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Short communication

Simultaneous determinations of paeonol and palmatine hydrochloride in Shangshi Aerosols by HPLC method

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Abstract

A simple and rapid HPLC method was described for the simultaneous determination of paeonol and palmatine hydrochloride in Shangshi Aerosols. The optimum separation for these analytes was achieved using the mixture of 0.025 M sodium dihydrogen phosphate–acetonitrile–diethylamine (64:35:1, v/v/v) as the mobile phase and a Nova-Pak® C8 column. The linear ranges of paeonol and palmatine hydrochloride were 0.2–80 and 0.06–60 μg/ml with the regression equations being $Y = 11716.4 + 2.96 \times 10^6 X$ ($R = 0.99969$), $Y = -6388.8 + 1.89 \times 10^5 X$ ($R = 0.99976$), and limit of quantifications (LOQ) for paeonol and palmatine hydrochloride were 0.2 and 0.06 μg/ml, respectively ($n = 6$). Other validation parameters: intra-day precision (R.S.D.: 0.71–1.65%) and inter-day precision (R.S.D.: 0.89–2.11%), and reproducibility (recoveries values: 94.6–98.2% for paeonol, 94.85–97.58% for palmatine hydrochloride) were found to be satisfactory. The proposed HPLC method had been applied for the determination of paeonol and palmatine hydrochloride in Shangshi Aerosols; R.S.D. values were 1.45 and 1.13%, respectively. In short, this method was rapid and convenient, which could be used for the routine control of paeonol and palmatine hydrochloride in Shangshi Aerosols.

Keywords: HPLC; Paeonol; Palmatine hydrochloride; Shangshi Aerosols; Diethylamine

1. Introduction

For some years, HPLC method had been a very powerful technique offering possibility of qualitative and quantitative determination of different drugs. Shangshi Aerosol was produced by Pharmacy Company, the main components were as follows: panicled swallowwort root, radix tinospoae, dahurian angelica root, camphor, menthol, zedoary and so on. It was used for the treatment of wrench, rheumatism, lumbago and backache. Both paeonol and palmatine hydrochloride could relieve pain and activate blood flow and so on [1,2], so the contents of paeonol and palmatine hydrochloride were regarded as the main index of Shangshi Aerosols quality. The structures were illustrated in Fig. 1. Several methods had been developed to determine paeonol and palmatine hydrochloride, such as thin-layer chromatography (TLC) [3], gas chromatography (GC) [4], GC–MS [5] and high-performance capillary electrophoresis (HPCE) [6], capillary zone electrophoresis (CZE) [7]. HPLC methods and HPLC methods associated with other apparatuses were also reported [8–12]. However, there was no method reported for simultaneously determining paeonol and palmatine hydrochloride. Hence the aim of the study was focused on finding a method for the simultaneous determination of paeonol and palmatine hydrochloride in Shangshi Aerosols. In our study, paeonol and palmatine hydrochloride were successfully separated on Nova-Pak® C8 column with a mixture of 0.025 M sodium dihydrogen phosphate–acetonitrile–diethylamine (64:35:1, v/v/v). The retention times of paeonol and palmatine hydrochloride were only 4.323 and 6.273 min, the peak asymmetries were 1.09 and 1.05, so it was better than others described before [13,14]. Furthermore, the results indicated that the linearity, intra-day precision, inter-day precision and re-

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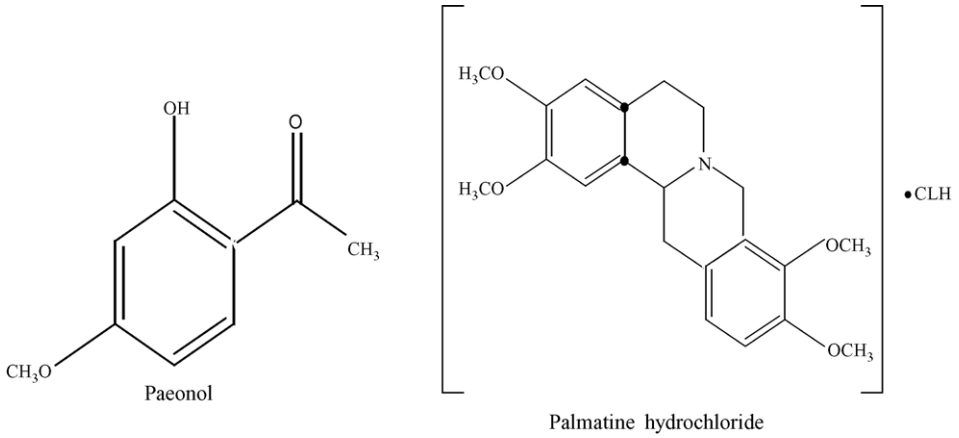

Fig. 1. Chemical structures of paeonol and palmatine hydrochloride.

producibility were good. In sum, this method was rapid and simple, which could be used for simultaneously determining paeonol and palmatine hydrochloride in Shangshi Aerosols.

