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High-performance liquid chromatographic method for the determination and pharmacokinetic study of oxypeucedanin hydrate and byak-angelicin after oral administration of *Angelica dahurica* extracts in mongrel dog plasma

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Abstract

A high-performance liquid chromatographic method was developed and validated for the determination and pharmacokinetic study of oxypeucedanin hydrate and byak-angelicin after oral administration of *Angelica dahurica* extracts in mongrel dog plasma. The coumarin components and the internal standard isopsoralen were extracted from plasma samples with the mixture of *tert*-butyl methyl ether and *n*-hexane (4:1, v/v). Chromatographic separation was performed on a C_{18} column (200 mm × 4.6 mm, 5 μm) with the mobile phase acetonitrile–methanol–water–acetic acid (20:15:65:2, v/v/v/v) at a flow-rate of 1.0 ml/min. Only the peak of oxypeucedanin hydrate and byak-angelicin could be detected in dog plasma after oral administration of ethanol extracts of *A. dahurica* mainly containing xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate and byak-angelicin. The calibration curves of oxypeucedanin hydrate and byak-angelicin were linear over a range of 22.08–8830.00 and 6.08–2430.00 ng/ml in dog plasma, respectively. The quantification limit of oxypeucedanin hydrate and byak-angelicin in dog plasma was 22.08 and 6.08 ng/ml, respectively. The intra- and inter-day precision was less than 7.6% and 8.5% and the accuracy was from 91.9% to 106.1%. The lowest absolute recoveries of oxypeucedanin hydrate and byak-angelicin were 85.7% and 87.0%, respectively. The method was successfully applied to the pharmacokinetic studies of oxypeucedanin hydrate and byak-angelicin in dog plasma after oral administration of ethanol extracts from *A. dahurica*.
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Keywords: *Angelica dahurica*; Oxypeucedanin hydrate; Byak-angelicin; HPLC; Pharmacokinetic study

1. Introduction

Traditional Chinese medicine (TCM) has been widely used to prevent and cure many diseases under the guidance of the theory of traditional Chinese medical science. *Radix Angelicae dahuricae* (AE), the dried radix of *Angelica dahurica* (Fisch. Ex hoffm.) Benth. Et Hook. f. and *A. dahurica* (Fisch. Ex hoffm.) Benth. Et Hook. f. var. formosana (Boiss.) Shan et Yuan, is a well-known Chinese herbal medicine which was originally described in Shennong Materia Medica, the earliest Pharmacopoeia of China in Eastern Han (24–220 AD) [1]. AE has been used for the treatment of cold, headache, toothache, nose congestion and the reduction of pain in ancient times of China [2], and now AE also has been reported as having the protective activity against dexamethasone-induced disorders, liver protective activity, antimutagenic activity [3], antimicrobial activity [4–7], anti-inflammatory, analgesic and antipyretic activity [8–11]. Main active components of AE are coumarins such as xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byak-angelicin (Fig. 1), which have been reported to have pharmacological effects such as activation of ACTH-induced lipolysis [12], inhibition of insulin-induced lipogenesis, inhibition of compound 48/80-induced histamine release

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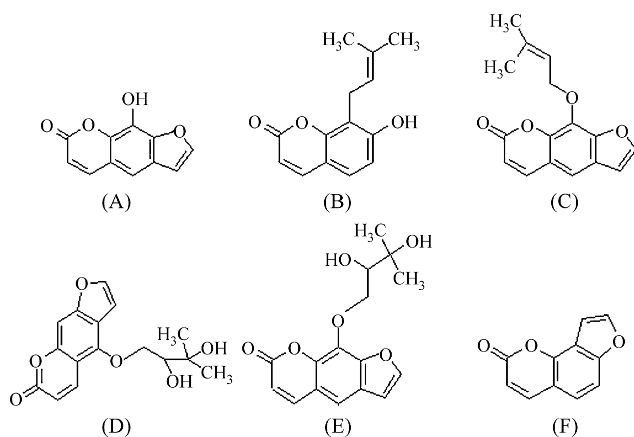

Fig. 1. Chemical structures of (A) xanthotoxol, (B) osthenol, (C) imperatorin, (D) oxypeucedanin hydrate, (E) byak-angelicin and (F) isopsoralen (internal standard).

[13,14] and inhibitory effect on cytochrome P-450 activity [15].

