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Determination of arotinoid acid in human plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

Arotinoid acid (Ro 13-7410) is the third generation of synthetic retinoid, which was developed for the treatment of psoriasis and other hyperkeratotic skin disorders. The therapeutically active dose is less than 0.5 μg/kg body weight/day. To investigate the pharmacokinetics of arotinoid acid, a sensitive and selective liquid chromatographic–tandem mass spectrometric method (LC–MS/MS) for the determination of arotinoid acid in human plasma was developed and validated. The sample processing was carried out in the dark to minimize photodegradation of the analytes. Arotinoid acid and the internal standard (IS), acitretin, were extracted from plasma samples using solid phase extraction with Oasis HLB cartridges. Chromatographic separation was achieved on a Zorbax Extend C_{18} column (150 mm × 4.6 mm, i.d., 5 μm) using methanol:acetonitrile:5 mM ammonium acetate (48:32:20, v/v/v) as the mobile phase at a flow rate of 0.8 mL/min. The detection was carried out in multiple reaction monitoring (MRM) mode via negative electrospray ionization (ESI) interface. The lower limit of quantification (LLOQ) achieved was 37.1 pg/mL with intra-day and inter-day precision (RSD) of 8.7% and 8.5%, and accuracy (RE) of 2.0%. Inter-day and intra-day RSD for three quality control levels (QCs) across validation runs were less than 11.0% and the accuracy ranged from 1.9% to 3.3%. The validated LC–MS/MS method was applied to a phase I clinical pharmacokinetic study after a single oral administration of 40 μg arotinoid trometamol to healthy subjects.

1. Introduction

The efficacy of systemic retinoid therapy in a number of dermatologic diseases is well established [1–4]. Until now, four oral retinoids have been approved by the Food and Drug Administration for use in the United States: isotretinoin, etretinate, acitretin and bexarotene [1,5]. Arotinoid acid [Ro 13-7410, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid] is among the most potent retinoids ever synthesized and has a high affinity to cellular retinoic acid-binding proteins. This accounts not only for its antipsoriatic activity in the treatment of a variety of skin diseases including severe acne, psoriasis and genodermatosesone [6,7], but also for its toxicity as a teratogen [8,9]. It belongs to the third generation of synthetic retinoids that may be effective in extremely low doses, and it is currently undergoing clinical investigation. It has been reported that the therapeutically active dose of arotinoid acid is less than 0.5 μg/kg body weight/day in humans [10]. Consequently, the plasma concentration of arotinoid acid is extremely low.

Arotinoid acid is a retinoidal benzoic acid derivative, which contains an aromatic ring replacing the unsaturated bonds of the tetraene side-chain of the retinoid skeleton. Retinoids are well documented as unstable compounds when exposed to light [11]. Therefore, sample processing is best carried out under light protection. Previously described analytical methods for the determination of retinoic acids [12–16] and arotinoids [17,18] in biological samples include high-performance liquid chromatography (HPLC) coupled with UV detectors [12,17,18], electrochemical detector [16] and mass spectrometers [13–15]. However, few methods have been published concerning the determination of arotinoid acid. Until now, only a two-dimensional gas chromatography–negative ion chemical ionization mass spectrometry method has been reported [19].

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is a powerful analytical technique that combines the resolving power of HPLC with the detection specificity and low detection limits of MS. In this paper, a sensitive and selective

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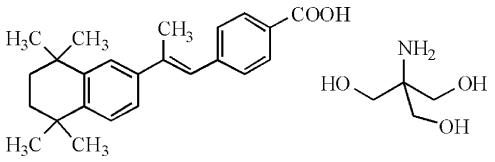

Fig. 1. Chemical structure of arotinoid trometamol.

LC–MS/MS method for the determination of arotinoid acid in human plasma using solid phase extraction (SPE) was developed and validated. SPE proved to be a useful sample preparation technique in this experiment, which provided relative high recovery, concentrated analytes and less matrix interference. The LC–MS/MS method was applied to a pharmacokinetic study of arotinoid acid after a single oral administration to healthy human volunteers of 40 μg arotinoid trometamol (arotinoid acid tromethamine salt, Fig. 1), equivalent to 29.68 μg arotinoid acid.