2. Experimental

2.1. Reagents and chemicals

Paeonol, palmatine hydrochloride, cinnamyl aldehyde and aconitine were purchased from the National Institute for Control of Pharmaceutical and Products (Beijing, China). Acetonitrile was of HPLC grade (KeMiOu, China). The Shangshi Aerosols were friendly supported by Pharmacy Company. The water was doubly distilled pure water. Other chemicals were of analytical grade.

2.2. Apparatus and chromatographic conditions

Quantitive HPLC was performed on a high-pressure liquid chromatograph with two Shimadzu LC-10AD pumps, a Shimadzu SPD-10A UV/VIS detector, a CTO-10A column oven, a SIL-10A injector with a 50 ml loop was used for the injection of samples. The HPLC system was equipped with the software "N2000 workstation" (Zhejiang University). Nova-Pak® C8 column (3.9 mm × 150 mm, 4 μm; Waters).

The mixture of sodium dihydrogen phosphate (0.025 M, pH 4.5)–acetonitrile–diethylamine (64:35:1, v/v/v) was used as the mobile phase at the flow rate of 1 ml/min. UV detection was at 270 nm with a sensitivity of 0.05 AUFS. The mobile phase was filtered through a 0.45 μm membrane filter and degassed by a Shimadzu DGU-4A degasser. The performance was carried out at 40 °C.

2.3. Preparation of standard solutions and calibration graphs

Stock solutions of paeonol and palmatine hydrochloride (1 mg/ml) were prepared by dissolving 50 mg standard paeonol and palmatine hydrochloride in 50 ml mobile phase. The standard solutions were diluted with the mobile phase to obtain a serial of concentrations of paeonol and palmatine hydrochloride. Triplicate 10 μl injections were made six times for each concentration and chromatographed under the conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.4. Assays of samples solutions

Accurately measured 2 ml Shangshi Aerosols to 100 ml volumetric flask, diluted with the mobile phase, then filtered through a 0.45 μm organic Millipore membrane filter to the vials for injection. The experiments were performed according to above chromatograph conditions and chromatograms were recorded by N2000 workstation.

3. Results and discussion

3.1. The optimization of separation

The choice of the chromatographic conditions was guided by the need to obtain chromatograms with better resolution of adjacent peaks within a short analysis time. The aim of the study was to develop a simple and rapid method, which could be used in the routine analysis and control of paeonol and palmatine hydrochloride in Shangshi Aerosols. Therefore, the work was concentrated on the optimization of separating conditions. For the determination of palmatine hydrochloride, some reports [13,14] used the mobile phase including (A) KH_2PO_4 buffer (containing triethylamine or sodium dodecyl sulfonate) and (B) acetonitrile with gradient elution HPLC systems. However, the retention time was as long as 38 min or the peak asymmetry was more than 1.3. In our study, it was found that the retention time of paeonol was linearly related to the percentage of acetonitrile, which was irrelevant to the pH value of mobile phase and the percentage of diethy-

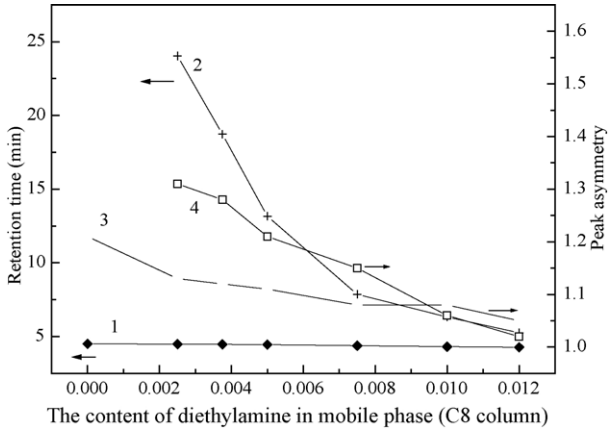

Fig. 2. The relationship of the diethylamine content vs. the retention time and peak asymmetry of paeonol and palmatine hydrochloride in mobile phase on C8 column; 1 and 3 were the curves of paeonol, 2 and 4 were the curves of palmatine hydrochloride.

