Simultaneous Determination of Urinary Metabolites of Methoxypsoralens in Human and *Umbelliferae* Medicines by High-Performance Liquid Chromatography

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Abstract

A high-performance liquid chromatography method has been developed for the determination of coumarins and furocoumarins (psoralens). Nine coumarins and furocoumarins are separated simultaneously on a Hypersil C_8 (25 cm × 4.6-mm i.d.) column with a gradient of methanol and acetonitrile aqueous solution as mobile phase at 1.0 mL/min with two-channel UV–vis absorbance detection. The limits of detection are 0.366, 0.219, 0.317, 0.440, 0.536, 0.300, 0.531, 0.531, 0.237, and 0.280 ng/mL for coumarin, 7-hydroxycoumarin, 7-methoxycoumarin, citropten (5,7-dimethoxycoumarin), 7-ethoxy-4-methylcoumarin, psoralen, xanthotoxin (8-methoxypsoralen), bergapten (5-methoxypsoralen), isopimpinellin (5,8-dimethoxypsoralen), and imperatorin (9-isopenteneoxypsoralen), respectively. Human urine is analyzed 1–6 days after ingestion of the oral Chinese medicines. This lead to the conclusion that the concentration of coumarins and furocoumarins is higher than that of the control urine. The coumarins and furocoumarins are detected at 312 and 249 nm, respectively.

Introduction

Coumarin (1,2-benzopyrone) and furocoumarin (psolarens) compounds are common to the *Umbelliferae* medicines (including *Radix Angelicae Dahuricae, Radix Glehniae, Fructus Cnidill, Foeniculum Vulgare Mill, Saposhnikovia Divaricata, Radix Prucedani,* and *Glycyrrhiza Uralensis Fisch*). The linear furocoumarins that have the most biochemical importance are psoralen, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 5,8-dimethoxypsoralen (5,8-MOP) (1). The metabolism of 8-MOP is catalyzed by the P450 enzyme in rodents (2). Methods for the determination of coumarin and 7-hydroxycoumarin (7-HC) in biological fluids have been published extensively (3,4), but few have dealt with furocoumarin determination in biological fluids. Each subject received one of the doses of 5- or 8-MOP in 0.9% sodium chloride. The 5- or 8-MOP plasma concentrations were studied (5–9). Different methods of analysis for furocoumarins in plants of the *Umbelliferae, Rutaceae,* and *Moraceae* families have been published (10–15). Gas chromatographic (GC)–mass spectrometric analysis can identify some psolaren derivatives, bergapten, oxypeucedarin, and phellopterin; the other compounds do not have enough data because of their very low concentration or some thermal degradation during the GC analysis (10–11). A convenient method that involves supercritical fluid extraction (SFE) and reversed-phase liquid chromatographic (LC) analysis was reported to quantitate the furocoumarins in celery (12–15). The high water content of the plant matrices and, particularly, of plant tissue cultures has been a technical and a practical problem in SFE. It has been partly resolved by the drying or freeze drying of material prior to SFE, but this may lead to losses in the recovery of the more volatile components. Separation has been achieved on a C_{18} stationary phase and methanol or acetonitrile mobile phase. Detection systems used here included UV and mass spectral detection. Most coumarins exhibit a broad absorption band in the vicinity of 335 nm, and this is the most generally useful wavelength to detect coumarins. However, furanocoumarins and a few others have rather weak absorption at 335 nm and a stronger peak close to 310 nm. These *Umbelliferaes* were examined by high-performance liquid chromatography (HPLC) for their coumarins and furanocoumarins content and detected at 312 and 249 nm, respectively. To our knowledge, there is no literature on the specific distribution of linear furanocoumarins in Umbelliferae medicines and on the determination of human urine concentrations. The primary objective of this study was to develop a sensitive HPLC method for the simultaneously determination of urinary metabolites of methoxypsoralens in human urine after oral administration of *Umbelliferae* medicines.