2. Experimental

2.1. Materials and chemicals

Arotinoid trometamol (99.7% purity) and acitretin (99.7% purity) were supplied by Chongqing Huapont Pharm. Co., Ltd. (Chongqing, China). Acetonitrile and methanol (HPLC grade) were purchased from Sigma (St. Louis, MO, USA). Ammonium acetate was obtained from Tedia (Fairfield, OH, USA). Blank (drug free) human plasma was obtained from Shanghai Shuguang Hospital (Shanghai, China). Pure water (18.2 mΩ and TOC ≤ 50 ppb) from Milli-Q system (Millipore SAS, Molsheim, France) was used.

Oasis HLB cartridges (1 cc, 30 mg) used in SPE were purchased from Waters Corporation (Milford, MA, USA).

2.2. Preparation of standard and quality control (QC) samples

All laboratory manipulations involving arotinoid acid and acitretin were performed in dark rooms under dim yellow light to prevent photodegradation.

Two stock solutions of arotinoid acid were prepared separately by dissolving an accurately weighed reference standard in methanol to give a final concentration of 742 μg/mL (calculated as arotinoid acid) for calibration and QC standards. The stock solutions were then serially diluted with methanol:water (50:50, v/v) to obtain working solutions over the concentration range of 0.185–37.1 ng/mL and QC solutions of 0.445, 3.71, and 29.68 ng/mL. The stock solution of acitretin at 400 μg/mL was also prepared in methanol and then diluted with methanol:water (50:50, v/v) to obtain an IS working solution of 100 ng/mL. Ultrasonication was used for complete dissolution. Stock and working solutions were stored under refrigeration (∼4 °C) when not in use, and were freshly prepared on a weekly basis.

The analytical standard samples were prepared at concentrations of 37.1, 111, 297, 742, 1484, 3710, and 7420 pg/mL by spiking blank human plasma (500 μL) with standard working solutions (100 μL) during validation and each experimental run. The QC standard samples at concentrations of 89.0, 742, and 5936 pg/mL were prepared using QC solutions in the same manner.

2.3. Sample preparation

Because of the mucocutaneous toxicities associated with retinoids [1], all personnel involved in sample preparation wore suitable protective clothing and gloves to avoid skin contact with the retinoids. The susceptibility of retinoids to light necessitated care during sample handling to keep out sunlight, and sample preparations were performed in a dark room under dim yellow light. Frozen unknown samples were thawed at room temperature and thoroughly vortexed. To an aliquot of 500 μL of human plasma sample, 100 μL of methanol:water (50:50, v/v) and 50 μL of IS (100 ng/mL) were added. After vortex mixing for 1 min and centrifuging at 2000 × *g* for 5 min, the supernatant was loaded onto an Oasis HLB cartridge preconditioned with 1.0 mL methanol and 1.0 mL water. After washing with 1.0 mL water, arotinoid acid and IS were eluted with 1.0 mL methanol. The eluate was evaporated to dryness at 40 °C under a stream of nitrogen. Residues were dissolved in 100 μL of the mobile phase and vortexed briefly. A 20 μL aliquot of the resulting solution was injected onto the LC–MS/MS system for analysis.

2.4. Liquid chromatographic and mass spectrometric conditions

An Agilent 1100 liquid chromatographic system equipped with a G1379A vacuum degasser, a G1311A quaternary pump, a G1316A column oven and a G1313A autosampler was used. Chromatography was performed on a Zorbax Extend C_{18} column (150 mm × 4.6 mm, i.d., 5 μm, Agilent Technologies, Palo Alto, CA, USA) protected by a SecurityGuard C_{18} column (4 mm × 3.0 mm, i.d., 5 μm, Phenomenex, Torrance, CA, USA). The column was thermostated at 40 °C. The isocratic mobile phase consisted of methanol:acetonitrile:5 mM ammonium acetate (48:32:20, v/v/v), which was delivered at a flow rate of 0.8 mL/min.