lamine; while the pH value of mobile phase, the percentage of acetonitrile and diethylamine had the great effect on the peak shape and retention time of palmatine hydrochloride, especially the percentage of diethylamine. When the percentage of acetonitrile exceeded 35%, paeonol could not be separated from the mixture. While the percentage of acetonitrile was less than 35%, the retention time of paeonol became longer. So the optimized acetonitrile concentration was 35%. The function of diethylamine was investigated under this condition. As were shown in Fig. 2. The retentions and peak asymmetries of palmatine hydrochloride decreased with the percent of diethylamine increasing. Palmatine hydrochloride and paeonol could be completely separated when the percent of diethylamine was in the range of 0.25–1%. However, when the percent of diethylamine was more than 1.2%, the peaks of palmatine hydrochloride and aconitine overlapped each other. So the optimum diethylamine concentration was 1%. As was illustrated in Figs. 2 and 3, the retention times of paeonol and palmatine hydrochloride were 4.323 and 6.273 min, the peak asymmetries were 1.09 and 1.05, respectively. So the mobile phase containing 35% acetonitrile and 1% diethylamine was selected in all subsequent experiments.

3.2. Validation of the method

The linearities of paeonol and palmatine hydrochloride in standard solutions were investigated at nine concentration levels. The calibration curves for paeonol and palmatine hydrochloride were linear in the range of 0.2–80 and 0.06–60 μg/ml, and the representative linear equations were $Y = 11716.4 + 2.96 \times 10^6 X$ ($R = 0.99969$), $Y = -6388.8 + 1.89 \times 10^5 X$ ($R = 0.99976$), respectively (Y and X represented as peak areas and the concentrations of standard solutions). Limit of quantitation (LOQ) was determined at a signal to noise ratio (S/N) 10, LOQ was experimentally verified by diluting known concentrations of paeonol and palmatine hydrochloride until the average responses were approximately 10 times the standard deviation of the responses for six replicate determinations, LOQ for paeonol and palmatine hydrochloride were 0.2 and 0.06 μg/ml, respectively ($n = 6$).

The intra-day precision of the method was determined by preparing the standards of paeonol and palmatine hydrochloride at four different concentrations and values for each compound were determined by six repeated analyses. Inter-day precision was check with the same concentration as intra-day assays, and the determination of each compound was repeated day by day during 5 days. The results were given in Table 1. The method was found to be precise with R.S.D. values within 0.71–1.65% for intra-day assays and 0.89–2.11% for inter-day assays.

To study the reliability and suitability of the above method, recovery experiments were carried out. Five samples of the same Shangshi Aerosols with known paeonol and palmatine hydrochloride contents were sampled and certain volumes of reference paeonol and palmatine hydrochloride solutions were added. The results were shown in Table 2. The recoveries values were within 94.6–98.2% for paeonol and 94.85–97.58% for palmatine hydrochloride. The average recovery and R.S.D. were 96.18, 96.29 and 1.45, 1.13%, respectively. It indicted that the proposed method for determination of paeonol and palmatine hydrochloride was highly accurate.

The specificity of the HPLC method was illustrated in Figs. 3 and 4. The results demonstrated that there was no interference from the other components (cinnamyl aldehyde and

Table 1
Precision of proposed HPLC method

Compound	Concentrations (μg/ml)	n	Intra-day precision		Inter-day precision	
			Peak area	R.S.D. (%)	Peak area	R.S.D. (%)
Paeonol	10	6	29517451	0.97	29674531	1.31
	15	6	44267876	1.11	44294599	1.27
	20	6	59024647	1.24	59421569	1.59
	25	6	73767619	1.51	73901541	2.11
Palmatine hydrochloride	0.5	6	63853	0.71	64171	0.89
	5	6	724983	1.05	725147	1.17
	10	6	1458067	0.91	1451899	1.11
	15	6	2193498	1.65	2201576	1.79

