Simultaneous Determination of Four Lignan Compounds in *Herpetospermum caudigerum* by Normal-Phase High-Performance Liquid Chromatography

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Abstract

A normal-phase high-performance liquid chromatographic method with diode array UV detection is developed for the simultaneous quantitation of four lignan compounds in *Herpetospermum caudigerum*. This analysis provides a good resolution and reproducibility. Chromatography is carried out with a mobile phase of *N*-hexane–dichlormethane–methanol (42.5:42.5:5, v/v) at a flow rate of 1.0 mL/min. UV detection is performed at 280 nm. The calibration curve for lignans concentration is linear over the range of 2.10 to 42.0 µg/mL, 15.26 to 305.2 µg/mL, 6.15 to 123.0 µg/mL, and 6.24 to 124.8 µg/mL, respectively. The limit of quantitation and detection for compounds 1, 2, 3, and 4 is 1.31, 2.74, 2.63, and 2.17 µg/mL and 0.28, 0.25, 0.27, and 0.31 µg/mL, respectively. The validation data show that the assay is sensitive, specific, accurate, and reproducible for the simultaneous quantitation of four compounds. This rapid method is therefore appropriate to quantitate these lignans in *Herpetospermum caudigerum*.

Introduction

Herpetospermum caudigerum is the dry and mature seed of *Herpetospermum caudigerum* Wall (1). It occurs chiefly in the southwest region of China and Nepal, and northeast of India, and it has been widely used for many liver diseases, cholic diseases, and dyspepsia treatments (2,3). The seed is known to produce many important compounds (mainly lignans) (4–10). The lignans are reported to have multiple pharmacological effects, such as anti-hepatitis virus and resisting experimental hepatic injury (12–14). Among its active compounds, compounds 1, 2, and 4 have attracted attention from scientists (4,6,9). Compound 3 is a new component obtained from *Herpetospermum caudigerum* at this institute. Up to the present, a high-performance liquid chromatography (HPLC) method has not been applied to quantitate these compounds. As the four compounds (1–4) are present in minor quantities in plant seeds, an accurate and rapid method of analysis with reproducible results is required. The major constraint is the lack of an accurate analytical procedure because of the resemblance of the two compounds (1 and 2). The available reversed-phase (RP)-HPLC methods have been investigated. The simultaneous quantitation of the previously mentioned lignans in *Herpetospermum caudigerum* remains a question because of the poor resolution of the two compounds (1 and 2).

In this paper, an accurate and rapid analytical procedure for the simultaneous quantitation of the four lignans using normal-phase (NP)-HPLC is reported. The analysis provides a good resolution of the four compounds (1–4) and reproducible results. This analysis is validated for the simultaneous quantitation of the previously mentioned lignans.

Experimental

Materials and chemicals

The dry and mature seeds of *Herpetospermum caudigerum* Wall. were obtained from Tibet, China. The compounds 1, 2, 3, and 4 (99.27%, 98.65%, 99.06%, and 99.12%, respectively) were prepared at this institute (Institute of Chinese Medicine, 302 hospital of PLA, Beijing, China). *N*-hexane, dichlormethane, and methanol were of HPLC grade. Other chemicals were of analy tical grade. Double distilled water was used after filtering through a 0.45-µm filter.

Chromatographic instrument and conditions

An HP 1100 chromatographic system consisting of a quaternary pump (G1100A QuatPump, Agilent, Palo Alto, CA), degasser, diode array detector (G1100A DAD, Agilent), and HP Chemstation Data system (Agilent Technologies) was used. Separation was achieved on an NP silica column (250 × 4.6-mm i.d., 5 µm) (Apollo Silica, spherical, pore size: 100 Å, SN:

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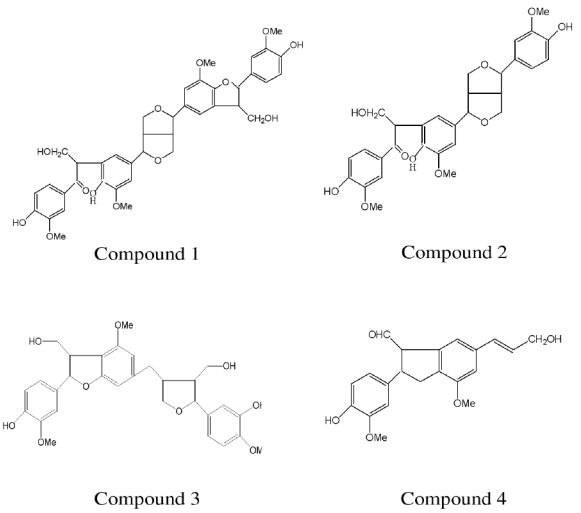

Figure 1. Chemical structures of the lignan compounds 1–4.