Mass spectrometric detection was conducted on an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ont., Canada) equipped with an ESI interface operated in the negative ionization mode. The tuning parameters were optimized separately for arotinoid acid and IS. This was done by infusing solutions of arotinoid acid and IS at a flow rate of 20 μL/min into the mobile phase (0.5 mL/min) using a post-column "T" connection. The nebulizer, auxiliary and curtain gases (nitrogen) were set at 50, 50 and 10 psi, respectively. For collision activated dissociation (CAD), nitrogen was used as the collision gas set at 4 psi. The values of collision energy (CE) were set at −30 and −21 eV for arotinoid acid and IS, respectively. The declustering potential (DP) was set at −82 V. Quantitation was performed by multiple reaction monitoring (MRM) using the transitions m/z 347 → m/z 303 for arotinoid acid and m/z 325 → m/z 266 for IS, respectively, with a dwell time of 200 ms per transition. The mass spectrometer was operated at unit mass resolution (peak width at half-height set at 0.7 Da) for both Q1 and Q3. Data were collected and processed using Analyst 1.4.1 software (Applied Biosystems).

2.5. Method validation

The selectivity of the method was evaluated by analyzing six blank plasma samples and six spiked plasma samples at the LLOQ level from six different sources. The MRM chromatograms of these blank plasma samples were compared with LLOQ MRM chromatograms from the same source. The peak area of endogenous compounds co-eluting with the analyte should be less than 20% of the peak area of the LLOQ standard.

Calibration curves were constructed by analyzing spiked calibration samples (each concentration in duplicate) on three separate days. Samples were quantified using the ratio of the peak area of the analyte to that of IS. Peak area ratios were plotted against analyte concentrations, and standard curves were calculated using weighted ($1/x^2$) least squares linear regression.

Accuracy and precision were assessed by the determination of QC samples at three concentration levels in six replicates (low, 89.0 pg/mL; medium, 742 pg/mL; high, 5936 pg/mL) in three validation days. The precision was expressed by relative standard deviation (RSD) and the accuracy by relative error (RE). The intra-

day and inter-day precisions were required to be below 15%, and the accuracy to be within ±15%. The LLOQ is strictly a figure of merit for an integrated analytical method. It refers to the lowest concentration of arotinoid acid in plasma matrix that can be analysed quantitatively by the analytical method with precision less than or equal to 20% and accuracy within ±20%. The LLOQ was evaluated by analyzing samples prepared in six replicates on three separate days.

To evaluate the matrix effect, i.e., the potential ion suppression or enhancement due to co-eluting plasma components, six different lots of blank plasma were extracted and then spiked with the analyte at 742 pg/mL. The corresponding peak areas of the analyte in spiked plasma post-extraction were then compared to those of the solution standards in mobile phase at the equivalent concentration. The same evaluation was performed for the IS at 10.0 ng/mL.

The extraction recoveries of arotinoid acid at three QC levels ($n = 6$) were determined by comparing peak area ratios of the analyte to the IS in samples that were spiked with the analyte prior to extraction with samples to which the analyte was added after extraction. The IS was added to both of these sets of samples after extraction. The extraction recovery of the IS was determined in a similar way using the QC samples at medium concentration as a reference.

The stabilities of arotinoid acid in human plasma were evaluated by analyzing replicates ($n = 3$) of plasma samples at the concentrations of 89.0 and 5936 pg/mL, which were exposed to different conditions (time and temperature) while protected from light. The analyte was considered stable in the biological matrix when 85–115% of the initial concentrations were found. The short-term stability was determined after the exposure of the spiked samples at room temperature for 2 h, and the ready-to-inject samples (after extraction) in the HPLC autosampler at room temperature for 24 h. The freeze/thaw stability was evaluated after three complete freeze–thaw cycles (−20 to 25 °C) on consecutive days. The long-term stability was assessed after storage of the standard spiked plasma samples at −20 °C for 49 days.