Pharmacokinetic studies of active components in Chinese herbs have considerable effects on clarifying their mechanism of action and promoting the development of modern TCM. By now three methods have been developed for the pharmacokinetic studies of AE in rat plasma. Firstly, a sensitive HPLC method, established by Ishihara et al. [16], was developed for the determination of the plasma concentrations of byak-angelicin and oxypeucedanin hydrate using a column-switching technique and applied to their pharmacokinetic study after administration of the hot water extracts of AE to male rats. Secondly, Gong et al. [17] studied nasal administration of AE emulsion, extracted by supercritical CO_2, to Wistar rats by investigating the pharmacokinetic parameters and the contents in rat brain tissue of imperatorin and isoimperatorin with liquid–liquid extraction (LLE). Recently, Li [18] reported a simple HPLC method for the determination of imperatorin in rat plasma with LLE and a pharmacokinetic study in Wistar rats after administration of the ethanol extracts of AE. A comparison of these three methods indicated that visible differences in plasma profiles of different active components were made by various pre-processing methods, administration routes, experimental animals and only rats were chosen. Furthermore, the analysis methods were not fully validated according to FDA guidelines [19]. Therefore, our study aims to develop a simple HPLC method with ultraviolet detection for the determination of active part of AE in plasma for further studies on the pharmacokinetics and action mechanism of active part of AE ethanol extracts orally administered to mongrel dogs.

2. Experimental

2.1. Materials and reagents

AE root (collected from Hangzhou, PR China) was obtained from Shanghai Lei Yun Shang Pharmaceutical Co. Ltd. Xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate and byak-angelicin were extracted and obtained from AE root by preparative HPLC, and identified with NMR, IR, UV, and MS comparing with literature data [20–22]. The purities of these compounds were up to 98% by RP-HPLC determination. 1300 Macroporous resin was from Shanghai Institute of Pharmaceutical industry. HPLC grade methanol and acetonitrile were purchased from Caledon Laboratories Ltd. (Georgetown, Ont., Canada). Acetic acid was purchased from China Medicine (Group) Shanghai Chemical Reagent Corporation. Isopsoralen (purity 99.1%, used as internal standard, IS) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pure water prepared by Milli-Q System (Millipore, Bedford, MA, USA) was used for the mobile phase.

2.2. Instruments

The analysis was performed on a HPLC system consisting of the Shimadzu LC-10 series (Shimadzu, Kyoto, Japan), i.e., two LC-10A pumps, a SIL-10AXL autosampler, a CTO-10A column oven, a DGU-12A degassing unit, and a SPD-10A UV detector. Data collection and integration were accomplished using N-2000 chromatography workstation (Zhejiang University Zhineng Information Engineering Institute, Zhejiang, China).

2.3. Chromatographic conditions

Chromatographic separation was performed on a Diamonsil C_{18} column (200 mm × 4.6 mm i.d., 5 μm; Dikma, Beijing, China). The mobile phase consisting of acetonitrile–methanol–water–acetic acid (20:15:65:2, v/v/v/v) was delivered at a flow-rate of 1.0 ml/min. The following HPLC parameters were used for the analysis of active part of AE: injection volume, 20 μl; detective wavelength, 311 nm; column temperature, 40 °C.

2.4. Preparation of the active part of AE

Pulverized dried root of AE (1338 g) was extracted twice with 70% ethanol (12,000 ml) by refluxing for 2 h in a water-bath at 70 °C. After filtration, the extraction was combined and evaporated to dryness by rotary vaporization at 60 °C under reduced pressure and redissolved in water. The solution (900 ml) was then chromatographed on 1300 macroporous resin by eluting stepwise with water, 10% ethanol, 30% ethanol and 70% ethanol. Each collection elution was analyzed by TLC, using chloroform–methanol (10:1, v/v) as the mobile phase. Consequently, 70% ethanol was used to yield active components. The fraction was evaporated to dryness by rotary vaporization at 60 °C under reduced pressure. Altogether 8.2 g of powder was obtained and the production rate was approximately 0.6%.