Experimental

Instrumentation

HPLC was performed with a Hitachi model L-7100 pump (Tokyo, Japan) and model 7125 injector equipped with a 20-µL sample loop and a Shimadzu SPD10A spectrophotometric

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detector (Koyoto, Japan). Chromatograms were acquired and peak areas calculated by means of a Scientific Information Service Corporation chromatograms data integrator (Taipei, Taiwan). Absorbance measurements were acquired with a Cary UV–vis spectrophotomer (Varian, St. Helens, Australia). A matched quartz cell with a 1-cm path length was used to store all solutions for measurement.

Reagents and materials

The methoxypsoralens tested were: coumarin, 7-HC, 5,7-dimethoxycoumarin (5,7-DMC) (citropten), and 5-MOP, obtained from Aldrich (Milwaukee, WI); 7-ethoxy-4-methylcoumarin (7-E-4-MC) and 7-methoxycoumarin (7-MOC), obtained from Lancaster (Eastgate, White Lund, and Morecambe, UK; 8-MOP, obtained from TCI (Tokyo, Japan); and psoralen, 5,8-MOP (isopimpinellin), and 9-isopenteneoxypsoralen (9-IOP) (imperatorin), obtained from Extrasythesr (Genay, France). *Umbelliferae* Chinese medicines (content 0.64–0.68 g/g) were purchased from Sun Ten Pharmaceutical Co. (Taipei, Taiwan).

Drug preparations

Umbelliferase medicines (0.25–0.50 g) were individually weighed and stired for 90 min with 30 mL methanol–water (1:1, v/v). After centrifugation, the supernatant was extracted with hexane–ethyl acetate (3:1, v/v) and evaporated to dryness with a stream of nitrogen. Methanol (1 mL) was immediately added to the residue after mixing for 5 s. The final solution was filtered through 0.45- and 0.2-µm membrane filters prior LC analysis.

Drug administration

Each subject (average height = 165 ± 5 cm; average weight = 60 ± 10 kg) received 12 g of *Umbelliferase* medicines (Sun Ten Pharmaceutical product) per day for 1–6 days. Approximately 500 mL of urine was obtained from five human volunteers who had not been treated with medicines. Urine samples were collected at specific time intervals (1, 4, 8, 16, 24, 48, 72, 96, and 120 h, respectively) and used as background. Urine samples were collected (~ 500 mL) after medicinal intake on days 1, 2, 3, 4, 5, and 6, respectively. The remainder of the samples was stored in high-density polyethylene containers in a freezer (–20°C) for further determination of metabolites of methoxypsoralens by HPLC.

Extraction of urinary metabolites of methoxypsoralens

Samples of human urine (3 mL) were centrifugated at 2000 × *g* for 30 min. Two milliters of supernatant urine was transfered to another centrifugal tube containing 5 mL ethanol and centrifugated for 30 min to sediment aggregates. The deproteinized sample was then treated with 50 µL of β-glucuronidase (Sigma, St. Louis, MO) at 119,000 units/mL in 1M sodium acetate buffer (pH 4.5). The mixture was gently mixed and incubated at 37°C for 30 min. The resulting solution was extracted two times with 10 mL of chloroform, and the organic layer was evaporated at 40°C under dry nitrogen. The dried extract was reconstituted with 0.5 mL of 50% methanol–water (v/v) and loaded onto a Waters Sep-Pak C_{18} cartridge (Milford, MA), which had been conditioned with 2 mL of methanol and 2 mL water prior to sample loading. An additional 0.5 mL of methanol was used to rinse the sample vial and was also loaded onto the C_{18} cartridge. The sample on the C_{18} cartridge was washed with 2.0 mL of water (eluent discarded), 2.0 mL of 22% acetonitrile–water solution (eluent discarded), 1.0 mL of 30% acetonitrile–water solution (eluent collected, contained 7-HC), and 1.0 mL of 40% acetonitrile–water solution (eluant collected, contained coumarin, 7-HC, 7-MOC, and 5,7-DMC), and 1.0 mL of 55% acetonitrile–water solution (eluent collected, contained 7-HC, 7-MOC, 5,7-DMC, and 5,8-MOP). These three fractions were combined and dried under nitrogen at 45°C. The dry extract was reconstituted with 500 µL of pure methanol and filtered through 0.45-µm membrane filters prior to LC analysis.