2.6. Application of the method

The developed LC–MS/MS method was used to investigate the plasma profiles of arotinoid acid after a single oral dose of 40 μg arotinoid trometamol to nine healthy subjects. The pharmacokinetic study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. Venous plasma samples (3 mL) were collected in heparinized tubes before and 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 24, 48, 72, 96 and 120 h after the dose. Plasma samples were stored at −20 °C until analysis. The pharmacokinetic parameters of arotinoid acid were calculated by non-compartmental analysis using the computer program WinNonlin (WinNonlin V5.0.1, Pharsight Corporation, Mountain View, CA, USA). The maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) were both obtained directly from the measured data. The area under the plasma concentration–time curve from time zero to the time of the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method. The terminal elimination rate constant (k_e) was estimated by linear least squares regression of the terminal portion of the plasma concentration–time curve. The corresponding elimination half-life ($T_{1/2}$) was then calculated as $0.693/k_e$.

3. Results and discussion

3.1. MS/MS detection

Precursor ions of arotinoid acid and IS were determined from Q1 scans during the infusion of neat solutions under both positive ionization (PI) and negative ionization (NI) mode with the ESI interface. The analytes showed good responses in NI mode, with $[M-H]^-$ ions at m/z 347 and m/z 325 for arotinoid acid and IS, respectively. The analytes could not be detected in PI. The deprotonated molecules were subjected to collision activated dissociation to determine the resulting product ions. When the CE was adjusted from −20 to −40 eV, only one intense fragment ion at m/z 303 was produced, which corresponded to the loss of CO_2. As the CE was further increased, more fragment ions were produced, but their intensity were quite low and not stable, therefore, the m/z 303 product ion was used. The fragment ion at m/z 266 was chosen for the IS. The product ion scan spectra are shown in Fig. 2.

3.2. Liquid chromatography

Arotinoid acid produced only one major product ion at m/z 303 through the loss of CO_2, and many endogenous interferences in plasma are easy to lose one molecule of CO_2 in the MRM mode. As a result, the transition of $[M-H]^-$ to $[M-H-CO_2]^-$ is susceptible to endogenous interferences and lack of reasonable selectivity for the analysis of arotinoid acid in biological samples. Therefore, chromatographic retention and separation were important, because prominent interference peaks were observed during the plasma sample analysis. Attempts were made to resolve chromatographic interferences caused by unidentified compounds.

Arotinoid acid has a large lipophilic "left-hand" group connected by a lipophilic tether to the polar carboxylic acid terminus. Since a good correlation between the chromatographic retention data ($\log k'$) and the octanol–water partition coefficients ($\log P$) were found [20], $\log P$ values of arotinoid acid and IS were calculated using the computer program ChemBioOffice (ChemBioOffice Ultra 2008 V11.0, CambridgeSoft Corporation, Cambridge, MA, USA). The high $\log P$ obtained (6.87 for arotinoid acid and 5.16 for IS) may imply strong retention of arotinoid acid and IS on commonly used reversed phase columns. Among the reversed phase columns tested, the best performance was obtained with the Zorbax Extend C_{18} column (150 mm × 4.6 mm, i.d., 5 μm) with regard to separation efficiency and relative high column recovery. When using a mobile phase consisting of methanol:acetonitrile:5 mM ammonium acetate (40:30:30, v/v/v), the retention time of arotinoid acid was 11 min. Therefore, further optimization of the mobile phase was done with the purpose of shorting the chromatographic run time without compromising separation efficiency and sensitivity.

