RESEARCH ARTICLE

A Gas Chromatography Flame Ionization Detector Method for Rapid Simultaneous Separation and Determination of Six Active Ingredients of Anticold Drug

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Abstract: Aims: To establish a rapid and simultaneous determination of multiple effective ingredients in anti-cold drugs.

Background: Anti-cold drugs are stock medicines at home, and most anti-cold formulations are compound preparations. Although the active ingredients of compound preparations have significant effects on the treatment of colds, the excessive dosage or long-term use can produce a series of adverse reactions, including dependence, liver and kidney function damage, digestive system reaction, blood system damage. Now, there are many mature methods for analyzing the active ingredients of anti-cold drugs. However, these methods may have shortcomings, such as a long analysis time or a small number of analysis components.

Objective: Establish a gas chromatography-flame ionization detector method for the simultaneous determination of six active ingredients, including acetaminophen, dextromethorphan hydrobromide, pseudoephedrine hydrochloride, chlorpheniramine maleate, diphenhydramine hydrochloride, and caffeine in anti-cold drugs.

Method: After the standard was accurately weighed, dissolved in ethanol, filtered by 0.22 µm membrane and ultrasonically degassed, the gas chromatograph was used for detection. After the actual sample was removed from the coating, ground and crushed, accurately weighed, dissolved in ethanol, filtered by 0.22 µm membrane and ultrasonically degassed, the gas chromatograph was used for detection.

Result: The six components can be completely separated within 7.0min. This method has good sensitivity, precision, accuracy and recovery rate. Under the optimum testing conditions, the limit of detection was 0.360-2.50µg/mL, the limit of quantification was 1.20-8.30µg/mL. The calibration curves showed good linearity (R²≥0.9932) over the investigated concentration range between 1.20 and 400µg/mL. The recoveries were 89.2% to 109.2%. The RSD of intra-day precision was less than 1.0%. The RSD of inter-day precision was less than 3.2%. The established method was used to determine the ingredients of three anti-cold drugs on the market, and the results showed that the method can accurately determine the ingredients.

Conclusion: The method can quickly and simultaneously determine multiple active ingredients in anti-cold medicines. Compared with the published methods in literature, the proposed method has the advantages of fast, the number of analysis components wide application range, convenience, low cost, etc. It provides a reference method for quality control of active ingredients of anti-cold drugs.

Keywords: Gas chromatography, flame ionization detector, active ingredients, anti-cold drug.

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1. INTRODUCTION

Most anti-cold formulations are compound drugs [1]. The active ingredients of anti-cold drugs are mainly acetaminophen (APAP), dextromethorphan hydrobromide (DN), pseudoephedrine hydrochloride (PE), chlorpheniramine maleate (CPM), diphenhydramine hydrochloride (DE) and caffeine (CA). Although these active ingredients have significant effects on the treatment of colds, excessive dosage or long-term use can give rise to a series of adverse reactions, including dependence, liver and kidney damage, digestive system reactions and blood system damage [2-7].

At present, the active ingredients in anti-cold drugs can be detected and commonly analyzed by means of capillary electrophoresis (CE) [8], high-performance liquid chromatography (HPLC) [9], fluorescence spectroscopy (FS) [10], gas chromatography (GC) [11], and spectrophotometric method [12].

Moreira AB et al. [10] determined APAP by FS in powdered pharmaceutical samples. Song A.Y. et al. [13] determined CA by hollow fiber liquid phase microextraction-GC (HF-LPME-GC) in anti-cold drugs. Costa Bet et al. [14] rapidly determined DE by CE capacitive coupling noncontact conductance method. Avram, N et al. [15] determined DN by HPLC with ultraviolet (UV) detector in syrup. However, only one single component in anti-cold formulations was determined in these published works.