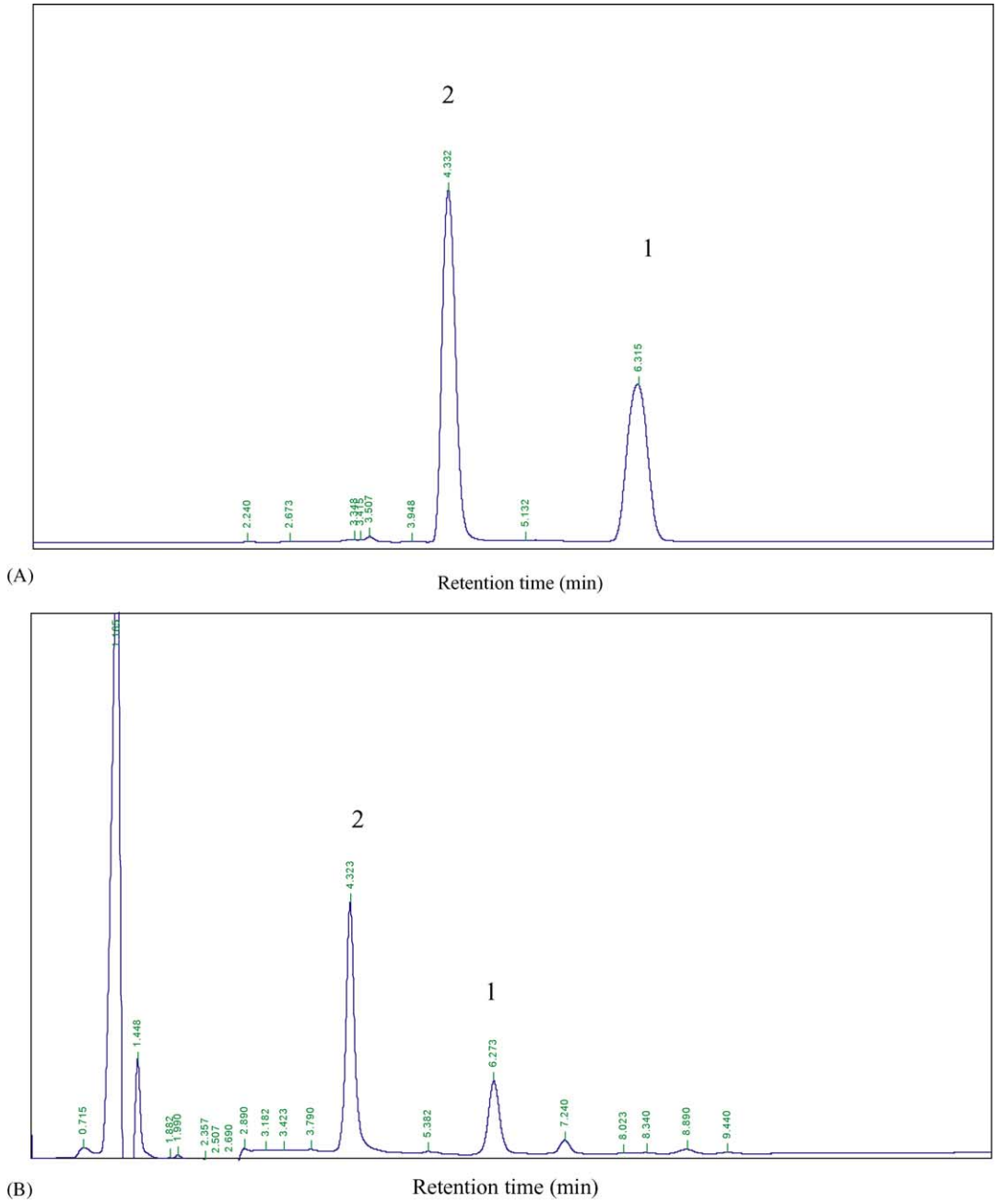

Fig. 3. HPLC chromatograms of standard solutions (A) and Shangshi Aerosol (B) on C8 column (1 denotes palmatine hydrochloride; 2 denotes paeonol); conditions: mobile phase, 0.025 M sodium dihydrogen phosphate:acetonitrile:diethylamine (64:35:1, v/v/v); flow rate, 1 ml/min; detection wavelength, 270 nm; column temperature, 40 °C; injection volume, 10 μl.

aconitine) in Shangshi Aerosols, and therefore conformed the specificity of the proposed method.

3.3. Analysis of real samples

Different batches of paeonol and palmatine hydrochloride in Shangshi Aerosols were analyzed by proposed HPLC method. The results were given in Table 3. The concentrations of paeonol and palmatine hydrochloride were within 0.7745–0.9456 mg/ml and 4.35×10^{-3} to 5.15×10^{-3} mg/ml, respectively. R.S.D. values within 0.75–2.11% were found to be satisfactory.