Arotinoid acid contains a carboxyl group in its structure, which was thought to chromatograph better and respond higher under basic conditions when the terminal carboxyl group was deprotonated. When the pH value of the aqueous phase was adjusted to 8.5 with ammonia solution in the mobile phase consisted of methanol:acetonitrile:5 mM ammonium acetate (40:30:30, v/v/v), although an improved signal response was obtained, the retention time was shortened to appropriately 3.0 min. This was undesirable because the resolution of arotinoid acid from endogenous compounds could not be achieved. The addition of 0.1% acetic acid also failed to enhance retention because the peak broadened severely and decreased sensitivity was also observed. As a result, the pH modifiers used in the mobile phase appeared to be unsuitable for the analysis of arotinoid acid. Since organic modifiers could shorten retention time, methanol and acetonitrile were investigated by varying their percentage in the mobile phase to obtain the best results in terms of peak shape, retention time, and sensitivity on the Zorbax Extend C_{18} column. The oven temperature was set at 40 °C in order to improve the peak shape. The mobile phase was finally optimized as methanol:acetonitrile:5 mM ammonium acetate (46:34:20, v/v/v), which was delivered using a ternary pump at the flow rate of 0.8 mL/min. The retention times for arotinoid acid and IS were 5.5 and 4.7 min, respectively. Ammonium

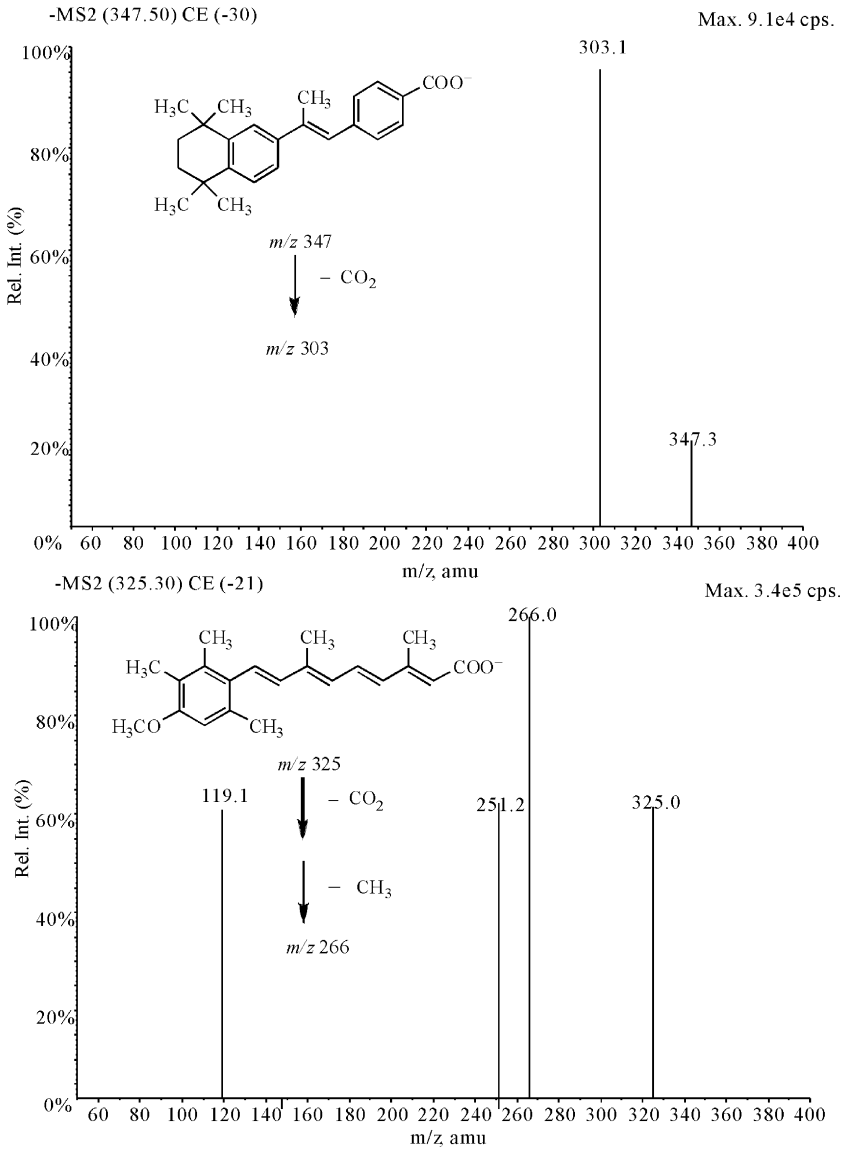

Fig. 2. Full scan product ion spectra of $[M-H]^-$ of arotinoid acid (A) and acitretin (IS, B) dissolved in the mobile phase.

