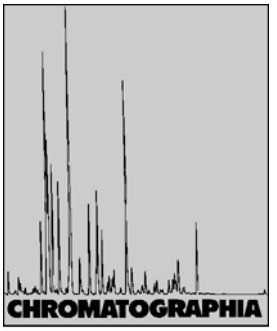
Simple HPLC Method for Simultaneous Determination of Acetaminophen, Caffeine and Chlorpheniramine Maleate in Tablet Formulations



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Key Words

Column liquid chromatography
Pharmaceutical analysis
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Summary

A simple, rapid and accurate, routine-HPLC method is described for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in a new tablet formulation. Chromatographic separation of the three pharmaceuticals was achieved on a Hypersil CN column (150 × 5.0 mm, 5 μm) using a mobile phase comprising a mixture of acetonitrile, an ion-pair solution and tetrahydrofuran (13:14:87, *v/v*, pH 4.5). The flow-rate was changed from 1.0 mL min^{-1} (in 0 ~ 7.5 min) to 1.8 mL min^{-1} (after 3.5 min). Detection was 223 nm. Separation was complete in < 10 min. The method was validated for system suitability, linearity, accuracy, precision, limits of detection and quantitation, and robustness. Linearity, accuracy and precision were found to be acceptable over the ranges 31.6 ~ 315.8 μg mL^{-1} for acetaminophen, 9.5 ~ 94.6 μg mL^{-1} for caffeine and 1.4 ~ 13.8 μg mL^{-1} for chlorpheniramine maleate.

Introduction

Anfenwanan Dispersible Tablets is a newly developed, over-the-counter cold medication, which achieves immediate dissolution of the active components after administration and can be used for the rapid relief of symptoms of coughs and colds. Three of the main active substances are acetaminophen (AMP, 250 mg), caffeine (CAF, 15 mg) and chlorpheniramine maleate (CHM, 2 mg) in each tablet. The properties of these active substances differ greatly and the problem of analysis is further complicated by the presence of 250 mg AMP compared to only 2 mg CHM. It is hard therefore, to find a simple assay for determining these three pharmaceuticals simultaneously.

An LC method seems to be the first choice due to its high separation efficiency, sensitivity and reproducibility. Of the papers concerned with determining the three pharmaceuticals by LC [1–4], only a few methods achieved simultaneous determination of all three [3–4]. One [3] describes a mixed ion-pair liquid chromatographic method for simultaneous assay of the three pharmaceuticals as well as two other components. The determination was by using a mobile phase comprising 50 mM monosodium phosphate, 125 mM TBA and 1 mM PSA by weight in acetonitrile-water (5:95, *v/v*, pH* 2.5) on a Shandon Hypersil Phenyl-2 column using two detection wavelengths (210 nm, 290 nm), constant high flow-rate (2 mL min^{-1}) and high column temperature (40 °C). The other method [2] uses a LiChrospher 100CN column and a mixture of acetonitrile-ion-pair solution [aqueous 5 mM HAS, 10 mM DBA, 0.8% AA (*v/v*) and 0.12% PA (*v/v*)] (15:85, *v/v*, pH* 3.3) as mobile phase. Three wavelengths were adopted separately: 265 nm for CHM, 298 nm for CAF and 310nm for AMP. In both methods, a photodiode array (PDA) detector was needed to achieve simultaneous detection of multi-components at individual maximum wavelengths.

Since the chromatographic conditions of both the above methods were complicated and two or three detection wavelengths were needed to achieve simultaneous detection of the three components, it was not easy to put these methods into practical use in standard laboratories. Therefore a simpler and more practicable LC method was needed for routine analysis of the three pharmaceuticals in Anfenwanan Dispersible Tablets. A simple, rapid and accurate LC method is described here, which can achieve simultaneous determination of the three active compounds in the above tablets using a Hypersil CN column (150 × 5.0 mm, 5 μm) at a single detection wavelength, 223 nm,

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in < 10 min. The method described can be used for routine analysis of Anfenwanan Dispersible Tablets in ordinary laboratories.