2.5. Content of five main coumarins in the active part of AE

To calculate the administered dose, the contents of five main active components of AE were quantitatively determined. One hundred milligrams of the dry powder was extracted by 100 ml of 1:100 mixture of acetic acid and methanol at 60 °C for 5 min, ultrasounded for 10 min and then diluted 10 times with

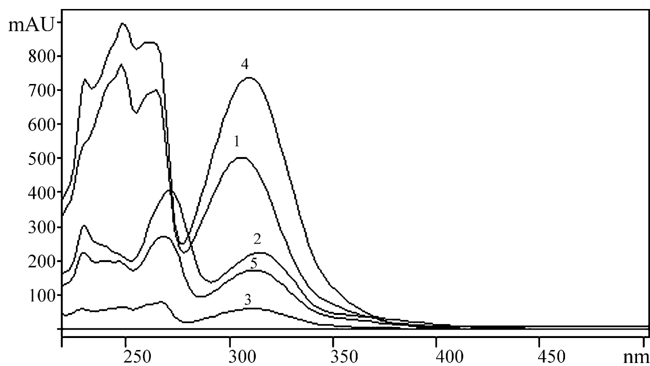

Fig. 2. UV absorption spectra of (1) xanthotoxol, (2) osthenol, (3) imperatorin, (4) oxypeucedanin hydrate and (5) byak-angelicin.

the mobile phase. One hundred microliters of the mixture was centrifuged for 10 min at 6207 × *g*. Twenty microliters of the supernatant was injected into the HPLC system. The maximum UV absorption wavelength of 311 nm was selected as shown in Fig. 2. The representative HPLC chromatogram of sample in vitro is shown in Fig. 3. The contents of five main coumarins were quantified using a modified HPLC method [23], and determined to be 15.6, 12.1, 3.1, 44.2 and 12.2 g/100 g active part for xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate and byak-angelicin, respectively.

2.6. Animal experiment

Six mongrel dogs (10.0–12.0 kg) were purchased from the Experimental Animal Center of Second Military Medical University. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of Second Military Medical University.

After being fasted for 12 h, each dog ($n = 6$) was orally administrated with active part of AE in capsule dosage form at the dosage of 30 mg/kg. The blood samples (1 ml) were collected from saphenous vein in a 5 ml tube containing heparin at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 h after administration. The

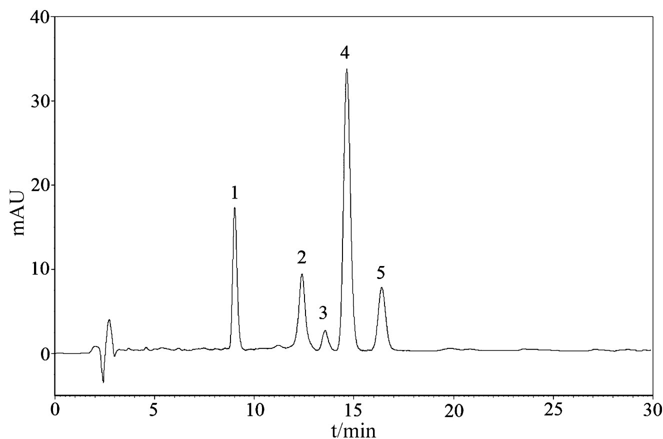

Fig. 3. Representative HPLC chromatogram of active part of AE (100 μg/ml) in vitro; (1) xanthotoxol; (2) osthenol; (3) imperatorin; (4) oxypeucedanin hydrate; (5) byak-angelicin.

plasma was isolated by centrifugation for 10 min at 2124 × *g*. Blank plasma was collected by the same method before dogs were orally dosed with active part of AE. Plasma samples were stored at −20 °C until analyzed.

2.7. Sample preparation

To 200 μl aliquot of sample and blank plasma, 100 μl of internal standard (isopsoralen, 2.00 μg/ml) was added. Then 50 μl of acetic acid was added to the sample and vortexed for 30 s. The sample was extracted with 1 ml of the mixture of *tert*-butyl methyl ether and *n*-hexane (4:1, v/v). After vortexing for 5 min and centrifuged at 2124 × *g* for 10 min, 0.8 ml of the supernatant was transferred to another tube. The supernatant was then evaporated to dryness at 45 °C under a gentle stream of nitrogen gas and the residue was reconstituted in 80 μl of mobile phase. The mixture was vortexed and centrifuged at 6207 × *g* for 10 min. Twenty microliters of the supernatant was injected into the HPLC column for analysis.

2.8. Standards and controls

The stock solutions of oxypeucedanin hydrate (1.766 mg/ml), byak-angelicin (1.215 mg/ml) and the internal standard (1.00 mg/ml) were prepared in methanol to obtain working standard solutions of oxypeucedanin hydrate (220.80 ng/ml–88.30 μg/ml) and byak-angelicin (60.75 ng/ml–24.30 μg/ml) by further dilution with distilled water. The stock solution of IS (isopsoralen) was also further diluted with distilled water to yield a working solution of 2.00 μg/ml. All the solutions were stored at 4 °C and brought to room temperature before use.

