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Journal of Pharmaceutical and Biomedical Analysis
33 (2003) 39–43
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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# HPLC determination of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in compound dosage forms with an aqueous-organic mobile phase

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Received 4 March 2002; accepted 18 February 2003

**Abstract**

A high-performance liquid chromatography procedure for the simultaneous determination of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in commercially available compound capsule dosage forms has been developed and validated. The separation and quantification were achieved on an Ultrasphere™ C18 column using a mobile phase of dichloromethane–methanol–0.25% (v/v) diethylamine aqueous solution (20:60:20, v/v/v) at a flow rate of 1 ml $min^{-1}$ with detection of all analytes at 264 nm. The separation was achieved within 6 min for each drug mixture. The method showed good linearity for the aminophylline, noscapine, chlorphenamine maleate and methoxyphenamine hydrochloride mixture in the 125–750, 35–210, 10–60 and 62.5–375 μg $ml^{-1}$ ranges, respectively. The intra- and inter-day R.S.D.s ranged from 0.4 to 0.5%, 0.4–0.6%, 0.5–0.7% and 0.4–0.6% for aminophylline, noscapine, chlorphenamine maleate and methoxyphenamine hydrochloride, respectively. The recoveries (mean ± S.D.) of low, middle and high concentrations were 99.9 ± 0.9, 100.4 ± 1.3 and 99.7 ± 0.7% for aminophylline; 99.9 ± 1.1, 100.4 ± 0.7 and 100.1 ± 0.8% for noscapine; 99.8 ± 1.1, 99.7 ± 1.0 and 100.7 ± 0.8% for chlorphenamine maleate; and 99.8 ± 0.9, 100.4 ± 1.6 and 99.9 ± 0.9% for methoxyphenamine hydrochloride, respectively.
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*Keywords:* HPLC; Aminophylline; Methoxyphenamine hydrochloride; Noscapine; Chlorphenamine maleate; Compound capsule dosage forms

## 1. Introduction

Compound aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate capsules, formulated with four different drugs that act synergistically for asthma, are

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0731-7085/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00352-2

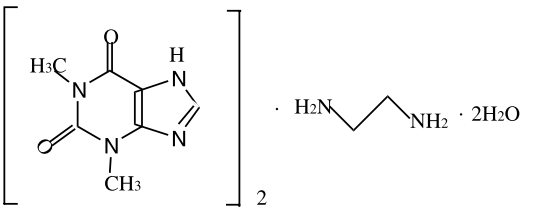
effective with little resistance and few side effects in the treatment of both adult and infantile asthma, as well as other symptoms, such as coughs associated with cold, bronchitis, etc. Since its launch, the drug has been extensively used in Japan, China and Southeast Asia countries. Therefore, it is necessary to establish a simple and accurate method to identify and quantify its active ingredients.

Several attempts have been tried in order to determine the active ingredients in the compound dosage forms. In the volumetric analysis method (Quality Control for Compound Methoxyphenamine Hydrochloride capsule, Sankyo Co. Ltd., Japan) and the UV-spectrophotometry method [1], many complex pretreatment processes are needed in order to prevent interference with other components. Previous HPLC methods [2] require measuring aminophylline, methoxyphenamine hydrochloride and noscapine together, and then chlorphenamine maleate separately. Since its procedures require more than one column, mobile phase and an increased flow rate, this method is both time-consuming and uneconomical. In this study, an isocratic HPLC assay is proposed, which can simultaneously analyze aminophylline, noscapine, chlorphenamine maleate and methoxyphenamine hydrochloride with only one injection. The compounds are separated on an Ultrasphere™ C18 column using an aqueous-organic eluent. The separation can be achieved within 6 min for all analytes in the compound dosage forms.