Experimental

Chemicals and Reagents

Anfenwanan dispersible tablets, each containing 250 mg AMP, 15 mg CAF and 2 mg CHM and two other components were from Shenyang PharmTech Institute of Pharmaceuticals (Shenyang, China). Standard samples for AMP, CAF and CHM were from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). HPLC-grade methanol, acetonitrile and hexanesulphonic acid sodium salt (HAS) were used. All other chemicals and reagents used were of analytical grade.

Instrumentation

Chromatographic separation was achieved on an LC system consisting of an HP1100 solvent delivery system, an HP variable UV:VIS detector, an HP integrator and an injection valve with 20 μL loop (Agilent, USA). The analytical column, Hypersil BDS C18 (150 ×4.6 mm, 5 μm), was packed and supplied by Elete (Dalian, China). A Shimadzu UV-2201 UV:VIS double-beam spectrophotometer was used for scanning and selecting the detection wavelength. Chromatograms and data were recorded on an EChrom Chromatographic Workstation.

Chromatographic Conditions

Separation was at ambient temperature (20 °C). The mobile phase comprising a mixture of acetonitrile, an ion pair solution and tetrahydrofuran (13:14:87, *v/v*, pH 4.5) was delivered at a flow rate of 1.0 mL min^{-1} (in 0 ~ 3.5 min) followed by 1.8 mL min^{-1}. The ion pair solution was an aqueous solution of HAS (5 mM), TEA (10 mM) and AA (1.0%). Prior to use, the mobile phase was filtered through 0.45 μm membrane filters and degassed in an ultrasonic bath. The analytical column was equilibrated with the eluent used. After an acceptably stable baseline was achieved, the standards and then the samples were analyzed. Absorbance was monitored at 223 nm where CHM had maximum absorption in the mobile phase. Under these conditions, the retention time was 2.6 min for CAF, 3.1 min for AMP and 7.2 min for CHM.

Preparation of Stock and Standard Working Solutions

The stock solution of AMP (1263.0 μg mL^{-1}) was prepared by dissolving 126.3 mg AMP in the mobile phase to make 100 mL of solution (Stock Solution 1). A mixed stock solution of CHM (221.0 μg mL^{-1}) and CAF (1514.0 μg mL^{-1}) was prepared by dissolving 22.1 mg CHM and 151.4 mg CAF in the mobile phase to make 100 mL of solution (Stock Solution 2). 25 mL of Stock Solution 2 was quantitatively transferred into a 100 mL volumetric flask and diluted to volume with mobile phase (Stock Solution 3). 25 mL each of Stock Solutions 1 and 3 was quantitatively transferred to a 50 mL volumetric flask and mixed well (Stock Solution 4). Standard working solutions were prepared by quantitatively transferring 0.5, 1, 2, 3, 4 and 5 mL of Stock Solution 4 separately into 10 mL volumetric flasks and made to volume with mobile phase. The concentration range for each of the three pharmaceuticals in the standard working solutions was 31.6 ~ 315.8 μg mL^{-1} for AMP, 9.5 ~ 94.6 μg mL^{-1} for CAF, 1.4 ~ 13.8 μg mL^{-1} for CHM, respectively.

Sample Preparation

Twenty tablets were accurately weighed and ground to a fine powder. An accurately weighed portion of the powder equivalent to 250 mg of AMP was transferred to a 100 mL volumetric flask. After about 40 mL of the mobile phase was added to the flask, the mixture was sonicated for 5 min, brought to volume with mobile phase, and filtered. The first 10 mL of filtrate was rejected, and 5.0 mL of the following filtrate was quantitatively transferred to a 50 mL volumetric flask, and diluted to volume with mobile phase.