acetate as a buffer in the mobile phase was important to maintain peak symmetry and retention time reproducibility over hundreds of injections, which ensured the ruggedness of this method. Under the present chromatographic conditions, the sample peaks were compared with that of the standard, and the retention times were within the time frame of 5.53 ± 0.2 min, which ensured the specificity of the method. Moreover, interfering peaks from matrix could be well resolved from the analyte during plasma sample analyses, which indicated that reasonable selectivity was obtained after the combination of MRM transition of $[M-H]^-$ to $[M-H-CO_2]^-$ with chromatographic separation.

3.3. Sample preparation

The reported extraction methods of retinoids from biological matrix include protein precipitation (PP) [13], liquid–liquid extraction (LLE) [14] and SPE [12,21]. In the present experiment, different sample preparation procedures were attempted. LLE gave clean extracts but the recovery was low and not quantitative in the extraction solvents studied. After PP with acetonitrile, significant chromatographic interference and great degree of ionization suppression were observed. This led to negative peak identification and incomplete resolution from interfering compounds. This was expected because of the minimum clean-up of the plasma samples. It has been well recognized that SPE can selectively extract and concentrate the target analyte from a liquid matrix onto a suitable solid phase that does not bind matrix interferences, and the analyte could be eluted using a stronger solvent [22]. Because arotinoid acid is present at very low concentrations in plasma samples, the SPE strategy could provide an efficient means of enrichment of the analyte. Further results suggested that SPE gave consistent recovery with minimum matrix interference and would therefore provide a more robust assay than LLE or PP for arotinoid acid. Different kinds of SPE cartridges were evaluated in terms of extraction recovery, including Bond-Elute C_{18}, Strata-X, and Oasis HLB. The best result was obtained with the latter, whose sorbent is a copolymer of hydrophilic N-vinylpyrroridone and lipophilic divinylbenzene. It provides higher retention capacity than silica-based reversed phase sorbents. SPE clean-up procedures were optimized to simultaneously achieve the concentration of arotinoid acid and the selective removal of interfering matrix contaminants. The result was that a single step of 1 mL methanol elution was efficient in delivering the most consistent recovery for arotinoid acid and IS.

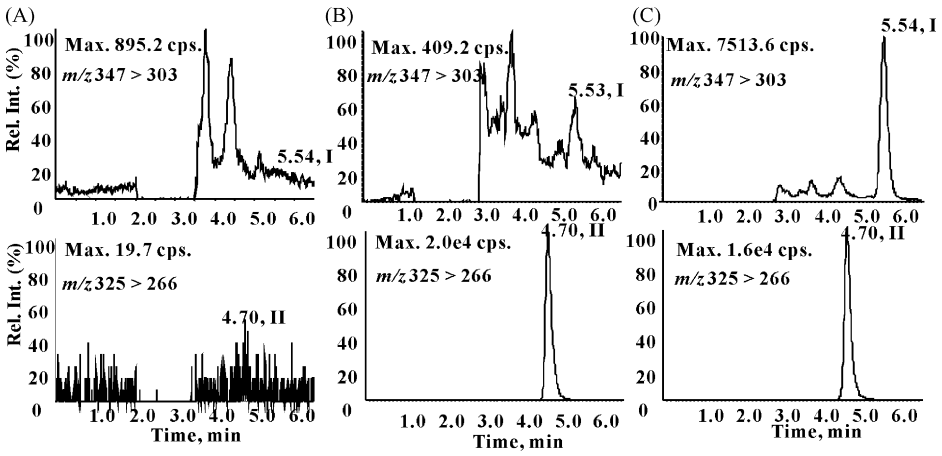

Fig. 3. Typical MRM chromatograms of (A) a blank human plasma sample; (B) a blank human plasma sample spiked with arotinoid acid at LLOQ (37.0 pg/mL) and IS at 10.0 ng/mL; (C) a human plasma sample obtained at 2.0 h after a single oral administration of 40 μg arotinoid trometamol, equivalent to 29.68 μg arotinoid acid. Peak I, arotinoid acid; Peak II, IS.