Determination by LC

A Hypersil C_8 (25 cm × 4.6 mm i.d., 5 µm) (Thermo, Cheshire, UK) column was used. The mobile phase consisted of a three solvent system: eluent A, methanol; eluent B, acetonitrile; and eluent C, water. The eluent was monitored simultaneously both at 312 and 249 nm. The combination of B and C (30:70) was used as the initial condition in 10 min. At the 1-mL/min gradient (I), the methanol concentration was increased from 0% to 20% A, acetonitrile was decreased from 30% to 20% B, and water was decreased from 70% to 60% C in 12–22 min. The gradient was then returned to the initial conditions in 40 min. Gradient (II) was 0% A, 30% B, and 70% C at 0–10 min; 25% A, 25% B, and 50% C at 10–12 min. The gradient was then returned to 0% A by 22 min, with the next injection at 30 min.

Results and Discussion

Optimization of the HPLC separation

It is the balance between the polarity and lipophilicity of a neutral compound that will determine the time required to reach elution form on an HPLC column. In the case of a reversed-phase column, the more lipophilic a compound is the more it will be retained. A very large number of packings for reversed-phase LC are now commercially available. These materials can be quite different in performance or nature because they may be prepared from various silica gels, bonded with diverse chlorosilianes, end-capped or not, and so on. Consequently, a given separation cannot be strictly transposed from one column of a particular brand to another. Hypersil WP300 columns (Thermo) are wide-pore, reversed-phase columns that are microspheres with a very narrow particle size distribution but with a pore diameter of 300 A. This results in improved precision for impurity and stability assays, more accurate quantitation, and adaptability to more rugged methods. The chromatographic conditions described in Figure 1 could be transposed, however, to other packings with minimum workup and unaffected overall selectivity. When coumarins and furocoumarins were eluted from a mobile phase containing acetonitrile–water (30:70, v/v), the 9-IOP had a large lipophilic group [–OCH_2–CH=C $(CH_3)_2$], which had a retention time of 40 min. When an HPLC system with a binary or ternary gradient system is used, the instrument can be programmed to increase the gradient in the methanol content in the mobile phase to expedite the elution of the later-running 9-IOP. A suitable solvent program might be as follows: methanol–acetoni-

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trile–water (0:30:70, v/v/v) for 10 min, then the solvent composition of methanol–acetonitrile–water (25:25:50, v/v/v) is ramped to 22 min. This type of program greatly reduced the retention time of the 9-IOP, was considered satisfactory with respect to separation and total retention time, and was used for further studies. For optimum UV detection, the wavelength of the detector was set at 312 nm, which was close to the λ_{max} of the coumarins. Figure 1 shows typical chromatograms of authentic standards. The gradient elution allowed the successful retardation of 9-IOP on the column. It was well-resolved from the other coumarins and psoralens.