Compared to the determination of one single component, multiple components in anti-cold formulations were determined in other published works. Dong Y.M et al. [8] established a non-aqueous CE-diode array ultraviolet (NACE-DAD-UV) detection method for the determination of PE, DN, CPM and DE in four anti-cold drugs within 8 minutes. In spite of its efficiency in time consumption, this method was developed for the identification and determination of four basic nitrogenous compounds, yet the APAP, the main ingredient in anti-cold drugs, was not determined. Dong’s group [9] developed a novel nonionic micellar HPLC to simultaneously separate and determine APAP, PE and CPM in cold compound preparations within 10 minutes. This work made up for the lack of APAP measurement in its NACE-DAD-UV study. At stake was the fact that only APAP, PE and CPM were measured in this method, which, therefore, brought about limitations in the determination of most compound anti-cold drugs.

Recently, Dongala T. et al. [17] developed a reverse-phase high-performance liquid chromatography-photo diode array (RP-HPLC-PDA) detector method for the simultaneous separation and quantification of APAP, DN, CA, doxylamine (DOX), phenylephrine (PHE), guaifenesin (GUA) and aspirin (ASP) in cold and cough medicines within 17.00 min. The type and analysis of the anti-cold active ingredient determined by this method took a time shorter than other liquid chromatographic methods. However, the amount of organic reagents that were used in this method was fairly large. It was not green and might cause safety hazards to the experimenter. Dong’s group [16] established a micellar per aqueous liquid chromatography (MPALC) method for the determination of PE, APAP, DN, CPM, DE and CA in two compound anti-cold drug preparations within 25.00 min. This MPALC method was a green one but took too much time to analyse.

GC has become one of the most widely used analytical methods in the pharmaceutical analysis [18]. GC can accurately quantify complex compounds, including active ingredients of drugs, the degradation products of drugs and impurities [19-21]. It is essential in the field of pharmaceutical analysis. The flame ionization detector (FID) has high sensitivity, fast response and wide linear range [22]. Therefore, GC-FID enjoys the advantages of high separation efficiency, high selectivity, high sensitivity, good reproducibility, and fast analysis [18].

The determination of the active ingredient of anti-cold drugs by GC-FID has been reported. Mario E. et al. [23] determined phenylpropanolamine (PME), GUA, CPM and DN by GC-FID in a commercially available cough-cold preparation within 30.0 min. Guo X. et al. [24] reported a capillary GC method for the determination of APAP, CA and CPM in Suxiao Shangfeng Capsules (Paracetamol, Caffeine, Atificalia Cow-bezoar and chlorpheniramine Maleate) within 15.00 min. Although these two methods were good for the determination of the active ingredients in the target sample, only three or four components were determined, and the analysing process took quite a long time. Indrayanto T. et al. [11] determined APAP, DN, PE, CPM, CA, GUA and PME in cough and cold preparations by GC-FID within 22.00 min. This method was well fit for the determination of the active ingredients of most anti-cold formulations, yet time-consuming. In addition, the sample processing of this method involved many steps and thus became quite complicated. Therefore, a GC-FID method for rapid simultaneous separation and determination of six active ingredients of anti-cold drugs can be established on the basis of this study.

In this work, we initiated and validated a new method for rapid simultaneous determination of six active ingredients without derivatization within 7.00 min by GC-FID, and applied this method in the assay of PE, CPM, APAP, DE, CA and DN in three anti-cold drug formulations.

2. METHODS

2.1. Chemicals

Anhydrous ethanol (HPLC grade. Lot number:20151201) was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Standard substances of PE (purity>99%). Lot number: 171237-200505), CPM (purity>98%). Lot number: W13M6D1) and APAP (purity>99%. Lot number: 018-8503) were supplied by China National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard substances of DE (purity>99%. Lot number:171237-200505) and DN (purity>99%. Lot number: F26M7E1810) were bought from Shanghai Yuanye Biotechnology Co. Ltd (Shanghai, China). Standard substances of CA (purity>98%, Lot number: WKQ16052505) was purchased from Sichuan Victory Biological Technology Co,. Ltd (Sichuan, China). Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride Tablets (Bayer Medical Care Co. Ltd, Jiangsu, China) were labeled to contain APAP 325 mg, PE 30 mg, DE 15 mg and DN 25 mg per tablet. Compound Acetaminophen and Amanadine Hydrochloride Tablets (Wutai Group Gankang
Pharmaceutical co. Ltd, Jilin, China) were labeled to contain APAP 250 mg, CPM 2 mg, CA 15 mg per tablet. Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, and Chlorpheniramine Maleate Tablets (Johnson and Johnson Pharmaceutical Co, Ltd, Shanghai, China) were labeled to contain APAP 325 mg, PE 30 mg, DE 15 mg, CPM 2 mg.

