Quantitative and Qualitative Determination of Liuwei Dihuang Tablets by HPLC–UV-MS–MS

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Abstract

A method is developed for the analysis of allantoin, gallic acid, dihydromelittoside, loganin, paeoniflorin, benzoylpaeoniflorin, and paeonol in Liuwei Dihuang tablets by high-performance liquid chromatography (HPLC)–UV-mass spectrometry (MS)–MS. Gradient elution with methanol–acetonitrile–water–formic acid solvent system is employed in the HPLC–electrospray ionization-MS study. The positive-ion ESI mode is suitable for these compounds. The peaks of gallic acid, loganin, dihydromelittoside, paeoniflorin, benzoylpaeoniflorin, and paeonol are identified by their mass spectra and the fragments of their MS–MS spectra. Allantoin, gallic acid, loganin, paeoniflorin, and paeonol are simultaneously determined by UV detection at 210 nm for quantitative purposes.

Introduction

Liuwei Dihuang Tablets, one of the most important Chinese patent medicines are widely used in eastern Asia. They are composed of Radix Rehmannide Preparata, Rhizoma Dioscoreae, Fructus Cormi, Cortex Moutan, Rhizoma Alismatis, and Poria. They have the therapeutic function of protecting the liver, arresting bleeding, inducing diuresis, blocking inflammation and fungi, lowering blood sugar level, and reinforcing the function of the heart. In China, there are hundreds of medicinal manufacturers who produce Liuwei Dihuang Tablets and its derivative varieties, such as Zhibai Dihuang Tablets, Guifu Dihuang Tablets, Mingmu Dihuang Tablets, Qiju Dihuang Tablets, Maiwei Dihuang Tablets, and Guishao Dihuang Tablets, etc. Suitable assay methods are therefore needed urgently for quality control purposes. Several methods have been developed to determine one constituent in Liuwei Dihuang Tablets. Determination of paeonal by gas chromatography (GC) (1), and ursolic acid by thin layer chromatography (TLC) (2), and high-performance liquid chromatography (HPLC) (3) are commonly used. However, owing to a lack of knowledge about the key bioactive compounds, there are still some imperfections in the appraisal of the preparation quality by the methods described. Suitable assay methods are, therefore, urgently needed for quality control purposes. Currently, there is no reported method using HPLC–electrospray ionization (ESI)-mass spectrometry (MS) for Liuwei Dihuang Tablets. The use of a MS as a detector for HPLC has increasingly gained acceptance, complementing or replacing conventional detection methods such as UV absorbance, electrochemical detection, or laser-induced fluorescence, which proved to be less informative and less universal (4–6). Among the various MS approaches, ESI-MS is considered to be the best when coupled with HPLC. It has been successfully used in the determination of ginsenosides (6) and cardiac glycosides (7). Electrospray ionization has a great advantage in the determination of polar constituents. It is a gentle ionization method in MS. A positive ESI detection mode was employed resulting in a good response for gallic acid, loganin, dihydromelittoside, paeoniflorin, benzoylpaeoniflorin and paeonol. Addition of formic acid to lower pH yields improved sensitivity.

In the HPLC–ESI-MS experiment, a UV detector was coupled to the HPLC system and a gradient elution with methanol–acetonitrile–water–formic acid solvent system was used to separate the Liuwei Dihuang Tablets within 60 min. Gallic acid, loganin, dihydromelittoside, paeoniflorin, benzoylpaeoniflorin, and paeonol were identified by analyzing their MS fragments. In this condition, allantoin, gallic acid, loganin, paeoniflorin, and paeonol were simultaneously determined by UV detection at 210 nm. The structures of these constituents are shown in Figure 1.

Experimental

Sample preparation

Three grams of powder of Liuwei Dihuang Tablets were accurately weighed and extracted with 50 mL of 95% ethanol

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for 0.5 h in an ultrasonic bath. The extraction was repeated three times. The extracted solutions were combined and concentrated nearly to dryness. The extract was diluted to 25 mL with 95% ethanol and filtered through 0.45-µm filter before sample injection.

Reagents and materials

Loganin was purchased from the Zhongriyouhao Hospital (Beijing, China), allantoin, gallic acid, paeoniflorin, and paeonol from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China), and formic acid from Shenyang Chemical Reagent Factory (Shenyang,China). Acetonitrile and methanol were of the HPLC grade (Yuwang Chemical Reagent Factory, Shandong, China). All the aqueous solutions were made up with doubly distilled water. Other chemicals were of the analytical grade.