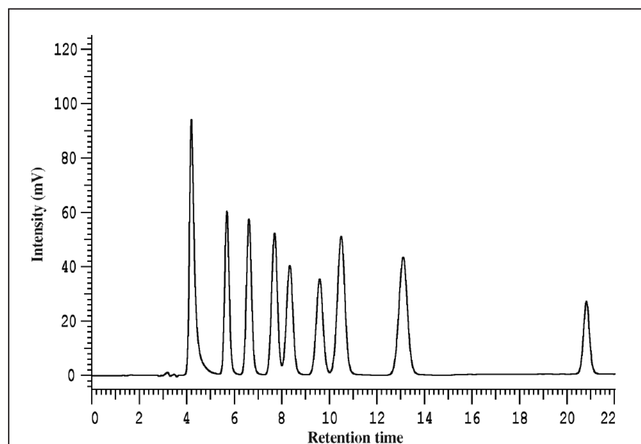

Figure 1. Separation of coumarins and psoralens standards on a Hypersil C_8 (25 cm × 4.6 mm) column. Gradient: 0% CH_3OH–30% CH_3CN–70% H_2O to 25% CH_3OH–25% CH_3CN–50% H_2O. The flow rate was 1.0 mL/min, and detection was at 312 nm. Peak identification: 7-HC (10 ppm), 1; coumarin (10 ppm), 2; 7-MOC (6 ppm), 3; psoralen (10 ppm), 4; 8-MOP (8 ppm), 5; 5,7-DMC (6 ppm), 6; 5,8-MOP (10 ppm), 7; 7-E-4-MC (10 ppm), 8; and imperatorin (6 ppm), 9.

Table I. Statistical Evaluation of Coumarin, 7-HC, 7-MOC, 5,7-DMC, 7-E-4-MC, Psoralen, 8-MOP, 5-MOP, 5,8-MOP, 9-IOP, and Calibration Data Obtained by HPLC with UV Absorbance Detection

Coumarins and psoralens	$y = a + bx$*	r†	Range of linearity‡ (µg/mL)	LOD§ (ng/mL)
Coumarin	$y = -736 + 4154x$	1.0000	2.0–8.0	0.366
7-HC	$y = -1153 + 7705x$	0.9999	1.0–10	0.219
7-MOC	$y = -302 + 6684x$	0.9999	2.0–6.0	0.317
5,7-DMC	$y = -222 + 4224x$	0.9999	2.0–6.0	0.440
7-E-4-MC	$y = -57 + 3417x$	0.9999	3.0–10	0.536
Psoralen	$y = -137 + 3600x$	0.9999	2.5–10	0.300
8-MOP	$y = -447 + 3306x$	1.0000	1.0–10	0.531
5-MOP	$y = 6 + 4000x$	0.9999	2.0–10	0.531
5,8-MOP	$y = -142 + 3613x$	0.9999	2.5–10	0.237
9-IOP	$y = -827 + 7583x$	0.9999	2.0–6.0	0.280

* a = intercept on the ordinate; b = slope.
† r = correlation coefficient.
‡ µg/mL, which correspond to injected amounts from 20 to 200 ng in 20 µL.
§ Limit of detection (µg/mL) at a signal-to-noise ratio of 3.

Linearity and limits of detection

The standard curves for all nine compounds were determined simultaneously at 312 nm. Their slopes, intercepts, correlation coefficients, and limits of detection (LOD) are shown in Table I. The UV intensity showed a linear relationship with the concentration of coumarins and psoralens over a wide range up to 1.0 µg/mL. The LOD was given by:

$$LOD = K\, So/S \qquad \text{Eq. 1}$$

where K is a numerical factor chosen according to the confi-

Table II. Recovery of Coumarins and Psoralens Fortified in Human Urine for HPLC–UV

Coumarins and psoralens	Sample subject's urine: Added (µg/mL)	Found (µg/mL)	Recovery (%, n = 6*)
Coumarin	5.49	5.38	98 (2.8%)†
7-HC	5.49	5.44	99 (2.2%)
7-MOC	5.56	5.62	101 (1.5%)
5,7-DMC	5.56	5.34	96 (0.7%)
Psoralen	5.26	5.19	99 (0.9%)
8-MOP	5.10	4.95	97 (1.0%)
5,8-MOP	5.10	5.15	101 (2.5%)

* Number of determination.
† Numbers in parentheses indicate relative standard deviation (RSD).