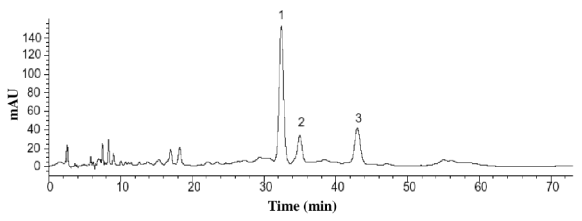

Figure 2. RP-HPLC separation of the extract of Herpetospermum caudigerum. Conditions were: RP-C18 column; acetonitrile–water contaning 1.0% glacial acetic acid (24:76, v/v); flow rate, 1.0 mL/min; and UV detection was performed at 280 nm. Peak 1, compound 1 and compound 2; peak 2, compound 3; and peak 3, compound 4.

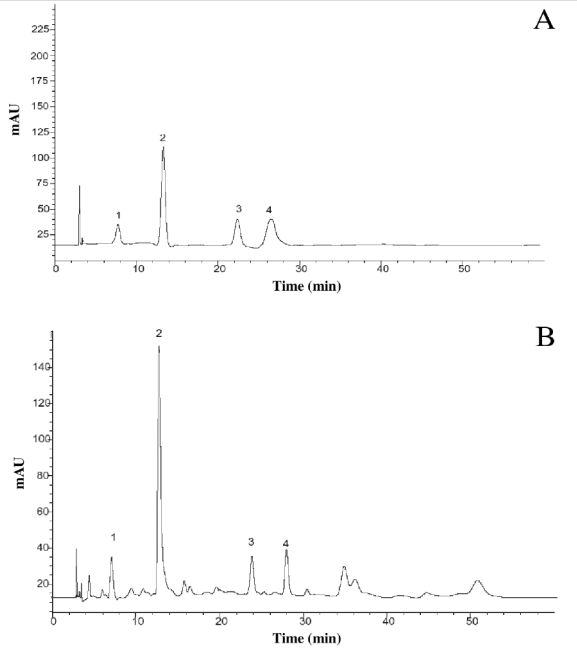

Figure 3. Chromatograms (NPLC) of four standard lignan compounds (A) and the extract of *Herpetospermum caudigerum* (B). Peak 1, compound 1; peak 2, compound 2; peak 3, compound 3; and peak 4, compound 4.

605061866.3, Alltech Associates, Deerfield, IL). The injection volume was 20 µL. Solvents were filtered before use by a Millipore filtration unit (Millipore, Billerica, MA). A constant flow rate of 1.0 mL/min was used during analysis. An optimum mobile phase composition was achieved by using different compositions of *N*-hexane–dichlormethane–methanol. The final composition was optimized as *N*-hexane–dichlor-methane–methanol (42.5:42.5:5, v/v). The other conditions were room temperature and detector wavelength at 280 nm.

Sample and calibration curve preparation

Brief, powdered dry seed (2.0 g) was extracted with methanol (1 × 50 mL, 3 h) at 80°C. It was cooled to room temperature, then methanol was added to 50 mL. The resultant was filtered through a 0.45-µm filter.

Stock solutions (1.0 mg/mL) of each of compound (1–4) were prepared in methanol, and different amounts of these were used for the preparation of calibration curves, linear in the range of the working concentration of each standard. The calibration curve for each analyte was built by injecting the standard solution, respectively.

Results and Discussion

The four lignan compounds are obtained from the extract of *Herpetospermum caudigerum*. The compound 3 is not previously found in this genus.