Plasma calibration standards, containing 22.08, 44.15, 88.30, 220.80, 441.50, 883.00, 2208.00, 4415.00 and 8830.00 ng/ml of oxypeucedanin hydrate and 6.08, 12.15, 24.30, 60.75, 121.50, 243.00, 607.50, 1215.00 and 2430.00 ng/ml of byak-angelicin, respectively, were prepared individually by spiking appropriate amount of the working standard solution in dog control plasma. Quality control (QC) samples, containing 44.15, 441.50 and 4415.00 ng/ml of oxypeucedanin hydrate and 12.15, 121.50 and 1215.00 ng/ml of byak-angelicin, were prepared independently in the same manner. All QC samples were stored at −20 °C until used.

2.9. Method validation

Calibration curves for the plasma assay were constructed by plotting the peak area ratio (y) of oxypeucedanin hydrate and byak-angelicin to isopsoralen against the nominal concentrations (x) of calibration standards. The concentrations of oxypeucedanin hydrate and byak-angelicin used were found to be linear over a range of 22.08–8830.00 and 6.08–2430.00 ng/ml, respectively, using weighted least square method, and the weight was $1/x^2$. The extraction procedure and HPLC analysis as described above were performed on the calibration samples.

QC samples at three concentrations containing 44.15, 441.50 and 4415.00 ng/ml of oxypeucedanin hydrate and 12.15, 121.50 and 1215.00 ng/ml of byak-angelicin, and the lower limit of quantification (LLOQ) samples including 22.08 ng/ml of oxypeucedanin hydrate and 6.08 ng/ml of byak-angelicin were analyzed to assess the accuracy (deviation from nominal values) and precision (relative standard deviation, R.S.D.) of the method. Intra-day precision was evaluated by analyzing QC and LLOQ samples in six replicates over 1 day, while inter-day precision was evaluated from the analysis of each control once on each of 6 days. The accuracy was estimated for each QC and LLOQ sample by comparing the measured concentration to the actual concentration. The criteria for acceptability of data included accuracy within ±15% deviation and precision within 15% R.S.D., while LLOQ of the assay could be quantitated with precision of 20% and accuracy of 80–120%.

Blank plasma of dogs ($n = 6$) was spiked with oxypeucedanin hydrate and byak-angelicin at low, medium and high concentration to evaluate the recovery, respectively. The absolute recovery was obtained by comparing the responses of the analytes extracted from three QC level samples with the response of analytes from non-extracted standard solutions at equivalent concentrations. The internal standard at the concentration of 666.70 ng/ml was determined in the same way.

The freeze-thaw stability was determined after three freeze and thaw cycles. In each cycle the QC samples were analyzed without being frozen at first, and then stored at −20 °C and thawed at the room temperature without any assistance. In short-term stability test, QC samples were kept at the room temperature for 0–4 h and the assay was performed at 0, 1, 2, 3, 4 h. Long-term stability of samples was analyzed after 2, 5, 10 and 15 days of storage (−20 °C).

3. Results and discussion

3.1. UV absorption spectra

The UV absorption spectra of xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate and byak-angelicin are presented in Fig. 2. Due to the possible interference of endogenous constituents at low wavelengths the long-wavelength maximum at 311 nm was selected for the assay.

3.2. Choice of internal standard and optimization of HPLC conditions

Other coumarins such as osthole, psoralen and isopsoralen were tried as potential internal standards (IS) with a mobile phase of methanol–water (45:55, v/v) at a flow-rate of 1.0 ml/min and column temperature of 30 °C [24,25]. The retention time of osthole was too long and hence the search was continued with psoralen and isopsoralen only. Due to the somewhat better separation from the analytes, isopsoralen was selected as the IS. To improve the peak shapes, acetonitrile and acetic acid were added to the eluent. Acetonitrile–methanol–water–acetic acid (20:15:65:2, v/v/v/v) with the column temperature setting at 40 °C was found to yield sharp and symmetric peaks. The retention behavior of isopsoralen was largely influenced by the proportion of acetonitrile while those of the five main coumarins were principally determined by methanol. The retention times of the five coumarins were from 9.0 to 16.5 min and that of isopsoralen was 20.3 min (Fig. 3).