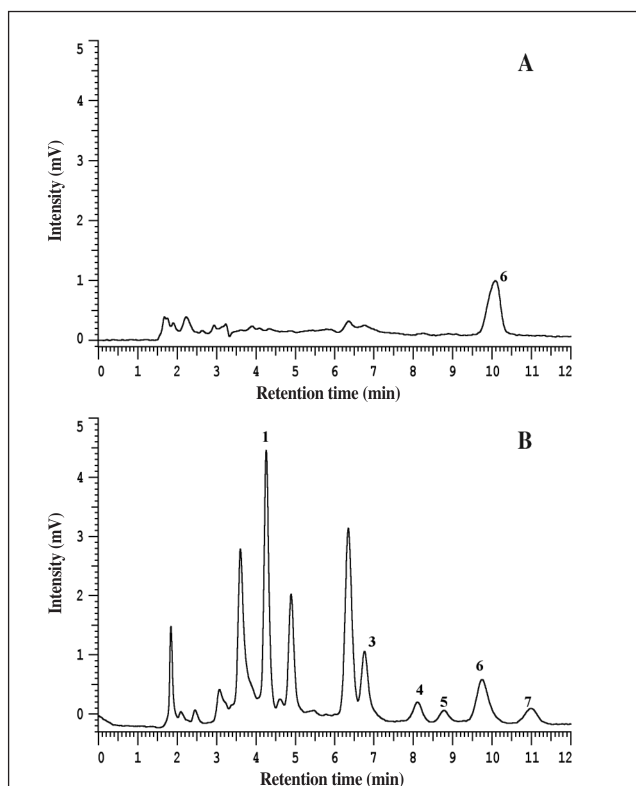

Figure 2. Coumarin and psoralen metabolites profile of human urine extract before (A) and after (B) intake of Umbelliferae medicines. The conditions are as given in Figure 1. Peak identification: 7-HC, 1; 7-MOC, 3; psoralen, 4; 8-MOP, 5; 5,7-DMC, 6; and 5,8-MOP, 7.

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dence level desired, So is the standard deviation of the blank measurement (n = 6), and S is the sensitivity of the calibration graph. Here, a K value of 3 was used, and the LODs are shown in Table I.

Recoveries and limit of quantitation (LOQ)

Coumarin and psoralen mixtures for fortification were prepared by mixing the stock solution and dilution with methanol. A 500-µL aliquot of the mixture was added to 0.5 mL of delipidated urine samples and to urine samples that contained known amounts of endogenous coumarins and psoralens. Extraction was carried out as described previously. To calculate percentage recovery, the amount of endogenous coumarins and psoralens was subtracted from the measured total amount, divided by the added amount, and multiplied by 100. Table II shows the LC–UV traces obtained for a control urine and volunteer sample spiked with coumarins, 7-HC, 7-MOC, 5,7-DMC, psoralen, 5-MOP, 8-MOP, 5,8-MOP, and 9-IOP, respectively. Excellent recoveries and precision were observed (recoveries ranging from 96% ± 0.7% to 101% ± 1.5%). The limits of quantitation were 7.32, 4.32, 6.34, 8.80, 10.7, 6.00, 10.6, 10.6, 4.74, and 5.60 pg for coumarin, 7-HP, 7-MOC, citropten (5,7-DMC), 7-E-4-MC, psoralen, xanthotoxin (8-MOP), bergapten (5-MOP), isopimpinellin (5,8-MOP), and imperatorin (9-IOP), respectively.

Table III. Mean Coumarin and Psoralens Metabolites Concentrations in Human Urine During and after Continuous Oral Administration of *Umbelliferae* Chinese Medicine by LC–UV