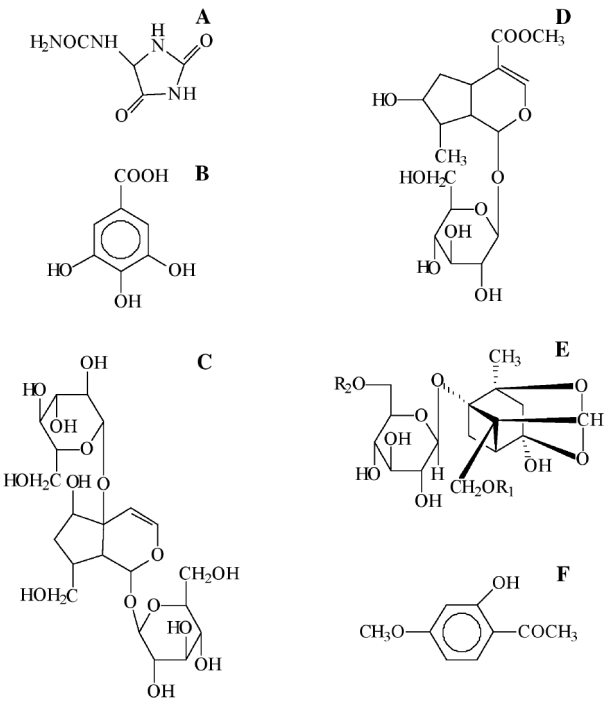

Figure 1. The structure of allantoin, gallic acid, dihydromelittoside, loganin, paeoniflorin, benzoylpaeoniflorin, and paeonol: allantoin (A), gallic acid (B); dihydromelittoside (C); loganin (D); paeoniflorin (E) (R1= Bz, R2= H); benzoylpaeoniflorin (E) (R1= Bz, R2= Bz); and paeonol, (F).

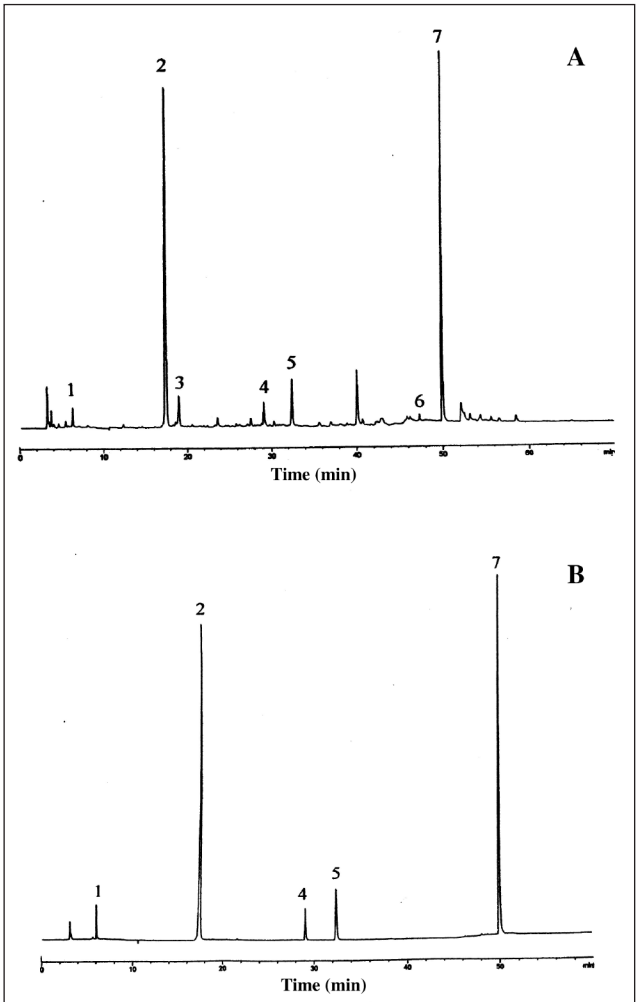

Figure 2. The UV (210 nm) chromatogram of the Liuwei Dihuang Tablets sample (A) and the standards (B). Peak numbers are: allantoin, 1; gallic acid, 2; dihydromelittoside, 3; loganin, 4; paeoniflorin, 5; benzoylpaeoniflorin, 6; and paeonol, 7.