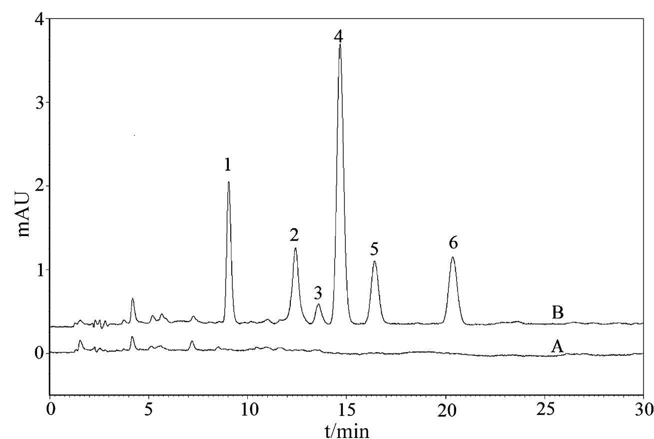

Fig. 4. Chromatograms of extracted blank plasma (A); blank plasma spiked with active part of AE (5.00 μg/ml) and internal standard (666.70 ng/ml) after LLE extraction with the mixture of *tert*-butyl methyl ether and *n*-hexane (4:1, v/v) (B); (1) xanthotoxol; (2) osthenol; (3) imperatorin; (4) oxypeucedanin hydrate; (5) byak-angelicin; (6) isopsoralen (IS).

3.3. Optimization of extracting solvent

In previous studies, the column-switching technique and the LLE method were developed in the purification step of the extracting procedure [16–18]. Although column-switching is an advanced technique, due to its simplicity LLE was used for the sample preparation in this work. In contrast to findings of Gong et al. [17] and Li [18], diethyl ether as extracting solvent was not found to be selective enough. After trying diethyl ether, ethyl acetate, *tert*-butyl methyl ether and *n*-hexane and their mixtures, LLE using a mixture of *tert*-butyl methyl ether and *n*-hexane (4:1, v/v) produced optimum results with average recovery of 87.3%, and without interference from endogenous substances (Fig. 4).

3.4. Method validation

The specificity of the method was demonstrated by comparing chromatograms of a blank sample and a spiked sample. Fig. 5 shows that no interference peaks from endogenous constituents were detected. Retention time was approximately 14.7 min for oxypeucedanin hydrate and 16.5 min for byak-angelicin.

The calibration curves of oxypeucedanin hydrate and byak-angelicin were linear from 22.08 to 8830.00 ng/ml and 6.08–2430.00 ng/ml, respectively. The regression equations were $y = 0.0004x + 0.0083$ ($r^2 = 0.9988$) for oxypeucedanin hydrate and $y = 0.0004x + 0.0063$ ($r^2 = 0.9984$) for byak-angelicin.

The intra- and inter-day precision and accuracy are summarized in Table 1. The intra- and inter-day precision of oxypeucedanin hydrate and byak-angelicin were less than 7.6%

