity of BOH. So, ammonium acetate and formic acid were not added into the mobile phase. The results showed that using pure water as the aqueous portion of the mobile phase could sufficiently achieve the symmetric chromatographic peak sharp and high MS sensitivity for BOH, B17P and the I.S. According to this, a mobile phase of water–methanol (15:85, v/v) was selected in the method. It is important to choose the suitable column temperature for the HPLC. The different column temperatures of 20, 25 and 30 °C were tested. The result showed that analytes could be separated from the interference of the endogenous substance when the column temperature was selected at 25 °C.

When using the ether or ethyl acetate as the extraction solvent in the plasma sample preparation procedure, the higher extraction efficiency of BOH, B17P and IS could be achieved, but the endogenous interference substances of the analytes were also extracted from the plasma. Using the mixture of ether–cyclohexane (4:1, v/v) as the extraction solvent can eliminate the interference of endogenous substances and meet the requirement of sensitivity for the assay.

### 3.2. Conditions for ESI

Because glucocorticoid had several hydroxy groups in their structure, they were medium-polarity compound. Usually, the electrospray ionization (ESI) was used for medium- to high-polarity analytes, so the ESI was adopted for the assay of analytes for their medium-polarity property. In preliminary experiments, each analyte was directly infused and an electrospray mass spectrum was acquired in positive and full scan mode. For BOH and B17P, the $[M+Na]^+$ ion was the most abundant, all MS parameters being fixed. Interestingly, under LC–MS conditions, they formed undesirable positive adduct ions with methanol from the LC mobile phase, for low-energy CID (2–10 eV), BOH and B17P formed comparatively stronger solvent adduct ion $[M+Na+CH_3OH]^+$. But the solvent adduct ions were not observed under higher energy CID (18 eV). The observations described above may indicate that the solvent adduct ions of B17P and BOH were not thermally unstable since they were observed under ESI conditions. The solvent adduct ion $[M+Na+CH_3OH]^+$ was so unstable that it was not used as the precursor ion in the SRM detection, so the energy of CID was set at 18 eV to reduce the formation of this solvent adduct ions. The precursor ion ($m/z$ 471) of B17P was the sodium adduct ion, but not $[M+Na+MeOH]^+$. Subsequently, acquisitions in SRM mode with different collision energies were performed in order to study the fragmentation of the $[M+Na]^+$ and find the highest response of product ion. For B17P, the collision-induced dissociation of the $[M+Na]^+$ precursor ion produced a main product ion, $[M+Na–HOPr]^+$ of $m/z$ 397.1. HOPr represent propionic acid. But for B21P, at low collision energy (6 V), the $[M+Na]^+$ ions of B21P fragmented to $[M+Na–HF]^+$ ions at $m/z$ 451.1 (see Fig. 2). The negative ion mode of B17P and BOH was also tested. Due to the higher ratio of the signal to noise (S/N), positive ESI ($ESI^+$) was chosen for B17P. But for BOH, $MS^2$ in positive ionization mode led to a large number of fragment ions, providing structural information but with disappointing sensitivity. So negative ESI ($ESI^-$) was chosen for BOH.

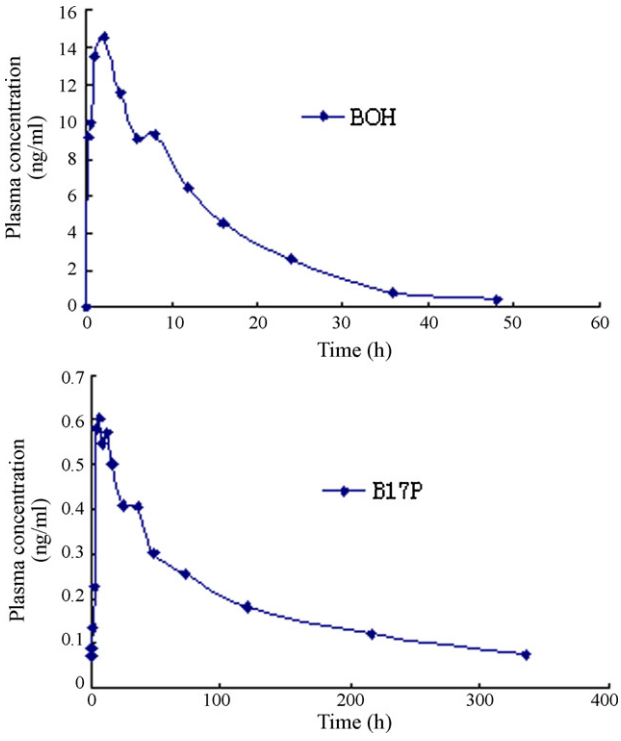


**Fig. 4.** Mean plasma concentration–time profile of BOH and B17P in 10 healthy Chinese volunteers after receiving a single intramuscular dose of betamethasone phosphate (2 mg)/betamethasone dipropionate (5 mg) injection.