As the chemical structures of the four lignan compounds are similar (Figure 1), they have similar solubility and spectral characteristics. This makes it difficult to detect and determine each of them in the presence of the others in plants such as

Table I. Regression Analysis of Calibration Curves of Four Lignan Compounds

Lignans	t_R (min)	Regression equation	Correlation coefficient (r)	Linear range (µg/mL)
1	7.388	$y^* = 118.80x^\dagger + 47.24$	0.9993	2.10 ~ 42.0
2	13.034	$y = 48.32x - 36.51$	0.9997	15.26 ~ 305.2
3	22.722	$y = 112.80x - 33.22$	0.9995	6.15 ~ 123.0
4	26.968	$y = 92.56x - 63.25$	0.9998	6.24 ~ 124.8

* y = Mass concentration (µg/mL).
† x = Peak area.

Table II. Peak Purity Test Results

Lignans	Peak purity		Similarity
	Up	Down	
1	0.99	0.99	0.99
2	0.99	0.98	0.99
3	0.98	0.99	0.99
4	0.99	0.99	0.99

Herpetospermum caudigerum. The RP-HPLC methods using different mobile phases and flow rates did not provide a good resolution of the two compounds (1 and 2) (Figure 2). As shown in Figure 2, there is a singlet in the peak 1. But the HPLC-diode array detector (DAD) test of the online UV spectra of the peak in Figure 2 shows two different UV spectra. Therefore, this peak could be confirmed as two compounds (1 and 2).

However, a good resolution was obtained by using NP-HPLC (Figure 3). As shown in Figure 3, there are four singlet peaks (peaks 1, 2, 3, and 4). And the HPLC-DAD test of the online UV spectra of each peak in Figure 3 shows one UV spectra, respectively. Therefore, the peak could be confirmed as one compound. This analysis was validated for the simultaneous quantitation of the four lignans. Analysis was carried out at a wavelength of 280 nm, close to the absorption maxima of all four lignans.

Precision

Precision of the method was measured by repeating each experiment three times. The relative standard deviation (RSD) percentages were 1.08%, 0.86%, 0.59%, and 0.70% for compounds 1, 2, 3, and 4, respectively.

Calibration and linearity

The calibration curves were obtained by plotting the peak area against the concentration of corresponding standards. Equations for a linear least square regression fit of each analyte of interest along with the concentration range and correlation coefficients are provided in Table I.

Recovery

For the estimation of recovery, a known amount of stock solution of the four lignans was added to *Herpetospermum caudigerum*. The values of recovery of the compounds 1, 2, 3, and 4 were 97.89%, 98.33%, 97.57%, and 99.12%, respectively.

Evaluation of peak purity

The identity of each peak and its homogeneity was checked by comparing the DAD-generated data with that of the reference compounds using library matching. All peaks were found pure both at up and down slopes of the peaks (Table II). Similarity of the four lignans in the samples checked by comparison of data in a library was greater than 0.99 (Table II).

Table III. Data of LOD and LOQ

Lignans	LOD (µg/mL)	LOQ (µg/mL)
1	0.28	1.31
2	0.25	2.74
3	0.27	2.63
4	0.31	2.17

Table IV. Percent Contents of the Lignans in the Dried Seeds of *Herpetospermum caudigerum* (%)

No.	Compound 1	Compound 2	Compound 3	Compound 4
1	0.0429	0.3853	0.0877	0.0953
2	0.0387	0.3987	0.0803	0.1008
3	0.0432	0.4119	0.0786	0.0856

Limit of detection and quantitation

The use of a DAD detector starting with the maximum noise assisted with the rapid and accurate investigation of the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ values were calculated for compounds 1–4 based on 3 and 10 times of noise level, respectively. The values of the LOD and LOQ are given in Table III.

Determination of contents

The NP-HPLC has been used to determine the content of four lignans in *Herpetospermum caudigerum* in three samples. The percent contents of compounds 1, 2, 3, and 4 in *Herpetospermum caudigerum* are reported in Table IV.

Conclusion

The content of compounds 1, 2, 3, and 4 in *Herpetospermum caudigerum* can be determined by NP-HPLC. This paper demonstrates that this method for the simultaneous quantitation of the four compounds is simple, accurate, sensitive, and rapid, and that this method can be applied for routine quality control of *Herpetospermum caudigerum*.

Acknowledgments

The authors would like to appreciate the financial support of the great Innovation Foundation of Beijing city of China under Grant No. D0204003040931.

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Manuscript received April 6, 2006;
revision received January 12, 2007.