2.2. Instrument

All experiments were carried out using the following instruments: separation and determination were done with a Scion 456C GC instrument (Techcomp (China) Ltd., Beijing, China) with FID; nitrogen was supplied from a high-purity nitrogen cylinder (Lanzhou Guolei Kefa Complete Equipment Co., Ltd., Lanzhou, China). Hydrogen was supplied by SPH-300 hydrogen generator (Beijing Institute of Analytical Technology, Beijing, China); air was supplied by SPB-3 fully automatic air source (Beijing Institute of Analytical Technology, Beijing, China). Chromatographic signals were collected and processed by means of the Compass CDS Basic license with GC-control data processing software. The capillary column was TM-1301 (30m×0.25 mm×0.25um).

2.3. Preparation of the Sample

2.3.1. Standard Preparation

5.00 mg of PE, CPM, APAP, DE, CA, DN standards were accurately weighted by LIBROR AEL-160 (Shimadzu (Shanghai) Experimental Equipment Co., Ltd., Shanghai, China), and were respectively dissolved in 5.00mL of absolute ethanol. The concentration of each standard stock solution was 1.00mg/mL. The stock solutions were stored at 4℃ before use. Different concentrations of the solution required in subsequent experiments were diluted from the stock solution. The solution was pipetted accurately by Eppendorf research plus single channel adjustable pipette (10-100 μL, Abbond China Ltd., Shanghai, China) and Thermo Scientific Finn pipette F3 pipette (1-10 μL, Thermo Fisher Scientific, Massachusetts, US). Before sample injection, all solutions were filtered through a 0.22 μm membrane filter, and the bubbles in the solutions were eliminated by KH-300DB ultrasonic (Kun Shan He Chuang Ultrasonic Instruments Co., Ltd, Kunshan, China).

2.3.2. Sample Preparation

Twenty tablets of Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride Tablets, Compound Acetaminophen and Amantadine Hydrochloride Tablets, Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, and Chlorpheniramine Maleate Tablets were taken and ground into powder, respectively. The amount of one piece was weighed precisely by LIBROR AEL-160. According to the indicated amount of the sample and the required sample concentration, an appropriate amount of ethanol was added to prepare the required concentration of sample solutions. All sample solutions were filtered through a 0.22μm membrane filter before injection and the bubbles were eliminated by KH-300DB ultrasonic.

2.4. GC-FID Condition

The system was set to a constant gas flow mode and the gas flow was 1.0 mL·min⁻¹. The initial temperature of the column was 200°C, and was maintained for 1.00 min. The temperature of the column was raised to 240°C at 50°C min⁻¹, and was maintained for 0.70 min. The temperature of the column was raised to 260°C at 40°C min⁻¹ and was maintained for 4.00 min. The inlet temperature was set at 260°C and the detector temperature was set at 280°C. The injection volume was 1.00 μL, and the split ratio was 10:1.

2.5. Method Validation

The proposed GC-FID method was validated in line with the guideline of the Pharmacopoeia of the People’s Republic of China (2015 version) [25].

2.6. Comparison with Other Analytical Methods for Determination of Anti-cold Drugs

Several well-reported and representative anti-cold drug assays were compared by applying the proposed method in this work to better characterize the method [8, 9, 11, 13, 15-17, 28-32].

3. RESULTS AND DISCUSSION

3.1. Optimization of GC Conditions

TM-1301 was a universal medium polarity stationary phase capillary chromatography column, which was made of high-purity polyacrylonitrile phenyl dimethyl siloxane cross-linked and mixed with 6% nitrile acrylic and phenyl. The fixed relative of TM-1301 to the inner wall of the quartz tube has good wettability and can obtain extremely high column efficiency. It was widely used in the analysis and determination of flavors and fragrances, esters, halogenated hydrocarbons, aldehydes, pesticides and aromatic hydrocarbons. During the optimization of GC conditions, the carrier gas flow rate was set to a constant current mode of 1.0 mL·min⁻¹ because of the stability of separation. It is, therefore, reasonable to say the temperature program of the column and split ratio was optimized.