Table I. Mobile Phase Gradient

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	2	0	98
3	2	0	98
4	0	2	98
12	0	6	94
26	0	20	80
30	0	20	80
40	0	30	70
50	0	100	0
60	0	100	0

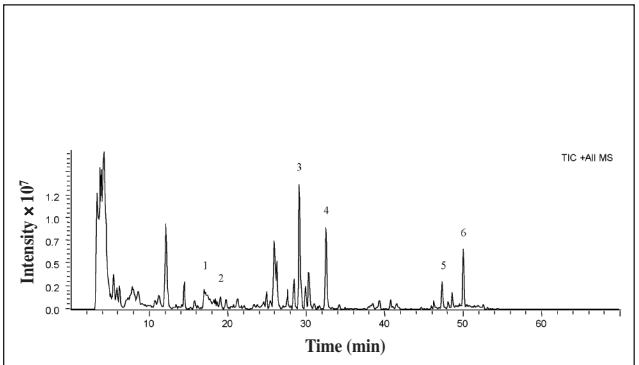

Figure 3. The total ion chromatogram (TIC) of the Liuwei Dihuang Tablets in the positive ion mass detection mode. Peak numbers are: gallic acid, 1; dihydromelittoside, 2; loganin, 3; paeoniflorin, 4; benzoylpaeoniflorin, 5; and paeonol, 6.

Apparatus and conditions

An Agilent 1100 series HPLC instrument (Waldbronn, BW, Germany) with a UV detector set at 210 nm was coupled with an Agilent ion trap MS. Mass spectra were obtained by acquiring data between 100 and 1000 atomic mass units. Chromatographic conditions were as follows: Column, Zorbax SB-C_{18}, 5 µm, 4.6 mm i.d × 250 mm. Gradient elution generated by the proportional mixing of methanol–acetonitrile–water–formic acid was used. The mobile phase consisted of solvent A (methanol), solvent B (acetonitrile), and solvent C (water–formic acid, pH 3.3) with the gradient is shown in Table I. Flow rate was 1 mL/min. After UV detection, 10% of the eluate was split off and introduced into the ESI-MS system. The MS was operated in the positive ion detection mode. The nebulizer nitrogen gas set at 40 psi, and the drying nitrogen gas set at 9 L/min. Drying temperature was 350°C.

Results and Discussion

In the ESI method under the positive ion detection mode, the addition of formic acid reduces the pH to improve sensitivity and the constituents analyzed gave a good response. In the present experiment, gradient elution with methanol–acetonitrile–water–formic acid was employed in different compositions. In this condition, allantoin, gallic acid, dihydromelittoside, loganin, paeoniflorin, benzoylpaeoniflorin, and paeonol gave a good separation in UV. The UV chromatogram is shown in Figure 2 and the total ion chromatogram (TIC) is shown in Figure 3.

The MS^2 spectra of gallic acid, loganin, dihydromelittoside, paeoniflorin, benzoylpaeoniflorin, and paeonol are shown in Figure 4. The MS fragment data and attribution of the 6 compounds are shown in Table II. The MS^2 fragments were analyzed as follows: Gallic acid (*m/z*), 171 (M^++H), and 127 (M^+-CO_2); loganin (*m/z*), 413 (M^++Na), 395 (M^+-H_2O), 381 (M^+-CH_3OH), 363 (M^+-CH_3OH-H_2O), and 251 (M^+-Glu); dihydromelittoside (*m/z*), 527 (M^++H), 509 (M^+-H_2O), 365 (M^+-Glu), and 347 (M^+-Glu-H_2O); paeoniflorin (*m/z*), 503 (M^++Na), 381 (M^+-C_6H_5COOH), and 341 (M^+-Glu); benzoylpaeoniflorin, 607 (M^++Na), and 485 (M^+-C_6H_5COOH); paeonol, 167 (M^++H), and 149 (M^+-H_2O).

In this condition, allantoin, gallic acid, loganin, paeoniflorin, and paeonol were simultaneously determined by UV detection at 210 nm. The results are shown in Table III.

Conclusion

The constituents of gallic acid, loganin, dihydromelittoside, paeoniflorin, benzoylpaeoniflorin, and paeonol were well separated using HPLC gradient elution and all of them had a good