**Table 5**
Mean pharmacokinetic parameters of BOH and B17P for 10 volunteers after intramuscular administration of betamethasone phosphate/betamethasone dipropionate (mean ± S.D., $n = 10$)

| Parameters | BOH | B17P |
|---|---|---|
| $t_{1/2}$ (h) | 9.6 ± 3.6 | 80.8 ± 22.7 |
| $C_{max}$ (ng/ml) | 14.54 ± 3.7 | 0.6 ± 0.2 |
| $t_{max}$ (h) | 2.8 ± 1.7 | 15 ± 9 |
| $AUC_{0-t}$ (ngh/ml)) | 177.2 ± 31.7 | 80.2 ± 18.3 |
| $AUC_{0-\infty}$ (ngh/ml)) | 198.6 ± 35.8 | 87.9 ± 19.5 |

*Note*: S.D., standard deviation; *n*, number of replicates.

It was observed in negative mode the formation of the molecular related ion $[M-H-CH_2O]^-$ of *m/z* 361.0 (Fig. 2). The formation of the ion $[M-H-CH_2O]^-$ is probably formed in the source by the neutral loss of formaldehyde ($CH_2O$), involving the cleavage of $C_{20}$–$C_{21}$ [8], a phenomenon probably to stabilization of the negative charge. For IS, the collision-induced dissociation of the $[M+Na]^+$ precursor ion of *m/z* 543.1 produced a main product ion, $[M+Na-HOPr-HCl]^+$ of *m/z* 433.2 (see Fig. 2).

Due to the high intensities of the *m/z* 361.0 → 307.2 (BOH), 471.1 → 397.1 (B17P), 471.1 → 451.1 (B21P), 543.1 → 433.2 (I.S.) transitions and no detectable interference in blank human plasma samples, these transitions were used in the present method.

### 3.3. Assay performance

The optimized method was validated by assessment of specificity, ME, recovery, linearity, the lower limit of quantification (LLOQ), precision and accuracy [22]. Coefficients of variation and relative errors of less than 15% were considered acceptable, except for LLOQ, whose values were extended to 20% for the analysis of biological samples for pharmacokinetic studies.

Acquisitions in SRM mode were used in the present method. Chromatography was specific for BOH, B17P and B21P, as metabolites and endogenous compounds did not interfere with the assay. The retention times were about 2.7 min for BOH, 3.3 min for B17P, 3.6 min for B21P and 5.2 min for the I.S. The typical SRM chromatograms were shown in Fig. 3.

The ME values of BOH, B17P and I.S. were between 103.2% and 111.5% (in the range of 85–115%), which showed there was no matrix effect of the analytes observed in this study (see Table 2).

Because of its high hydrophobicity, BOH, B17P and I.S. can be easily extracted from plasma with ether–cyclohexane without adjustment of the pH value of the plasma. The results of the experiment showed that there was no significant difference between the recovery values obtained by extraction of the plasma samples with ether–cyclohexane with or without adjustment of the plasma pH to the acidic value. The extraction recoveries based on comparison with direct injection of standards were 82.7–85.9% for BOH and 83.6–85.7% for B17P over a wide plasma concentration range.

The method was linear for BOH from 0.10 to 50 ng/ml ($r^2 > 0.9984$) and for B17P from 0.050 to 5.0 ng/ml ($r^2 > 0.9972$) on repeated calibration curves. The lower limit of quantification (LLOQ) was 0.10 ng/ml for BOH and 0.050 ng/ml for B17P. The accuracy, intra-run and inter-run precision for the quality controls are summarized in Table 3.

The stability test was studied under a variety of storage and handling conditions. The results (see Table 4) showed that no significant degradation occurred at ambient temperature for 5 h. The samples in autosampler were stable for at least 24 h. And there were also no significant degradation occurred during the three freeze–thaw cycles for plasma samples. BOH and B17P in plasma at −70 °C were stable for 2 weeks.

### 3.4. Application

The method described above was successfully applied to the pharmacokinetic study in which plasma concentrations of BOH and B17P in ten healthy Chinese volunteers were determined up to 48 and 336 h, respectively after intramuscular injection. The mean plasma concentration–time curves of BOH and B17P were shown in Fig. 4. The main pharmacokinetic parameters of BOH and B17P were shown in Table 5.

## 4. Conclusion

This is the first method to simultaneously quantifying two active metabolite, BOH and B17P using LC–ESI(+/−)–MS–MS in human plasma. The method achieved good sensitivity and specificity for the determination of BOH and B17P in human plasma. No significant interference caused by endogenous compounds was observed.

BOH and B21P represent also potential metabolites for BDP, but BOH was not detected in human plasma samples collected after 48 h post-administration, as previous pharmacokinetic studies suggested very low levels of this potential metabolite and activity much lower than that of betamethasone 17-monopropionate [15]. In addition, plasma was not assayed for the minor and inactive metabolite B21P, since it could not be detected in most samples following the administration of betamethasone phosphate/betamethasone dipropionate by intramuscular injection. The validation results have shown that the method is robust and is suitable to assess BOH and B17P levels after intramuscular injection. This method is suitable for the pharmacokinetic studies in human subjects.

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