3.1.1. Effect of Temperature of Column on the Separation

The temperature program of the column optimization chromatogram is shown in Fig. (S1). It can be seen from Fig. (S1) that the separation efficiency gradually increased with the increase of the gradient heating rate. As the initial temperature increased, the separation efficiency gradually increased, and the peak height and peak area were not affected. If the starting temperature of the temperature program was too high, detection would be unstable. The initial temperature of the temperature program was optimized to 200°C and it was maintained for 1.00 min to ensure the stability of the process.

3.1.2. Effect of Split Ratio on the Separation

Subsequently, the split ratios were optimized under the optimal temperature program of the column, and 5:1, 10:1, 15:1, 20:1, and 25:1 were selected. It was found that as the split ratio increased, the peak height and peak area gradually decreased. There was a clear peak overload when the split
ratio was less than 10:1. The split ratio optimized chromatogram was shown in Figure S2.

3.1.3. Optimized GC-FID Conditions

Finally, the overall optimization of temperature rise and split ratio, the best chromatographic conditions were as follows: The gas flow was 1.0 mL·min⁻¹. The initial temperature of the column was 200 °C, and was maintained for 1.00 min. The temperature of the column was raised to 240 °C at 5 °C·min⁻¹, and was maintained for 0.70 min. The temperature of the column was raised to 260 °C at 40 °C·min⁻¹ and was maintained for 4.00 min. The inlet temperature was 260 °C, the detector temperature was 280 °C, the injection volume was 1.00 µL, and the split ratio was 10:1. All components to be tested were completely separated (resolution > 1.5) under the best chromatographic conditions. The following work was carried out under optimal chromatographic conditions.

The chromatogram under optimal conditions was shown in Fig. (1). It can be seen from Fig. (1) that PE was detected at 2.42 min, APAP was detected at 4.11 min, DE was detected at 4.23 min, CA was detected at 4.50 min, and CPM was detected at 5.26 min. The DN was detected at 6.49 min. The six substances were completely separated in 7.00 min.

![Chromatogram of mixed standards under optimal conditions.](image)


3.2. Method Validation

The method was validated according to the requirement of the guideline of Chinese Pharmacopoeia (2015 version), and the validation parameters included accuracy, precision, Limit of Detection (LOD), Limit of Quantitation (LOQ), stability and linearity.

3.2.1. Linearity and Range

According to the requirements of actual measurement and the LOQ of six analytes, the concentration ranges of PE (8.30-200µg/mL), CPM (2.50-50.0µg/mL), APAP (2.70-400µg/mL), DE (2.40-200µg/mL), CA (5.30-200µg/mL) and DN (1.20-200µg/mL) were established. Under the optimal chromatographic conditions, the PE, CPM, APAP, DE, CA, DN stock solutions were diluted into five different concentrations of standard solutions of 12.5µg/mL, 25.0µg/mL, 50.0µg/mL, 100µg/mL and 200µg/mL according to the corresponding concentration range, and the standard curve was established according to the measured peak area and concentration. The results showed that the method had good linearity. The results were shown in Table I.

3.2.2. LOD and LOQ

In general, the LOD is determined by S/N. When the sample’s S/N is 3, the sample concentration is LOD. Under the optimal chromatographic conditions, the concentration of the sample was continuously diluted and injected until the S/N was 3. LOQ shows whether the test method has a sensitive, quantitative detection capability. Similarly, the LOQ is also determined by S/N. When the sample’s S/N is 10, the sample concentration is LOQ. Under the optimal chromatographic conditions, the concentration of the sample was continuously diluted and injected until the S/N was 10. The LOD and LOQ are shown in Table I. The LOD of the tested substances ranged from 0.36 to 2.50 µg/mL, and the LOQ ranged from 1.20 to 8.30 µg/mL.