Results and Discussion

Method Development

One of the two difficulties for the simultaneous determination of AMP, CAF and CHM in dispersible tablets was the resolution between AMP and CAF. The other was the large difference between AMP (250 mg) and CHM (2 mg), which resulted in off-scale peaks of AMP when the concentration of CHM in samples was measurable. The final mobile phase was chosen after several trials with different compositions and at different pH values. The ion-pair solution was added primarily to reduce interactions of the analytes with accessible residual silanol groups, which otherwise would result in tailing and:or poorly resolved peaks. Alteration of the concentration of acetonitrile over the range 6 ~ 14% (*v/v*) had an effect on retention and resolution. Increasing the concentration of acetonitrile led to significant decrease of retention time of CHM from 14–8 min, but no significant change with the other two drugs, and also resulted in a slightly reduced resolution of CAF and AMP from 1.7 to 1.0. The pH of the mobile phase was varied 3.5–6.0. The pH increase favored an increase in column efficiency for CHM (N increased 3500–5600 when the flow rate was 1.0 mL min^{-1}), but reduced resolution between CAF and AMP 1.7–0.9. All things considered, the final mobile phase was chosen as a mixture of acetonitrile-an ion pair solution-tetrahydrofuran (13:14:87, *v/v*, pH 4.5), which could achieve optimal resolution for AMP and CAF($Rs > 1.5$) and high column efficiency for CHM.

During analysis, flow programming was applied to accelerate elution of CHM and thus shorten analysis time. It could also increase the peak height of CHM. The flow-rate was increased from 1.0 mL min^{-1} (previously 3.5 min) to 1.8 mL min^{-1} (after 3.5 min). Without the flow programming, a flat peak for CHM was observed and its retention time was ca. 12 min. With flow programming, the CHM peak became sharper and retention was decreased to 7.2 min. Hence flow programming was necessary for achieving optimal peak shape in shorter time.

On account of the much smaller amount of CHM, the detection wavelength was set at 223 nm where CHM has maximum absorption. The tablet excipients were also determined to see if any interference from them existed. No peaks were observed in the chromatogram of blank sample, which showed that no interference from the excipients occurred. Typical chromatograms of the standard mixture of AMP, CAF and CHM and the tablet sample are in Figures 1a and b, respectively.

Suitability of Method

System performance parameters of the above method were determined by analyzing standard working solutions. Chromatographic parameters such as number of theoretical plates (N), resolution (Rs), selectivity (α) and capacity factor (k) were determined and are shown in Table I. The repeatability of the system was also determined by five replicate injections of the standard solution. Relative standard deviations (S_R) were 0.41% for AMP, 0.53% for CAF and 0.80% for CHM. These results indicate that the described method showed adequate column efficiency, good selectivity and repeatability and could be applied for simultaneous determination of the three pharmaceuticals.

Based on the experimental results, the acceptance criteria of the system performance parameters were set as follows for later application of the method. N should be $\lessgtr$4000 for CAF, 1000 for AMP and 6000 for CHM. Rs between each peak-pair, especially CAF and AMP, should be $\lessgtr$1.5 and $\alpha \lessgtr$1.0. S_R for the repeatability of the system was required to be $\gtrless$2.0%.