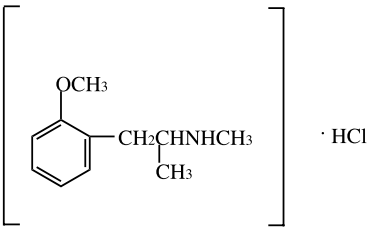
## 2. Experimental

### 2.1. Reagents and chemicals

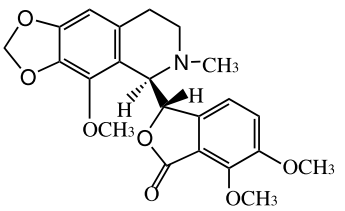
The structures and formulae of the compounds studied are shown in Fig. 1. The aminophylline was purchased from Shanghai Shenxing Pharmaceuticals, Inc. (Shanghai, China), the methoxyphenamine hydrochloride from Yangzhou No. 3 Pharmaceutical Factory (Jiangsu, China), the noscapine from Qinghai Pharmaceuticals, Inc. (Qinghai, China) and the chlorphenamine maleate from Shanghai Huaihai Pharmaceutical Works (Shanghai, China). The compound methoxyphenamine hydrochloride capsules (Lot: 010615, 010618 and 010620) were manufactured by Zhejiang Changxing Pharmaceuticals, Inc (Zhejiang, China) and bought from a local pharmacy. Methanol (BDH Laboratory Supplies, UK) was HPLC grade. Dichloromethane and diethylamine were analytical grade. The water used in the experiment was double distilled.



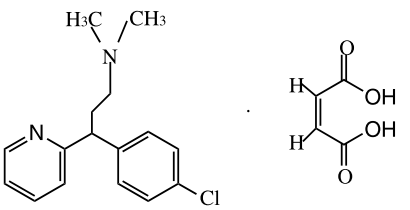

Aminophylline




Methoxyphenamine Hydrochloride




Noscapine




Chlorphenamine Maleate

Fig. 1. Chemical structures of compounds studied.

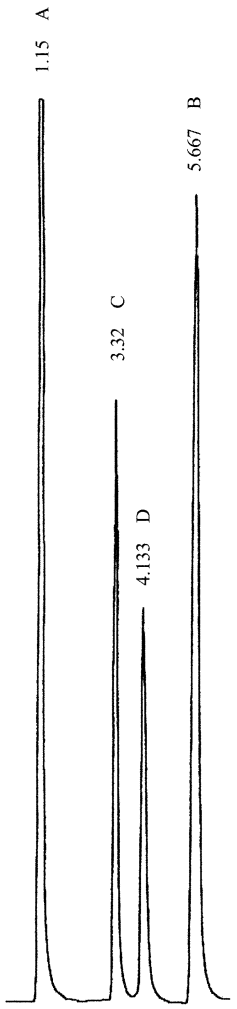



Fig. 2. Typical HPLC chromatogram of aminophylline (A), methoxyphenamine hydrochloride (B), noscapine (C) and chlorphenamine maleate (D) on Ultrasphere™ C18 column with dichloromethane–methanol–0.25% (v/v) diethylamine aqueous solution (20:60:20, v/v/v) mobile phase. See Section 2 for assay conditions.

### 2.2. Instrumentation

The HPLC system used in the study was made up of a Simadzu Model LC-10AT pump (Simadzu, Japan), a Rheodyne Model 7725 injection valve equipped with a 20 μl loop (Rheodyne, USA), a Simadzu SPD-10A UV-VIS detector (Simadzu) and a Simadzu C-R6A chromatographic integrator (Simadzu). Separation was accomplished on a 15 cm Ultrasphere™ C18 column (4.6 mm i.d., 5 μm particle size, Beckman, USA). The mobile phase was composed of dichloromethane–methanol–0.25% (v/v) diethylamine aqueous solution (20:60:20, v/v/v) at a flow rate of 1 ml $min^{-1}$. The mobile phase was filtered through 0.45 μm Nylon-66 filter and degassed by sonication before use. The UV detector was set at 264 nm.