3.2.3. Stability

Under the optimal chromatographic conditions, the mixed standard solution of 50.00 µg/mL (equivalent to 10.00 µg/g/ppm)-0.01% of the content of the component to be determined) was placed at room temperature for 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and then injected. The RSD of the peak area was calculated from the results to indicate the stability of the sample. The RSD results were 0.8% for PE, 0.8% for CPM, 0.9% for APAP, 1.4% for DE, 1.2% for CA, and 1.0% for DN. According to the acceptable range of RSD content and precision of components to be determined in Pharmacopoeia of the People’s Republic of China (Part IV, 2015 Edition) Pharmaceutical Quality Standard Analysis Method Verification Guidelines (General Principle 9101), the results showed that the test sample solution had good stability within 12 h.

3.2.4. Precision

Precision verification includes daytime precision and intraday precision. The same solution (50.00 µg/mL) was measured 6 times at different times in a day, and the RSD of the peak area of the test substance was calculated to express the intraday precision. The same solution (50.00 µg/mL) was measured for six consecutive days, and the RSD of the peak area of the test substance was calculated to express the daytime precision. The RSD results showed that the method had good daytime precision and intraday precision. The results were shown in Table I.

3.2.5. Recovery

Accuracy is generally expressed in terms of recovery. Within the specified range, the sample to be tested was prepared into a sample solution of a certain concentration according to the labeled amount, and then the standard solution was diluted to three levels of high, medium and low (120%, 100%, 80%) according to the concentration of the prepared sample. The sample solution was mixed with the high, medium and low concentration solutions in the same volume (1.0 mL), respectively. The solution was injected after mixing, and the recovery was calculated. The recovery rate of all substances was between 89.2%-109.2%. Among the results of the recovery rate, one of the results of DN and
PE were 89.2% and 109.2%, respectively. At this time, the content of the components to be determined in the DN and PE samples is between 10.00 µg/g (ppm)-0.01%. The recovery result was in line with the recovery limit of 85-110% when the content of the component to be determined in the Pharmacopoeia of the People's Republic of China (2015 edition) was 0.01%. The content of the components to be determined in other samples was less than 0.1%, and the recovery results were in line with the recovery limit when the content of the components to be determined was 0.1%. The results were shown in Table 2.

Table 1. Linearity, correlation coefficient, LOD, LOQ and precision of the method.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear Equation</th>
<th>Coefficient of Determination (R²)</th>
<th>Ranges (µg/mL)</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
<th>RSD (inter-day / intra-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>y = 0.1471x - 2.1833</td>
<td>0.9932</td>
<td>8.30-200</td>
<td>2.50</td>
<td>8.30</td>
<td>0.7 / 1.4</td>
</tr>
<tr>
<td>APAP</td>
<td>y = 0.2792x - 5.7161</td>
<td>0.9982</td>
<td>2.70-400</td>
<td>0.82</td>
<td>2.70</td>
<td>0.8 / 1.2</td>
</tr>
<tr>
<td>DE</td>
<td>y = 0.2363x - 1.4958</td>
<td>0.9990</td>
<td>2.40-200</td>
<td>0.72</td>
<td>2.40</td>
<td>0.7 / 3.1</td>
</tr>
<tr>
<td>CA</td>
<td>y = 0.0903x - 0.6417</td>
<td>0.9983</td>
<td>5.30-200</td>
<td>1.60</td>
<td>5.30</td>
<td>0.9 / 2.1</td>
</tr>
<tr>
<td>CPM</td>
<td>y = 0.1608x - 0.22</td>
<td>0.9948</td>
<td>2.50-200</td>
<td>0.75</td>
<td>2.50</td>
<td>0.6 / 1.7</td>
</tr>
<tr>
<td>DN</td>
<td>y = 0.2645x - 0.2167</td>
<td>0.9953</td>
<td>1.20-200</td>
<td>0.36</td>
<td>1.20</td>
<td>0.8 / 1.4</td>
</tr>
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Table 2. Results of the recovery experiments in three preparations.