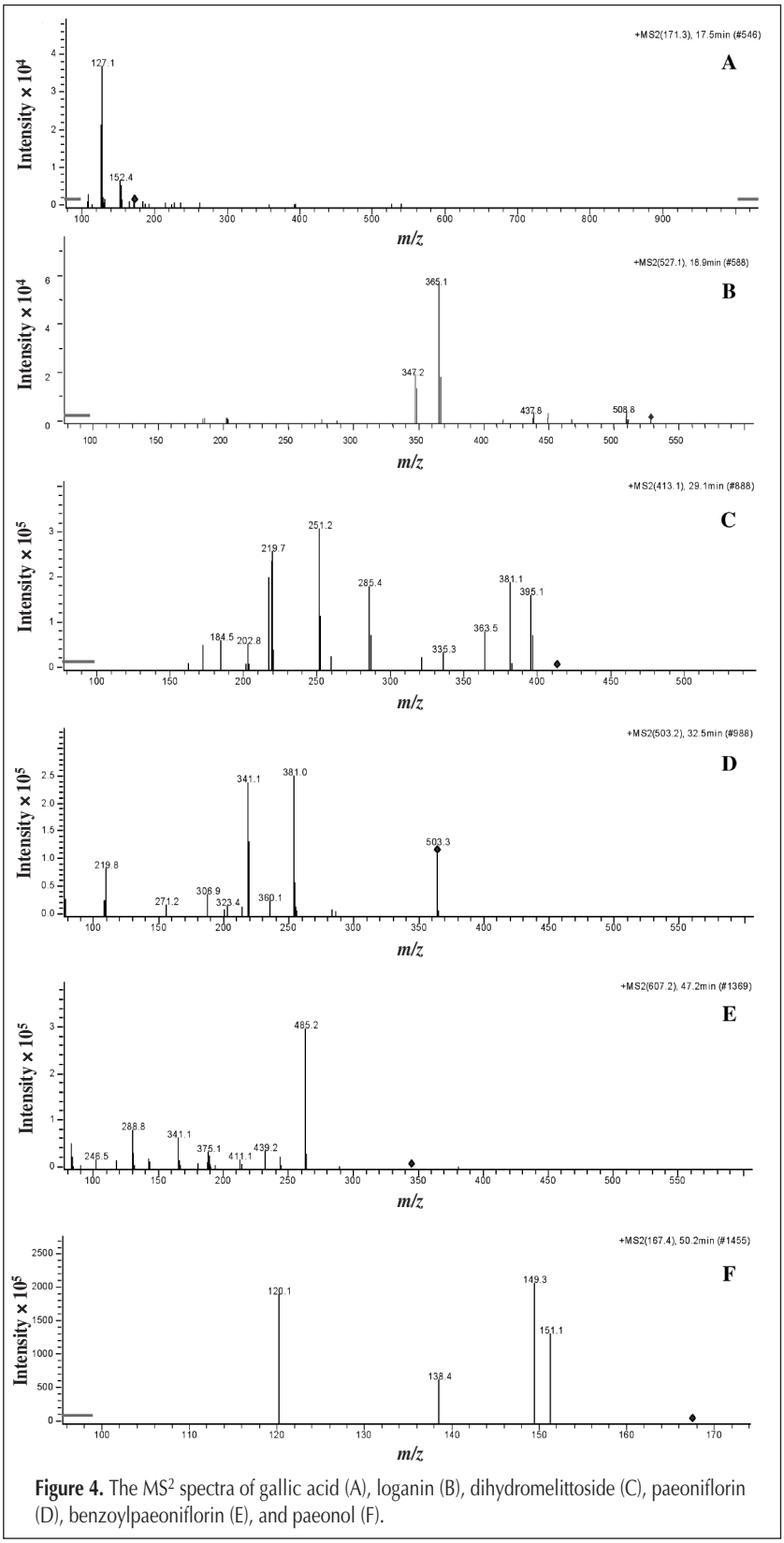

Figure 4. The MS^2 spectra of gallic acid (A), loganin (B), dihydromelittoside (C), paeoniflorin (D), benzoylpaeoniflorin (E), and paeonol (F).

Table II. The MS Fragment Data and Attribution of the Six Compounds in Liuwei Dihuang Tablets

MW	Attribution	$[M+H]^+$ or $[M+Na]^+$	MS–MS	Losses
170	Gallic acid	171	127	44
390	Loganin	413	395, 381, 363, 251	18, 32, 50, 162
526	Dihydromelittoside	527	509, 365, 347	18, 162, 180
480	Paeoniflorin	503	381, 341	122, 162
584	Benzoylpaeoniflorin	607	485	122
166	Paeonol	167	149	18

Table III. Determination Results for This Method (n = 5)

Compound	RSD (%)	Recovery (%)	Tailing factor	Plate number	Correlation coefficient	Content (%)
Allantoin	0.9	98.1	~ 1	3.4 × 104	0.9998	0.02
Gallic acid	0.6	98.8	~ 1	3.1 × 104	1	0.07
Loganin	0.8	98.7	~ 1	1.9 × 105	0.9999	0.04
Paeoniflorin	1.1	101.7	~ 1	1.1 × 105	0.9999	0.05
Paeonol	1.4	100.5	~ 1	8.3 × 105	0.9999	0.10

response in MS positive ESI mode. Allantoin, gallic acid, loganin, paeoniflorin, and paeonol were simultaneously determined by UV detection at 210 nm. The method is simple with excellent resolution and reproducibility which provided a good way for evaluating the quality of Liuwei Dihuang Tablets. The method can be more "accurate" in the appraisal of the quality of Liuwei Dihuang Tablets when compared with a single constituent determination method.

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