### 2.3. Preparation of standard solution

A combined standard solution was obtained by mixing 250 mg of aminophylline, 125 mg of methoxyphenamine hydrochloride, 70 mg of noscapine and 20 mg of chlorphenamine maleate with sufficient mobile phase in a 100 ml volumetric flask, shaking until dissolved and then adding more mobile phase to volume.

Six point calibration curves were constructed in each drug mixture by plotting analyte peak area versus analyte concentration in μg $ml^{-1}$.

Additional dilution (1:10) of the combined standard solution was made in mobile phase to serve as control sample for each analyte to calculate the contents.

Table 1
The results of linear regression for aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate

| Compound | Calibration equation[a] | Correlation coefficient $r$ | Concentration ranges (μg $ml^{-1}$) |
|---|---|---|---|
| Aminophylline | $C = -3.4132 + 2.1238 \times 10^{-3}A$ | 0.999 | 125–750 |
| Methoxyphenamine hydrochloride | $C = 2.1413 + 1.3080 \times 10^{-2}A$ | 0.998 | 62.5–375 |
| Noscapine | $C = 2.8044 + 1.7450 \times 10^{-2}A$ | 0.998 | 35–210 |
| Chlorphenamine maleate | $C = 0.8770 + 5.8370 \times 10^{-3}A$ | 0.999 | 10–60 |

[a] A, peak area; C, concentration of drug in μg per ml.

Table 2
Intra- and inter-day data for aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate

| | No. | The percent label (%) | | | |
|---|---|---|---|---|---|
| | | Aminophylline | Methoxyphenamine hydrochloride | Noscapine | Chlorphenamine maleate |
| Day | 1 | 99.2 | 99.8 | 100.1 | 101.5 |
| | | 99.6 | 98.9 | 100.6 | 102.4 |
| | | 98.4 | 98.9 | 99.9 | 101.8 |
| | | 98.7 | 99.5 | 99.7 | 101.6 |
| | | 99.1 | 99.5 | 100.5 | 101.0 |
| | 2 | 100.0 | 98.5 | 101.3 | 102.4 |
| | | 99.0 | 99.1 | 100.3 | 101.1 |
| | 3 | 98.9 | 99.4 | 99.9 | 100.7 |
| Intra-day | Mean | 99.0 | 99.3 | 100.2 | 101.7 |
| | R.S.D. (%) | 0.5 | 0.4 | 0.4 | 0.5 |
| Inter-day | Mean | 99.1 | 99.2 | 100.3 | 101.6 |
| | R.S.D (%) | 0.5 | 0.4 | 0.5 | 0.6 |

### 2.4. Preparation of analytical sample

One commercial gelatin capsule contains 25 mg of aminophylline, 12.5 mg of methoxyphenamine hydrochloride, 7 mg of noscapine and 2 mg of chlorphenamine maleate. Some 72.5 mg of sample out of ten capsules homogenate were dissolved in a 100 ml volumetric flask with mobile phase, shaken until dissolved and then added with more mobile phase to volume. The solution was then filtered through filter paper and the resulting filter liquor was so made for analysis.

## 3. Results and discussion

The goal of this study was to develop a single isocratic HPLC assay for the analysis of four anti-asthmatic drug mixtures: aminophylline–methoxyphenamine hydrochloride–noscapine–chlorphenamine maleate. Previous studies to develop a single isocratic HPLC method for the analytes in the drug mixtures required the use of C18 and phenyl columns with various mobile phases containing acetonitrile or methanol-aqueous phosphate buffer. However, almost all systems developed in the previous studied methods can separate only two compounds at a time, though $>25$ min of retention time was needed. In this study, our attention turned to the use of an Ultrasphere™ C18 column combined with reverse- and normal-phase mobile phases for the separation and quantification of the analytes in the drug mixtures. The final HPLC mobile phase containing dichloromethane–methanol–0.25% (v/v) diethylamine aqueous solution