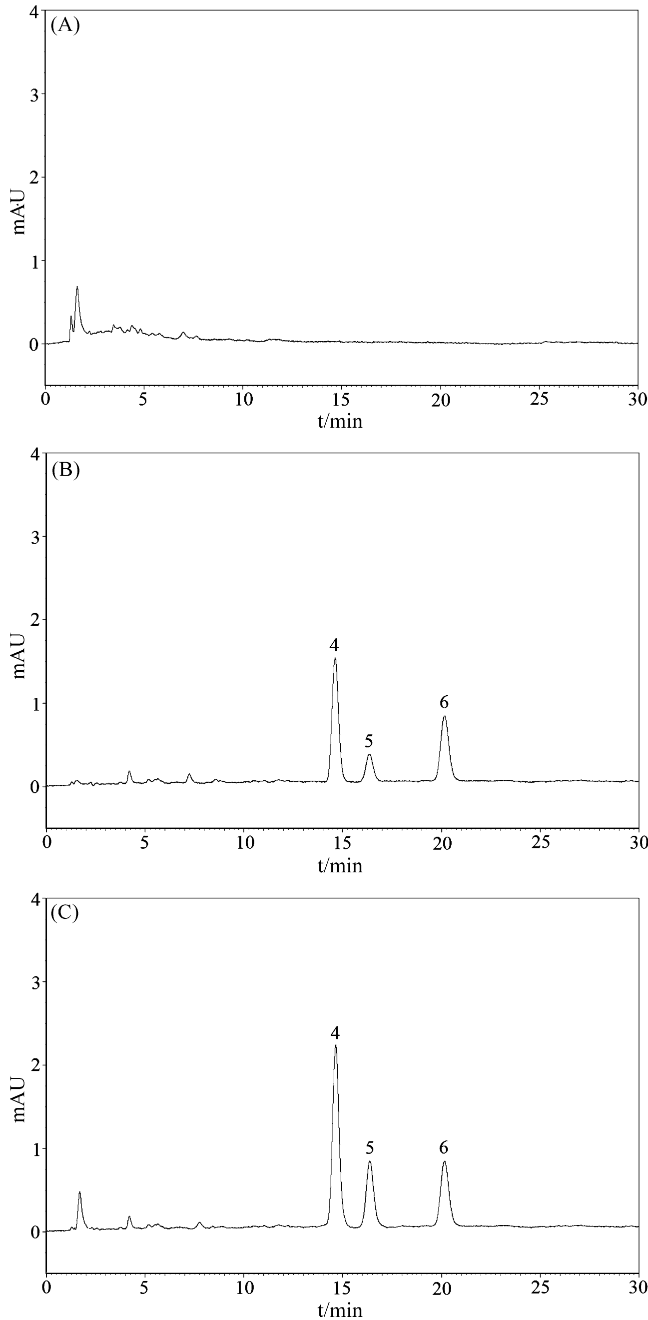

Fig. 5. Chromatograms of extracted blank plasma (A); blank plasma spiked with oxypeucedanin hydrate (883.0 ng/ml), byak-angelicin (243.0 ng/ml) and internal standard (666.7 ng/ml) (B); dog plasma sample at 90 min after oral administration of active part of AE (30 mg/kg) (C); (4) oxypeucedanin hydrate; (5) byak-angelicin; (6) isopsoralen (IS).

and 8.5% and the accuracy was from 91.9% to 106.1% for QC samples. The precision and accuracy of LLOQ were below 10% and 116%.

The results showed that the lowest absolute recoveries of oxypeucedanin hydrate and byak-angelicin were 85.7% and 87.0% (see Table 2).

After three freeze-thaw cycles, the corresponding relative errors for low, medium, high concentrations of oxypeucedanin hydrate were 5.0%, 3.2%, −6.6% and byak-angelicin were

Table 1
Intra-day and inter-day precision and accuracy of oxypeucedanin hydrate and byak-angelicin assay in dog plasma ($n = 6$)

Compounds		Nominal concentration (ng/ml)	Intra-day			Inter-day		
			Determined concentration (mean ± S.D., ng/ml)	Precision (%, R.S.D.)	Accuracy (%)	Determined concentration (mean ± S.D., ng/ml)	Precision (%, R.S.D.)	Accuracy (%)
Oxypeucedanin hydrate	LLOQ	22.08	25.40. ± 1.94	7.6	115.0	25.39 ± 2.02	8.0	115.0
	QC	44.15	46.84 ± 1.12	2.4	106.1	46.38 ± 1.16	2.5	105.0
		441.50	458.82 ± 3.17	0.7	103.9	455.73 ± 8.76	1.9	103.2
		4415.00	4057.99 ± 60.82	1.5	91.9	4121.84 ± 1.35	3.3	93.4
Byak-angelicin	LLOQ	6.08	7.17 ± 0.68	9.4	116.0	7.04 ± 0.70	10.0	116.0
	QC	12.15	12.76 ± 0.97	7.6	105.0	12.73 ± 1.08	8.5	104.8
		121.50	126.40 ± 2.69	2.1	104.0	127.27 ± 5.32	4.2	104.8
		1215.00	1155.67 ± 30.45	2.6	95.1	1140.64 ± 31.91	2.8	93.9

Table 2
Absolute recoveries of oxypeucedanin hydrate and byak-angelicin ($n = 6$)

Compounds	Nominal concentration (ng/ml)	Absolute recovery	
		Mean (%)	R.S.D. (%)
Oxypeucedanin hydrate	44.15	85.7	2.3
	441.50	86.5	1.6
	4415.00	87.7	4.0
Byak-angelicin	12.15	87.0	2.3
	121.50	87.6	2.7
	1215.00	89.2	1.2
Isopsoralen (IS)	666.70	86.4	3.8

4.8%, 4.7%, −6.1%, respectively. The short-term stability and long-term stability of oxypeucedanin hydrate and byak-angelicin were within ±7.9%. The present study indicated that oxypeucedanin hydrate and byak-angelicin were stable after three freeze-thaw cycles, or left at room temperature for 4 h and stored in the refrigerator and freezer for 15 days (see Table 3).