Subjects		Duration of intake (days) (µg/mL, n = 3*)					
		1	2	3	4	5	6
F1	Coumarin	–[†]	–	0.303 (4.5%)	0.748 (0.7%)	–	–
	7-HC	0.416 (3.3%)[‡]	0.674 (4.8%)	0.758 (0.9%)	1.157 (4.0%)	0.231 (4.9%)	0.124 (3.5%)
	7-MOC	–	0.138 (1.4%)	0.181 (3.4%)	0.164 (1.8%)	0.147 (3.5%)	0.110 (1.3%)
	5,7-DMC	0.152 (1.8%)	0.233 (2.8%)	0.321 (0.9%)	0.281 (3.7%)	0.199 (0.7%)	0.112 (3.5%)
	Psoralen	–	0.008 (4.6%)	0.010 (2.9%)	0.107 (3.0%)	0.079 (3.7%)	0.047 (4.5%)
	8-MOP	0.043 (1.8%)	0.161 (3.7%)	0.172 (5.2%)	0.063 (4.7%)	0.027 (2.5%)	0.031 (4.0%)
	5,8-MOP	–	0.008 (3.2%)	0.010 (0.9%)	–	–	–
F2	coumarin	1.036 (1.3%)	–	–	–	–	–
	7-HC	1.148 (1.9%)	1.665 (4.0%)	2.098 (0.4%)	0.155 (1.7%)	1.080 (1.2%)	0.351 (2.9%)
	7-MOC	0.119 (2.6%)	0.391 (4.8%)	–	–	–	–
	5,7-DMC	0.521 (2.8%)	0.301 (2.6%)	–	0.205 (3.3%)	–	0.307 (3.9%)
	Psoralen	–	–	–	–	–	–
	8-MOP	–	–	–	–	–	–
	5,8-MOP	–	–	–	–	–	–
F3	coumarin	1.116 (1.0%)	2.088 (2.1%)	1.114 (0.8%)	0.555 (1.2%)	0.229 (2.8%)	0.122 (4.2%)
	7-HC	1.11 (2.3%)	1.332 (0.3%)	1.457 (1.7%)	1.209 (0.5%)	1.269 (0.5%)	0.710 (2.9%)
	7-MOC	–	–	–	–	–	–
	5,7-DMC	0.112 (5.2%)	0.258 (1.3%)	0.299 (5.1%)	0.104 (2.1%)	0.066 (1.2%)	0.043 (3.3%)
	Psoralen	0.037 (0.9%)	0.037 (1.3%)	–	0.028 (4.7%)	0.013 (2.1%)	0.010 (4.2%)
	8-MOP	–	–	–	–	–	–
	5,8-MOP	–	–	–	–	–	–
M1	Coumarin	0.179 (3.7%)	0.086 (3.5%)	–	0.024 (3.6%)	–	–
	7-HC	0.111 (3.4%)	0.515 (0.8%)	0.755 (0.6%)	0.500 (1.1%)	0.786 (4.9%)	0.308 (5.0%)
	7-MOC	–	–	–	–	–	–
	5,7-DMC	0.032 (2.3%)	–	–	0.295 (2.7%)	0.169 (1.5%)	0.170 (3.6%)
	psoralen	–	–	–	–	–	–
	8-MOP	–	–	–	–	–	–
	5,8-MOP	–	–	–	–	–	–
M2	Coumarin	–	–	–	0.139 (3.3%)	0.059 (2.4%)	–
	7-HC	0.525 (1.9%)	0.710 (3.8%)	2.180 (5.1%)	0.478 (5.0%)	0.636 (2.7%)	191 (3.9%)
	7-MOC	–	–	–	–	–	–
	5,7-DMC	0.105 (0.6%)	0.148 (2.4%)	0.384 (5.1%)	0.375 (5.0%)	–	–
	Psoralen	–	–	–	–	–	–
	8-MOP	–	0.142 (1.1%)	0.126 (3.2%)	0.067 (5.3%)	–	–
	5,8-MOP	–	–	–	–	–	–

* Number of determination.
† Not determined.
‡ Number in parentheses indicates RSD.