Table 3
Recovery values obtained for the determination of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in model mixtures

| Model mixture (%) | Aminophylline | | Methoxyphenamine hydrochloride | | Noscapine | | Chlorphenamine maleate | |
|---|---|---|---|---|---|---|---|---|
| | Found | R.S.D. (%) | Found | R.S.D. (%) | Found | R.S.D. (%) | Found | R.S.D. (%) |
| I (90) | 99.9 | 0.9 | 99.8 | 0.9 | 99.9 | 1.1 | 99.8 | 1.1 |
| II (100) | 100.4 | 1.3 | 100.4 | 1.6 | 100.4 | 0.7 | 99.7 | 1.0 |
| III (110) | 99.7 | 0.7 | 99.9 | 0.9 | 100.1 | 0.8 | 100.7 | 0.8 |

Results are the average of five determinations and are expressed as a percentage of the drugs added.

(20:60:20, v/v/v) and an ultrasphere™ C18 column provided chromatograms (Fig. 2) with a steady baseline and the specificity required for the simultaneous quantification of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in compound dosage forms.

### 3.1. Linearity

Linearities were demonstrated for the aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate mixture from 20 μl injections of the standard solutions containing quantities of aminophylline (125, 250, 375, 500, 625 and 750 μg $ml^{-1}$), methoxyphenamine hydrochloride (62.5, 125, 187.5, 250, 312.5 and 375 μg $ml^{-1}$), noscapine (35, 70, 105, 140, 175 and 210 μg $ml^{-1}$) and chlorphenamine maleate (10, 20, 30, 40, 50 and 60 μg $ml^{-1}$). The resulting data (Table 1) was plotted as peak area versus concentration and studied by linear regression. The results indicated good proportionate linearity between the detector response and the concentration of the drugs.

### 3.2. Precision

To obtain the intra- and inter-day precision data of the aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate mixtures, eight determinations of drug mixture were examined over 3 days. The results of the precision studies are tabulated in Table 2.

### 3.3. Accuracy

The accuracy of the method was evaluated by using spiked samples containing known amounts of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate in blank matrices. The model mixtures, comparing to the labeled amount, contain 90 (I), 100 (II) and 110% (III) of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate. For each model mixture, five determinations of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate were made. The results, as shown in Table 3, indicate that the procedure provided acceptable accuracy and precision for all the analytes in the drug mixture.

### 3.4. Assay of commercial dosage forms

Three standard replicates for control experiment and the capsule solutions were injected into the column three times, respectively. The system suitability requirements were met and the data were calculated. The percent label claims for three batch of the commercial capsules were found to be 99.6, 99.1 and 102.5% ($n = 5$, R.S.D. = 0.4, 0.5 and 0.4%) per capsule for aminophylline; 100.9, 99.8 and 99.6% ($n = 5$, R.S.D. = 0.4, 0.5 and 0.4%) per capsule for noscapine; 100.3, 99.5 and 100.8% ($n = 5$, R.S.D. = 0.4, 0.4 and 0.6%) per capsule for methoxyphenamine hydrochloride; and 98.3, 99.1 and 102.5% ($n = 5$, R.S.D. = 0.4, 0.5 and 0.3%) per capsule for chlorphenamine maleate.

## 4. Conclusion

The proposed HPLC method in this study using common reagents and simple sample preparation procedures is particularly appropriate for the routine analysis of the compound capsule dosage forms. This assay has the advantages of simplicity, precision, accuracy, sensitivity and convenience for separation and quantification of aminophylline, methoxyphenamine hydrochloride, noscapine and chlorphenamine maleate and can be employed for their assay in compound dosage forms with a single injection. The selectivity of the chosen chromatographic system was ascertained. Excipients, for example, talcum powder, magnesium oxide, aluminum magnesium silicate, magnesium stearate and wheat starch, showed no peak in the retention times corresponding to the analytes.

## References

[1] Z.X. Li, Guangzhou Pharm. 6 (1986) 293–295.
[2] S.L. Zhang, Chin. J. Pharm. Anal. 19 (2) (1999) 130–132.