3.5. Pharmacokinetic study

The developed HPLC method was applied to determine concentrations of the selected coumarins in plasma samples obtained after oral administration of 30 mg/kg active part of AE to dogs. Different from those five peaks of sample in vitro (Fig. 3), only the peak of oxypeucedanin hydrate and byak-angelicin could be detected in the whole pharmacokinetic process (Fig. 5). The identification of oxypeucedanin and byak-angelicin was performed by the comparison with standard solutions. Since xanthotoxol, osthenol and imperatorin have not been found in mongrel dog plasma, further study will focus on the mechanism of absorption and metabolism of AE extracts. Following oral administration of active part of AE to dogs, log-transformed mean plasma concentration–time curves are shown in Fig. 6, which indicates the kinetic characteristic of elimination of the drug. The pharmacokinetic parameters

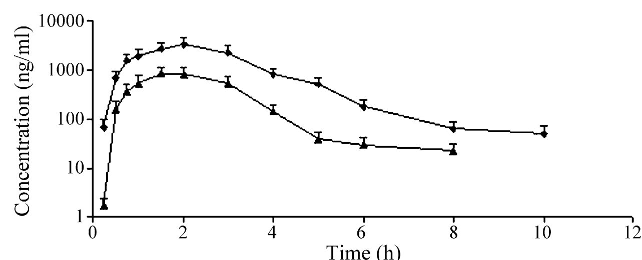

Fig. 6. Log-transformed mean (±S.D.) plasma concentration–time curve profiles of oxypeucedanin hydrate and byak-angelicin after oral administration of active part of AE (30 mg/kg) to dogs ($n = 6$).

Table 3
Stability of oxypeucedanin hydrate and byak-angelicin in dog plasma ($n = 6$)

Sample condition	Compounds	Nominal concentration (ng/ml)	R.S.D.[a] (%)	R.E.[b] (%)
Freeze-thaw stability	Oxypeucedanin hydrate	44.15	1.7	5.0
		441.50	1.8	3.2
		4415.00	3.2	−6.6
	Byak-angelicin	12.15	0.7	4.8
		121.50	3.7	4.7
		1215.00	2.8	−6.1
Short-term stability	Oxypeucedanin hydrate	44.15	2.0	4.6
		441.50	0.4	3.9
		4415.00	1.1	−7.4
	Byak-angelicin	12.15	0.7	5.1
		121.50	2.1	4.2
		1215.00	1.7	−3.7
Long-term stability	Oxypeucedanin hydrate	44.15	1.5	6.4
		441.50	0.8	3.9
		4415.00	2.7	−7.9
	Byak-angelicin	12.15	0.7	5.1
		121.50	4.2	5.3
		1215.00	2.1	−6.4

[a] Relative standard deviation (R.S.D.) is expressed as: (standard deviation between observed concentrations/mean observed concentration) × 100.
[b] Relative error (R.E.) is expressed as: (mean observed concentration/nominal concentration) × 100.

Table 4
Pharmacokinetic parameters of oxypeucedanin hydrate and byak-angelicin after oral administration of active part of AE (30 mg/kg) to dogs ($n = 6$)

Compounds	Parameters						
	t_{max} (h)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng h/ml)	$t_{1/2}$ (h)	MRT (h)	Cl_s (l/h kg)	Vd_{ss} (l/kg)
Oxypeucedanin hydrate	1.71 ± 0.81	4154.09 ± 1049.36	12439.92 ± 3008.17	3.06 ± 0.41	2.59 ± 0.29	1.21 ± 0.51	3.26 ± 1.13
Byak-angelicin	1.71 ± 0.81	1474.72 ± 400.48	2266.56 ± 716.05	2.77 ± 0.38	2.27 ± 0.58	3.23 ± 1.23	7.05 ± 2.40

calculated are summarized in Table 4. It was shown that absorption of oxypeucedanin hydrate and byak-angelicin with maximum concentration occurred approximately 1.71 h after oral administration of active part of AE. However, the parameters were different from those of Ishihara et al. [16], which might result from racial difference. The results demonstrated that this simple and rapid method was sufficiently sensitive to follow plasma levels of oxypeucedanin hydrate and byak-angelicin after oral administration of AE extracts to mongrel dogs.