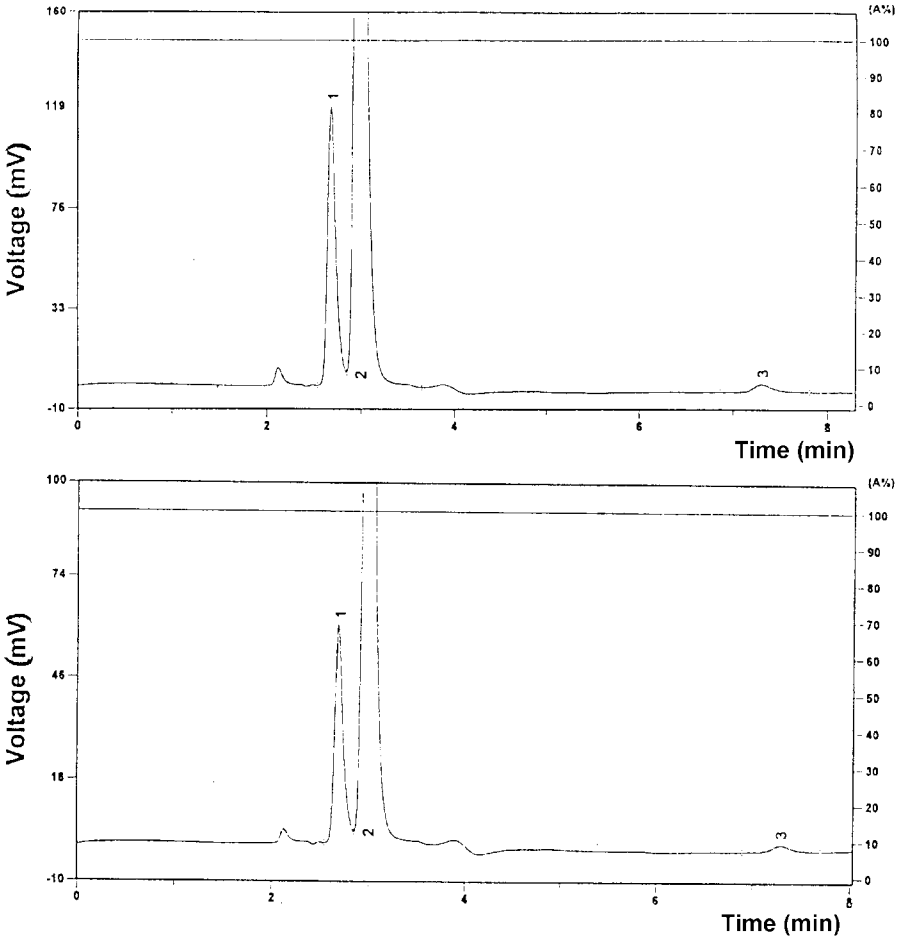

Figure 1. Chromatograms from standard mixture of (**a**) acetaminophen, caffeine, chlorpheniramine maleate and (**b**) tablet sample. Peaks: **1** = caffeine (2.6 min); **2** = acetaminophen (3.1 min); **3** = chlorpheniramine maleate (7.2 min).

Table I. System performance parameters for acetaminophen, caffeine and chlorpheniramine maleate (n = 5).

Peak no.	Drugs	t_R	N	k	Rs	α
1	Caffeine	2.65	4761	0.76	1.54	1.36
2	Acetaminophen	3.05	1783	1.03	11.45	3.71
3	Chlorpheniramine maleate	7.23	6500	3.82		

Linearity

Linearity was evaluated by determining five mixed, standard working solutions of AMP, CAF and CHM in triplicate. The peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the calibration equation and correlation coefficients. The regression equations obtained for the three pharmaceuticals separately were $A = -73.6 + 716.6C$ ($r = 0.9998$, $n = 6$) for AMP, $A = -148.8 + 399.6C$ ($r = 0.9999$, $n = 6$) for CAF and $A = 44.8 + 167.2C$ ($r = 0.9998$, $n = 6$) for CHM. The linear range was individually 31.6–315.8 μg mL^{-1} for AMP, 9.5–94.6 μg mL^{-1} for CAF and 1.4–13.8 μg mL^{-1} for CHM. The results show that within the concentration range tested, there was an excellent correlation between peak area and concentration of each drug.

Limits of Detection and Quantitation

Since CHM has the lowest concentration of the three pharmaceuticals, determination of limit of detection (LOD) and limit of quantitation (LOQ) of the method was limited to CHM. LOD and LOQ were established at a signal-to-noise ratio of 3 and 10, respectively. LOD and LOQ of CHM were experimentally verified by six injections of CHM at the LOD and LOQ concentrations. The LOD and the LOQ of CHM were found to be 0.78 μg mL^{-1} and 1.12 μg mL^{-1}, respectively.