Retinoids are well documented as unstable compounds that are readily oxidized and/or isomerized into altered compounds, especially in the presence of air, light, and excessive heat [11]. As a result, sample processing can be favorably carried out in the dark to minimize photodegradation of both arotinoid acid and acitretin.

It was found during plasma sample analysis that more interference peaks appeared in the MRM channel of arotinoid acid when the plasma samples were stored under refrigeration (−20 °C) for more than 3 months compared with the fresh one. Therefore, fresh plasma was used for the preparation of the standard and QC samples, and the incurred samples were analysed within 1 month after collection.

3.4. Method validation

3.4.1. Assay selectivity and matrix effect

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma samples. Fig. 3 shows the typical chromatograms of a blank plasma sample, a blank plasma sample spiked with arotinoid acid at the LLOQ and IS, and a plasma sample obtained at 2.0 h after a single oral administration of 40 μg arotinoid trometamol to a volunteer. No interfering endogenous substances were observed at the retention times of the analyte and IS. The chromatograms presented in Fig. 3 indicated that the method was selective.

A general concern on the reliability of any new LC–MS/MS methods is the ionization suppression or enhancement caused by co-eluting endogenous compounds in biological matrices. Matuszewski et al. [23] reported that matrix components, which co-elute with analytes, may adversely affect the reproducibility of analyte ionization in the MS. In this study, the matrix effect in six different batches of human plasma were investigated for arotinoid acid and IS at the respective concentrations of 742 pg/mL and 10.0 ng/mL. The matrix effect value was 106 ± 10.8% for arotinoid acid and 106 ± 6.7% for IS. This result indicated that no co-eluting substances significantly influenced the ionization of arotinoid acid and IS.

3.4.2. Lower limit of quantification and linearity of calibration curve

The LLOQ for determination of arotinoid acid in plasma was 37.1 pg/mL. The precision and accuracy at LLOQ are shown in Table 1. In the pharmacokinetic study of arotinoid acid, plasma samples were collected from nine volunteers at different time points after a single oral dose of 40 μg arotinoid trometamol. Generally speaking, the plasma concentrations were below LLOQ from 72 to 120 h. These results indicate that the method was sensitive enough to investigate the low dose pharmacokinetics of the drug.

Table 1
Precision and accuracy data for the analysis of arotinoid acid in human plasma (in prestudy validation, 3 days, 6 replicates per day).

Concentration (pg/mL)		RSD (%)		RE (%)
Added	Found	Intra-day	Inter-day	
37.1	37.8 ± 3.7	8.7	8.5	2.0
89.0	90.7 ± 8.5	9.7	6.9	1.9
742	766 ± 77	10.5	4.4	3.3
5936	6125 ± 661	11.0	8.7	3.2

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range of 37.1–7420 pg/mL for arotinoid acid in human plasma. The typical equation of the calibration curve was: $y = 2.67 \times 10^{-4}x + 6.46 \times 10^{-3}$, $r^2 = 0.9950$, where y represents the ratio of arotinoid acid peak area to that of IS, and x represents the plasma concentration.

3.4.3. Precision and accuracy

The precision of the method was determined by calculating RSD for QCs at three concentration levels over three validation days using a one-way analysis of variance (ANOVA) [24]. The intra-day precision was 11.0% or less, and the inter-day precision was 8.7% or less at each QC level.

The accuracy of the method, expressed in terms of RE, ranged from 1.9% to 3.3% at three QC levels. Assay performance data are presented in Table 1. The above results demonstrated that the values were within the acceptable range and the method was accurate and precise.