4. Conclusion

A simple, specific and sensitive HPLC method for the determination of oxypeucedanin hydrate and byak-angelicin after oral administration of AE extracts in mongrel dog plasma was developed. This method was fully validated with satisfactory accuracy and adequate reproducibility. It was successfully applied to the pharmacokinetic studies of active part of AE. Further research should be carried out to explain the mechanism that only two peaks could be detected in vivo, which was different from those five peaks of sample in vitro.

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References

[1] The Pharmacopoeia of the People's Republic of China. Part 1. The Pharmacopoeia Commission of PRC, 2005.
[2] M.Y. Wang, M.R. Jia, Shangh. J. Trad. Chin. Med. 38 (2004) 56–57.
[3] C.M. Kim, M.Y. Heo, H.P. Kim, K.S. Sin, P. Pachaly, Arch. Pharm. Res. 14 (1991) 87–92.
[4] G.S. Liu, J.Z. Kang, Y.L. Wang, Chin. Med. J. 68 (1950) 52–53.
[5] Z.Y. Liu, G.W. Zhang, Q.Z. He, Chin. J. Dermatol. 29 (1996) 349–351.
[6] T.S. Zheng, W.G. Han, X.J. Jiang, J. Chin. Pharm. Univ. 27 (1996) 277–279.
[7] Y. Dai, Chin. Pharm. 10 (2001) 61–62.
[8] C.Y. Li, Y.J. Dai, C.K. Xie, Chin. J. Chin. Mater. Med. 16 (1991) 560–562.
[9] H. Nie, Y.J. Shen, Chin. J. Chin. Mater. Med. 27 (2002) 690–693.
[10] H. Nie, Y.J. Shen, N. Zeng, J.M. Wu, Pharm. Clin. Chin. Mater. Med. 18 (2002) 11–14.
[11] H. Nie, Y.J. Shen, J.M. Wu, R.A. Tan, Y.X. Ren, Trad. Chin. Drug Res. Clin. Pharm. 13 (2002) 221–223.
[12] Y. Kimura, H. Ohminami, H. Arichi, H. Okuda, K. Baba, M. Kozawa, S. Arichi, Planta Med. 45 (1982) 183–187.
[13] Y. Kimura, H. Okuda, J. Nat. Prod. 60 (1997) 249–251.
[14] T. Okuyama, J.R.L. Gerstner, R.S. Fichera, Chem. Pharm. Bull. 37 (1990) 38–40.
[15] L. Guo, M. Taniguchi, Y. Xiao, K. Baba, T. Ohta, Y. Yamazoe, Jpn. J. Pharmacol. 82 (2000) 122–129.
[16] K. Ishihara, M. Fukutake, T. Asano, Y. Mizuhara, Y. Wakui, T. Yanagisawa, H. Kamei, S. Ohmori, M. Kitada, J. Chromatogr. B. 753 (2001) 309–314.
[17] Z.N. Gong, L.Y. Xu, J.Z. Song, J.S. Tao, S.R. Ma, Chin. J. Clin. Pharm. 10 (2001) 370–373.
[18] Y.B. Li, J. Pharm. Biomed. Anal. 40 (2006) 1253–1256.
[19] Guidance for Industry, Bioanalytical Methods Validation, U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001. http://www.fda.gov/cder/guidance/4252fnl.htm.
[20] N.I. Baek, E.M. Ahn, H.Y. Kim, Y.D. Park, Arch. Pharm. Res. 23 (2000) 467–470.
[21] M. Eeva, J.P. Rauha, P. Vuorela, H. Vuorela, Phytochem. Anal. 15 (2004) 167–174.
[22] J. Yang, Y. Deng, Z.D. Zhou, F.E. Wu, Chin. Chem. Res. Appl. 14 (2002) 227–229.
[23] G. Zhou, G. Li, X.F. Liu, Q.X. Liu, S.G. Li, P. Li, Chin. Arch. Trad. Chin. Med. 21 (2003) 320–321.
[24] H. Chen, T. Chen, J.X. Li, Q. Xu, Chin. J. Chin. Mater. Med. 29 (2004) 654–657.
[25] M.Y. Wang, M.R. Jia, Y.Y. Ma, G.H. Jiang, J. Chin. Med. Mater. 27 (2004) 826–828.