<table>
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<tr>
<th>Samples</th>
<th>Composition</th>
<th>Original Amount (µg/mL)</th>
<th>Added (µg/mL)</th>
<th>Found (µg)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<tbody>
<tr>
<td>APAP</td>
<td>-</td>
<td>100</td>
<td>80</td>
<td>175.4</td>
<td>94.2</td>
<td>2.2</td>
</tr>
<tr>
<td>-</td>
<td>100</td>
<td>100</td>
<td>197.4</td>
<td>97.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>100</td>
<td>120</td>
<td>218.9</td>
<td>99.1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>32</td>
<td>71.0</td>
<td>96.9</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>40</td>
<td>79.4</td>
<td>98.5</td>
<td>2.0</td>
<td></td>
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<tr>
<td>-</td>
<td>40</td>
<td>48</td>
<td>86.8</td>
<td>97.5</td>
<td>1.5</td>
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<td>-</td>
<td>5</td>
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<td>9.8</td>
<td>96.0</td>
<td>3.2</td>
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<td>-</td>
<td>5</td>
<td>6</td>
<td>10.9</td>
<td>98.3</td>
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<td>100</td>
<td>80</td>
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<td>218.1</td>
<td>98.4</td>
<td>1.2</td>
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</tr>
<tr>
<td>PE</td>
<td>-</td>
<td>45</td>
<td>36</td>
<td>84.3</td>
<td>109.2</td>
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<td>92.1</td>
<td>104.7</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>45</td>
<td>54</td>
<td>100.2</td>
<td>102.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>-</td>
<td>15</td>
<td>12</td>
<td>25.7</td>
<td>89.2</td>
<td>3.8</td>
</tr>
<tr>
<td>-</td>
<td>15</td>
<td>15</td>
<td>29.2</td>
<td>94.7</td>
<td>2.2</td>
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<tr>
<td>-</td>
<td>15</td>
<td>18</td>
<td>32.5</td>
<td>97.2</td>
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</tr>
<tr>
<td>DE</td>
<td>-</td>
<td>35</td>
<td>28</td>
<td>63.2</td>
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<tr>
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<td>69.9</td>
<td>99.7</td>
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<tr>
<td>-</td>
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<td>42</td>
<td>76.3</td>
<td>98.3</td>
<td>1.1</td>
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</tbody>
</table>
3.3. Application

After validation of this method, the three compound anti-cold medicine preparations of Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride Tablets, Compound Acetaminophen and Amantadine Hydrochloride Tablets, Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, Chlorpheniramine Maleate Tablets were employed in the application of this method that we established. Fig. (2) was a chromatogram of three compound anti-cold preparations. As can be seen from Fig. (2), the anti-cold medicine components in the compound preparation can be measured and the absolute accuracy and the peak shape were good. In all compound preparations, the content of APAP is much higher than other components, and the chromatographic peaks of other components need to be visualized. Through the application, the method proved to be of high practical value. After that, the percentage of the labeled amount of the sample component was calculated according to the formula [18]. The results were shown in Table S1.

As a new method, the six effective ingredients of anti-cold medicine in two different pharmaceutical dosage forms determined by the proposed method must be compared with the results of the reference method [16] in terms of t- and F-values [26, 27]. The interval hypothesis test based on recovery experiments and calculating the lower and upper acceptance limits was applied by the following formula [26, 27].

\[
\theta_{L} = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \\
a = \bar{x}^2 - \frac{s_p^2 t^2}{n_1} \\
b = 2\bar{x}_1 \bar{x}_2 \\
c = \bar{x}^2 - \frac{s_p^2 t^2}{n_2}
\]

\(
\theta_{L} = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \\
\theta_{U} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}
\)

\(\bar{x}_1 \) and \(\bar{x}_2 \) are the mean values determined by the proposed and reference methods, respectively. \(S_p \) and \(t \) are the pooled standard deviation and one-sided t-value at the 95% confidence level, respectively. \(n_1 \) and \(n_2 \) are the number of measurements (five times) of the proposed and reference methods, respectively. The results were shown in Table S2. As can be seen from Table S2, it was concluded that no significant difference between the two methods was observed except for PE at the 95% confidence level. On the whole, the results of PE determined by the proposed method are slightly better than the result of the reference method. The true bias of all dosages of six active ingredients of anti-cold drug was found to be less than ±2%, the result was acceptable [26, 27].