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Application to human urine

The proposed LC–UV method was applied to the determination of coumarin, 7-HC, 7-MOC, 5,7-DMC, psoralen, 8-MOP, and 5,8-MOP in human urine. Representative LC–UV chromatograms of the metabolites for the coumarins and psoralens in a volunteer urine extract both before and after medicinal intake are shown in Figures 2A and 2B, respectively. Figures 2A and 2B compare with a chromatogram of pure standard (Figure 1). Sample constituents with retention characteristics identical to those of coumarin, 7-HC, 7-MOC, 5,7-DMC, psoralen, 8-MOP, and 5,8-MOP were identified and measured. Table III contained urine concentrations of metabolites in five groups of subjects that continuously took *Umbelliferae* medicines for 1, 2, 3, 4, 5, and 6 days. The previously described assay was used to elucidate the pharmacokinetics of methoxypsoralensin in human urine after continuous doses of *Umbelliferae* medicines.

Application to *Umbelliferae* medicines

Recovery tests were carried out on *Umbelliferae* medicines for evaluation of the reproducibility and accuracy of the proposed method. Seven commercial *Umbelliferae* medicines were spiked with the amounts of the agents reported in Table IV and subjected to the full extraction procedure. As can be seen, excellent recoveries and precision for *Umbelliferae* medicines were observed. A representative *Umbelliferae* medicine is shown in Figure 3A. Analytical results are given in Table V.

Table IV. Recovery of Coumarins and Psoralens for Umbelliferase Medicines

	Sample Chinese medicines		
Coumarins	Added (μg/mL)	Found (μg/mL)	Recovery (%, $n = 3$*)
Coumarin	2.00	2.02	101 (3.8%)†
7-HC	2.50	1.47	99 (1.5%)
Psoralen	1.00	0.99	99 (4.0%)
8-MOP	4.00	1.95	99 (1.0%)
5-MOP	1.00	1.02	102 (2.7%)
5,8-MOP	1.00	1.00	100 (1.8%)
9-IOP	2.00	1.98	99 (2.8%)

* Number of determinations.
† Number in parentheses indicates RSD.

Robustness and ruggedness

This method is relatively free from chemical interferences, and it can be applied to the determination of analytes of lime oil in *Rutaceae*, which is shown in Figure 3B. Random variations in experimental conditions also introduce uncertainty. To determine the ruggedness of the method, the effect of a change in level for the following factors was studied using the experimental