3.4.4. Extraction recovery

Mean extraction recoveries of arotinoid acid were 62.5 ± 5.7%, 62.0 ± 7.1% and 57.8 ± 6.2% (n = 6) at the concentrations of 89.0, 742 and 5936 pg/mL, respectively. The extraction recovery of the IS was 71.4 ± 5.4% (n = 6).

3.4.5. Stability

The stability tests of the analyte were designed to cover anticipated conditions for the preservation of the clinical samples. All tests were carried out under the premise of avoiding direct light. The stability results showed that arotinoid acid spiked into human plasma was stable for 2 h at room temperature, for 49 days at −20 °C, and during three freeze–thaw cycles. The stability of arotinoid acid

Table 2
Stability of arotinoid acid exposed to various storage conditions under light protection ($n = 3$).

Condition	Concentration (pg/mL)		RSD (%)	RE (%)
	Added	Found		
Ambient, 2 h	89.0	91.0 ± 6.5	7.1	2.2
	5936	5411 ± 348	6.4	−8.8
−20 °C, 49 days	89.0	89.2 ± 6.7	7.5	0.2
	5936	5851 ± 186	3.2	−1.4
Three freeze–thaw	89.0	84.7 ± 7.5	8.9	−4.8
	5936	5959 ± 440	7.4	0.4
Autosampler	89.0	98.0 ± 5.1	5.2	10.1
Ambient, 24 h	5936	5941 ± 722	12.2	0.1

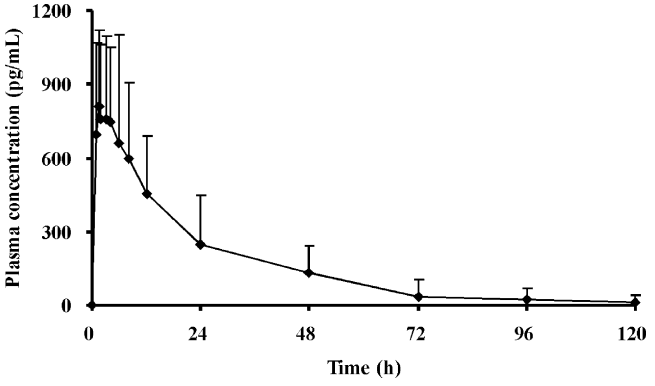

Fig. 4. Mean plasma concentrations of arotinoid acid after a single oral dose of 40 μg arotinoid trometamol to nine healthy volunteers.

extracts in the sample solvent on autosampler was also observed over a 24 h period. The results of the stability experiments are shown in Table 2.

3.5. Application of the method to a pharmacokinetic study in healthy volunteers

The validated analytical method was applied to the assay of arotinoid acid in human plasma after a single oral administration to healthy male volunteers of 40 μg arotinoid trometamol, equivalent to 29.68 μg arotinoid acid. The plasma samples were processed based on the proposed extraction protocol for the quantification of arotinoid acid. The mean plasma concentration versus time profile is presented in Fig. 4. The main pharmacokinetic parameters of arotinoid acid in nine volunteers were calculated. After oral administration of 40 μg arotinoid trometamol, T_{max} and C_{max} of arotinoid acid were found to be 2.5 ± 1.7 h and 985 ± 369 pg mL^{-1}, respectively. Plasma concentration declined with $T_{1/2}$ of 16.2 ± 9.0 h. The AUC_{0-t} and $AUC_{0-\infty}$ values obtained were 18.6 ± 12.3 and 19.8 ± 13.3 ng h mL^{-1}, respectively.

4. Conclusions

A sensitive and selective LC–MS/MS method for the determination of arotinoid acid in human plasma was developed and validated, and the SPE was adopted in plasma sample preparation. Besides advantages regarding clean sample and consistent extraction recovery using SPE, it also provides sample enrichment and separation efficiency with minimum endogenous interference and matrix effect. The LLOQ of this method is low enough to meet the needs of pharmacokinetic study of low dose arotinoid trometamol with good intra-day and inter-day reproducibility for the QCs. The applicability of the method was demonstrated in a pharmacokinetic study of arotinoid acid in healthy human volunteers.

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