The proposed HPLC method could be used to simultaneously determine paeonol and palmatine hydrochloride in Shangshi Aerosols. The retention times of paeonol and palmatine hydrochloride were only 4.323 and 6.273 min, and the peak asymmetries were 1.09 and 1.05, so it was better than others described before. In sum, it was simple and rapid, and the results obtained were accurate and precise, and no significant interfering peaks were detected. So it was suitable

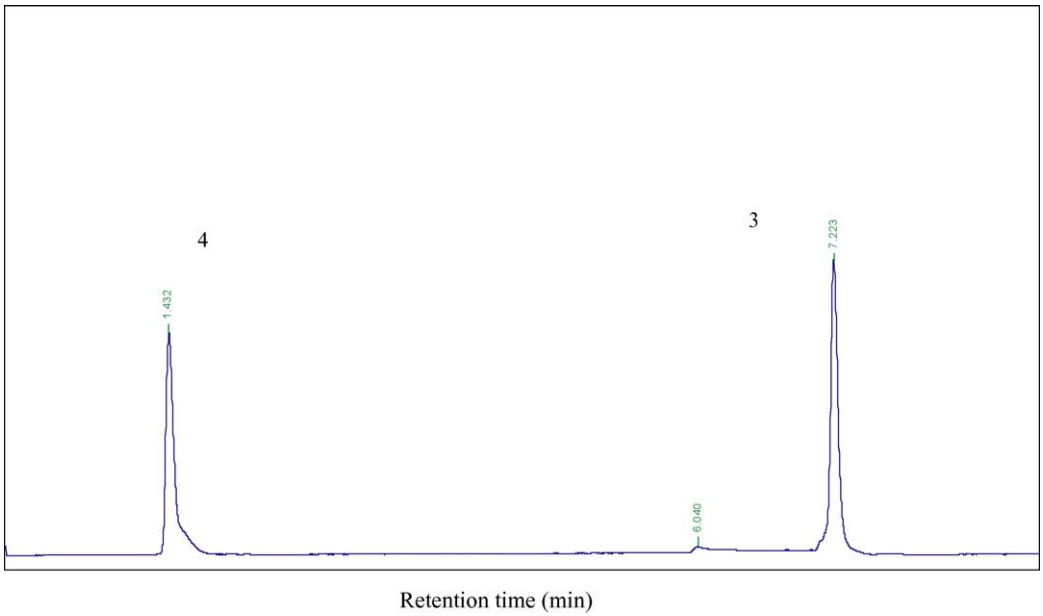

Fig. 4. HPLC chromatograms of aconitine (3) and cinnamyl aldehyde (4).

Table 2
Recovery tests of paeonol and palmatine hydrochloride in Shangshi Aerosols

Compound	Added (μg)	Measured (μg)	Recovery (%)	Average (%)	R.S.D. (%)
Paeonol	1.008	0.963	95.54		
	1.113	1.093	98.20		
	1.218	1.165	95.65		
	1.323	1.282	96.91		
	1.428	1.351	94.60	96.18	1.45
Palmatine hydrochloride	0.096	0.093	96.88		
	0.112	0.107	95.54		
	0.124	0.121	97.58		
	0.136	0.129	94.85		
	0.148	0.143	96.62	96.29	1.13

Table 3
Contents of paeonol and palmatine hydrochloride in batches of Shangshi Aerosols (mg/ml)

Batches	Paeonol		Palmatine hydrochloride	
	Concentration	R.S.D./(%) ($n=5$)	Concentration	R.S.D./(%) ($n=5$)
20030401	0.8522	0.96	4.65×10^{-3}	1.23
20030602	0.7745	1.52	4.9×10^{-3}	0.89
20030701	0.8579	0.75	4.8×10^{-3}	1.51
20040301	0.8240	0.89	4.35×10^{-3}	1.78
20040303	0.9456	1.38	5.15×10^{-3}	2.11
20040401	0.8743	1.15	4.7×10^{-3}	1.61
20040503	0.8651	1.27	5.07×10^{-3}	1.91
20040601	0.8597	1.41	4.95×10^{-3}	1.43
20040603	0.8861	1.19	4.67×10^{-3}	1.35

for the routine analysis and control of paeonol and palmatine hydrochloride in Shangshi Aerosols.

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