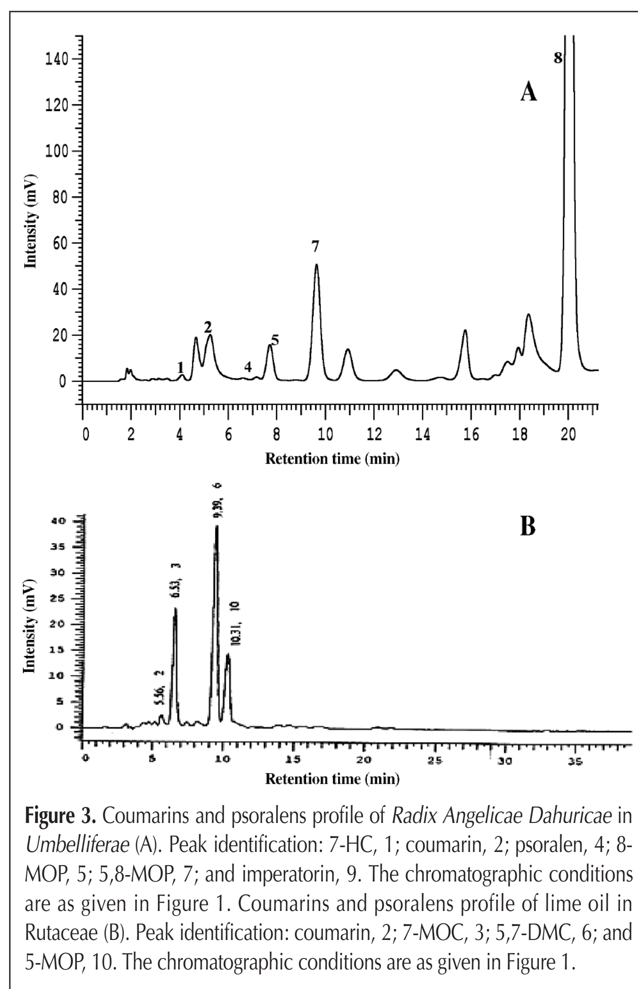

Figure 3. Coumarins and psoralens profile of *Radix Angelicae Dahuricae* in *Umbelliferae* (A). Peak identification: 7-HC, 1; coumarin, 2; psoralen, 4; 8-MOP, 5; 5,8-MOP, 7; and imperatorin, 9. The chromatographic conditions are as given in Figure 1. Coumarins and psoralens profile of lime oil in Rutaceae (B). Peak identification: coumarin, 2; 7-MOC, 3; 5,7-DMC, 6; and 5-MOP, 10. The chromatographic conditions are as given in Figure 1.

Table V. Analytical Results of Determination of 7-HC, Coumarin, Psoralen, 8-MOP, 5-MOP, 5,8-MOP, and 9-IOP in Umbelliferase Medicines by HPLC–UV*

Sample	7-HC	Coumarin	Psoralen	8-MOP	5-MOP	5,8-MOP	9-IOP
Radix Angelicae Dahuricae	0.24 (0.9%)†	1.04 (1.0%)	0.06 (2.4%)	0.91 (3.3%)	–‡	2.82 (1.8%)	23.068 (1.2%)
Radix Glehniae	–	–	0.02 (1.7%)	0.02 (4.0%)	–	0.03 (2.1%)	0.021 (2.7%)
Fructus Cnidii	–	1.12 (0.3%)	–	6.92 (3.6%)	15.04 (3.4%)	36.428 (0.5%)	
Foeniculum vulgare Mill	–	0.23 (4.0%)	0.08 (1.8%)	0.06 (3.4%)	–	0.27 (4.3%)	0.258 (4.9%)
Saposhnikovia divaricata	0.41 (2.6%)	0.28 (5.5%)	0.43 (3.3%)	1.09 (2.7%)	–	1.58 (0.8%)	0.557 (3.0%)
Radix Prucedani	1.27 (1.2%)	–	1.34 (2.0%)	2.96 (0.3%)	3.05 (3.1%)	–	3.755 (4.7%)
Glycyrrhiza uralensis Fisch	0.29 (6.9%)	0.86 (1.4%)	–	0.53 (0.9%)	–	0.76 (2.7%)	0.931 (2.7%)

* Number of determinations ($n = 6$).
† Number in parentheses is the RSD.
‡ Not indicated.

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design shown in ruggedness testing:

Factor A, mobile phase compositions: gradient (I), A; and gradient (II), a. Factor B, column temperature: 25°C, A; 35°C, a. Factor C, storage position: light, A; dark, a. Factor D, storage temperature: 4°C, A; 30°C, a. Factor E, storage time: 7 h, A; 28 h, a.

The method sensitivity is highly independent of (i.e., unaffected by) experimental conditions, such as variations of mobile phase compositions, column temperature, analyte storage in the dark, temperature, and time. The exception is psoralen, which increased by approximately 25%. The method's estimated relative standard deviation was approximately 0.5%.

Conclusion

Chromatographic separations were carried out with gradient elution on a reversed-phase Hypersil C_8 column. Acetonitrile–water showed good extraction efficiency, with a 1:1 mixture being optimal for liquid–solid extraction. LC–UV was applied to the determination of the metabolites in the urine sample from five subjects who had been treated with *Umbelliferae* medicine. This allowed quantitation of coumarin and psoralen metabolites in human urine by a single determination. The results of this study support the notion that 7-HC is the major metabolite of coumarin in humans that is largely excreted in the urine as the glucuronide conjugate. Although minor quantities of psoralen metabolites have been identified in human urine, the presence of psoralen in urine has not been reported before.

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