3.4. Comparison of this Method with the Reported Method for Measuring Anti-cold Drugs

As recapitulated in the literature review, the determination of the active ingredients of anti-cold drugs is mainly based on HPLC. The method proposed in this work was compared with the other reported methods respectively in the number of analysis components and analysis duration. Table 3 showed the comparison between the method proposed in this work and the other methods in the number of analysis components. Analysis duration of the compared

(a) and (b) show chromatograms of different samples. The peaks are labeled with numbers, and the retention times are indicated on the x-axis. The peak heights are shown on the y-axis. The y-axis scale for peak heights is 200 mV.

(c) is another chromatogram with peaks labeled as 1, 2, 3, 4, and 7. The peak heights are shown on the y-axis, and the retention times are indicated on the x-axis. The y-axis scale for peak heights is 250 mV.
methods was less than or equal to 10.00 min. As can be seen from Table 3, the method proposed in this work has more analytical components than other reported methods. Moreover, besides that of the method of Song, A.Y. [14], the analysis duration of the method proposed in this work is also the shortest in comparison. However, the method of Song, A.Y. only measured CA in anti-cold drugs and had certain limitations for the determination of other active ingredients in anti-cold drugs. Table 4 shows the comparison between the method proposed in this work and the other method in the analysis duration. As can be seen from Table 4, among several methods with a similar number of analysis components, the length of time for analyzing as in the method proposed in this work is the shortest. It can be seen from Table 3 and Table 4 that the main method for the determination of the active ingredients of anti-cold drugs was HPLC [9, 16, 17, 28, 30]. However, these methods have certain disadvantages, such as high mobile phase consumption, long mobile phase preparation time, and a large amount of organic reagents. Although Duan, X.Y. et al. [16] solved the problem of large consumption of organic reagents in HPLC and realized green chromatography, the time spent on analysis is a little longer. Other methods, such as HF-LPME-GC, GC and CE [8,11,13,31,32], have their own advantages, such as environmental protection, low cost, high detection sensitivity. However, these methods have a limited range of applications. Indrayanto's GC method is well suited for determining the active ingredients of most anti-cold formulations, but slow in analyzing. Furthermore, liquid chromatography-mass spectrometry (LC-MS) [33] can also be used to measure active ingredients in anti-cold medicine, but LC-MS is too expensive and most laboratories cannot afford them, so that it has not gained currency. In this work, we initiated a method for rapid simultaneous determination of six active ingredients within 7.00 min by GC-FID without derivatization. This method is simple in sample preparation and does not require complex derivatization. On top of that, it has a small injection volume, which does not require the consumption of a large amount of mobile phase and does not generate a large amount of waste liquid. The cost of the experiment is greatly reduced. On the strength of a comprehensive comparison, it is within reason to say that the method we have established to determine the active ingredients of anti-cold drugs has shown great advantages in terms of analysis duration, the number of analysis components, sample processing and experimental cost.

CONCLUSION

In this work, we initiated a method for rapid simultaneous determination of six active ingredients within 7.00 min by GC-FID and succeeded in using it for the detection of actual samples. Compared with the published methods in the literature, this proposed GC-FID method has shown an edge over them in various aspects, such as time consumption, the number of analysis components, operability, and cost. It not only detects the active ingredients of most anti-cold drugs to a comprehensive and complete degree, but also provides a reference method for quality control of active ingredients of anti-cold drugs. In the follow-up work, we hope to establish a more sensitive analysis GC-FID method that can be used for the analysis of biological samples such as blood samples and urine samples.
Table 3. Comparison of the proposed method with other methods in the number of analysis components.