Accuracy

Accuracy was determined by applying the described method to synthetic mixtures of excipients to which known amounts of each drug corresponding to 80, 100 and 120% of label claim had been added. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay. Mean recoveries for AMP, CAF and CHM from the specific formulations are in Table II. The results indicate good accuracy of the method for simultaneous determination of the three drugs.

Precision

The within-day precision and between-day precision of the method were determined by assaying tablets in triplicate each day for three consecutive days. The results for precision are in Table II, which

Table II. Accuracy and precision of described method.

Drug	Accuracy		Precision	
	Mean recovery ± SD	RSD (%, n = 9)	Within-day (%, n = 3)	Between-day (%, n = 9)
Acetaminophen	100.6 ± 0.52	0.5	0.46	0.55
Caffeine	99.5 ± 0.85	0.9	0.54	0.81
Chlorpheniramine maleate	99.5 ± 1.36	1.4	0.85	1.2

Table III. Assay results for acetaminophen, caffeine and chlorpheniramine maleate in Afenwanan Dispersible Tablets (Mean ± SD, %).

Batch No.	Acetaminophen	Caffeine	Chlorpheniramine maleate
1	104.5 ± 0.40	106.0 ± 0.52	104.7 ± 0.89
2	103.9 ± 0.43	104.6 ± 0.55	108.6 ± 0.82
3	101.9 ± 0.46	104.6 ± 0.60	107.7 ± 0.79

indicates that for each of the pharmaceuticals, acceptable precision was achieved as revealed by relative standard deviation data (0.46–0.85% for within-day precision and 0.55–1.2% for between-day precision).

Robustness

The robustness of a method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the present method was evaluated in terms of pH of the mobile phase, temperature, columns and instruments. To determine the robustness of the method, experimental conditions were altered and chromatographic characteristics were evaluated.

The pH of the mobile phase was changed by in increments of 0.2 from 4.2 to 4.8. At both ends of the pH range, the variations in resolution between CAF and AMP and retention times of the three drugs were within 1%. The effect of temperature on capacity factors (k) of the three drugs were studied by changing the temperature in steps of 2 °C from 18 to 22 °C. The increase in temperature leads to a slight decrease in k values and had no significant effect on resolution and peak shape. Column-to-column and instrument-to-instrument variations were also determined using two different CN columns from Elete (China) and Dikma (China) and two separate sets of HPLC delivery systems from Agilent (USA) and Elete (China). No significant differences were found in the results from the two columns and the two instruments. The above results demonstrate that separation of the three active substances was achievable under the correct conditions using the method developed, which was satisfactory for the simultaneous determination of AMP, CAF and CHM in Anfenwanan Dispersible Tablets.

Solution Stability

The stability of both standard and sample solutions was determined by monitoring the peak area responses of solutions of the standard mixture of AMP, CAF and CHM, and a tablet sample over a period of two weeks. The results showed that for both solutions, the retention times and peak areas of AMP, CAF and CHM remained almost unchanged and no significant degradation was observed within the given period, indicating that both solutions were stable for at least 14 days.

Method Application

The validated LC method was applied to determination of Anfenwanan Dispersible Tablets. Three batches of tablets were assayed the assay results of which, expressed as a percentage of the label claim, are in Table III. The results indicate that the amount of each drug in the tablets corresponds to requirements – 90 ~ 110% of the label claim.

Conclusions

The HPLC method developed for determination of acetaminophen, caffeine, chlorpheniramine maleate in Anfenwanan Dispersible Tablets has sufficient accuracy, precision and reproducibility, as well as sensitivity and selectivity, and also offers short analysis time. In conclusion, the method can be used for the assay of the three active substances in Anfenwanan Dispersible Tablets.

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