<table>
<thead>
<tr>
<th>Method</th>
<th>Samples</th>
<th>Analysis Time(min)</th>
<th>The Number of Analytes</th>
<th>Analytes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPC</td>
<td>Xiweifenming Dispersible tablets.</td>
<td>10.00</td>
<td>4</td>
<td>APAP PE DN DE</td>
<td>[29]</td>
</tr>
<tr>
<td>MLC</td>
<td>Compound Paracetamol Caffeine and Pseudoephedrine Hydrochloride Capsules.</td>
<td>10.00</td>
<td>3</td>
<td>APAP PE CPM</td>
<td>[9]</td>
</tr>
<tr>
<td>NACE-DAD-UV</td>
<td>Fufang Ganmaoling tablets, Black tablets and White tablets of Compound Pseudoephedrine HCl tablets, Compound Paracetamol and Amantadine HCl tablets, Capsules of Compound Pseudoephedrine HCl Sustained capsules.</td>
<td>8.00</td>
<td>4</td>
<td>PE DN DE CMP</td>
<td>[8]</td>
</tr>
<tr>
<td>HF-LPME-GC</td>
<td>999 Ganmao Ling Granules, Children's Version of Acetaminophen and Amantadine Hydrochloride granules, Acetaminophen, Aaffeine, Artificial Bezoar and Chlorpheniramine Maleate tablets.</td>
<td>4.50</td>
<td>1</td>
<td>CA</td>
<td>[13]</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>syrup</td>
<td>5.00</td>
<td>1</td>
<td>DN</td>
<td>[15]</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride tables, Compound Acetaminophen and Amantadine Hydrochloride tablets, Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, Chlorpheniramine Maleate tablets.</td>
<td>7.00</td>
<td>6</td>
<td>APAP DN PE CPM DE CA</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 4. Comparison of the proposed method with other methods in analysis time.

<table>
<thead>
<tr>
<th>Method</th>
<th>Samples</th>
<th>Analysis Time(min)</th>
<th>The Number of Analytes</th>
<th>Analytes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-DAD</td>
<td>Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, Chlorpheniramine Maleate tablets.</td>
<td>15.00</td>
<td>3</td>
<td>PE DE CPM</td>
<td>[28]</td>
</tr>
<tr>
<td>MPA LC</td>
<td>Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride tables, Compound Acetaminophen and Amantadine Hydrochloride tablets.</td>
<td>25.00</td>
<td>6</td>
<td>APAP PE DN DE CPM CA</td>
<td>[16]</td>
</tr>
<tr>
<td>RP-HPLC-PDA</td>
<td>Soft gel, Syrup and Tablet Formulations of the Over-The-Counter (OTC) US Marketed Products</td>
<td>17.00</td>
<td>7</td>
<td>APAP DN CA DOX PHE GUA ASP</td>
<td>[17]</td>
</tr>
<tr>
<td>RPLC</td>
<td>Vegaskine-D tablet, Power Cold tablet.</td>
<td>25.00</td>
<td>4</td>
<td>APAP</td>
<td>CA</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>CZE- MEKC</td>
<td>Cold Medicine Ingredients of Dextromethorphan, Chlorpheniramine Maleate, and Paracetamol.</td>
<td>11.38</td>
<td>3</td>
<td>APAP</td>
<td>DN</td>
</tr>
<tr>
<td>CZE</td>
<td>Cold medicine containing acetaminophen and dextromethorphan.</td>
<td>12.00</td>
<td>2</td>
<td>APAP</td>
<td>DN</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Laboratory Prepared Syrups and Tablets</td>
<td>22.00</td>
<td>7</td>
<td>APAP</td>
<td>PE</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Acetaminophen, Pseudoephedrine Hydrochloride, Diphenhydramine Hydrochloride and Dextromethorphan Hydrochloride tables, Compound Acetaminophen and Amantadine Hydrochloride tablets, Acetaminophen, Pseudoephedrine Hydrochloride, Dextromethorphan Hydrobromide, Chlorpheniramine Maleate tablets.</td>
<td>7.00</td>
<td>6</td>
<td>APAP</td>
<td>DN</td>
</tr>
</tbody>
</table>

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

No animals/humans were used for studies that are base of this research.

**CONSENT FOR PUBLICATION**

Not applicable.

**AVAILABILITY OF DATA AND MATERIALS**

Not applicable.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

**SUPPLEMENTARY MATERIAL**

Supplementary material is available on publisher’s website.

**REFERENCES**


A Gas Chromatography Flame Ionization Detector Method for Rapid Simultaneous Determination of Four Active Components in a Compound Formulation by Liquid Chromatography


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