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# Multi-Residues Analysis of Volatile Organic Solvents in Cefotaxime Products by Capillary Gas Chromatography

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A newly simple and sensitive method was established for the multi-residues analysis of two volatile organic solvents, acetone and isopropanol, in cefotaxime products by capillary gas chromatography with *n*-propanol as the internal standard material. A PEG-20M flexible fused quartz capillary column (30 m × 0.25 mm × 0.33 μm) was used as the analysis column with nitrogen carrier gas and FID detector. A variety of influencing factors for separation and detection of these volatile organic solvents were investigated and optimized. Good linearity were obtained in a range of 0.4-200 μg/mL with the correlation coefficients ($r^2$) of 0.9991 for acetone, 0.9993 for isopropanol. The average recoveries of acetone and isopropanol in three different spiked concentrations were ranged from 92.7-103.2 % with the relative standard deviation less than 3.5 % (n = 5). The proposed method was successfully applied for determination of acetone and isopropanol residues in different cefotaxime products with satisfied result. This method offers a good alternative for routine analysis due to its simplicity and reliability.

**Key Words: Capillary gas chromatography, Residual organic solvents, Cefotaxime, Acetone, Isopropanol.**

## INTRODUCTION

The residual solvents in drugs are the volatile organic compounds which were produced or used in the production of raw material drugs or excipients in the preparation of formulations[1,2]. The residual solvents have no treatment effect but probable toxic and carcinogenic effects on the human body when used, so all of them should be removed as far as possible in order to comply with the product specifications, GMP or other quality requirements[3]. Until now, the volatile organic solvents in synthetic drugs are impossible to remove them out absolutely in production. In recent years, the toxicity and carcinogenesis of residual organic solvents in drugs are attracting increasing attention from all areas. In order to protect workers from the harm of residual organic solvents in drugs, it is necessary to monitor the organic solvent residues introduced in the process of production[4]. To ensure drug safety, many countries had made strict rules on quality control in the exploitation and production of new drugs, which provide that the organic solvent residues in raw materials or drug formulations must be checked[5,6]. Since 1994, ICH (International Conference on Harmonization of Technical Requirements with Human drugs) initiated to compile the guiding principles on the solvent residues in human drugs. In 1997, American FDA issued in accordance with this principle, entitled "Guiding Principles on the impurities: residual solvents (Q3C)." This principle was passed by ICH program committee after extensive solicitation of opinions and came into effect after the signing of the European Union, the United States and Japan. China also provides for restrictions on seven kinds of solvent residues in the 1995 Pharmacopoeia and adjusted their residue limits in the 2000 edition according to the limit requirements of ICH.

With the deepening of the toxicity research on residual solvents, the cognition of its security is more and more profound and the means and methods of quality control have become more and more improved; especially in the quality study of raw material drugs, the control of residual solvents has become one key quality control project. In recent years, reports on analysis study of organic solvent residues in drugs are increasing gradually and the current methods to determine the residual organic solvents mostly are usages of gas chromatography, which can be divided according to the different sampling methods into direct injection method[7,8] and headspace method[9-11] or coupled with MS[12].

Antibiotic drugs, whether for human or for animals, are widely applied in recent decades, therefore, the determination of residual solvents in antibiotic drugs seems particularly important. Acetone and isopropanol are the mainly contained organic residual solvents in cefotaxime products, which were considering by ICH as Class III organic solvents and the safety limits are 0.5 %. So a simple and reliable method for the simultaneous determination of the two volatile organic solvents residues in cefotaxime materials and its products are desired. This work developed a simple and reliable separation method for the simultaneous determination of the two volatile organic solvent residues (acetone and isopropanol) in cefotaxime products by capillary gas chromatography with dibutyl phthalate and iso-octyl phthalate ester as an internal standard. This method is simple and sensitive and is therefore an alternative tool to the existing methods for analyzing the residuals of acetone and isopropanol in cefotaxime products.

## EXPERIMENTAL

Cefotaxime materials and its products were obtained from Shijiazhuang Pharmaceutical Group (Shijiazhuang, China). N,N-dimethyl formamide, isopropanol and N-propanol were purchased from Tianjin Ding-Sheng Chemical Material Co. (Tianjin, China). Acetone was purchased from Beifang-Tianyi reagent Co. (Tianjin, China). All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with 0.45 μm cellulose acetate filter membrane (Millipore, Billerica, MA, USA) before use.

GC analysis was performed using a Shimadzu GC-2014 system equipped with a split/splitless injector and an FID detector (Shimadzu, Japan). GA-2000A air generator and SH-300 high-purity hydrogen generator were purchased from Zhongxing Huili Co. Ltd. (Beijing, China). A N-2000 Chromatography data workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. The analytical column was PEG-20M flexible fused silica capillary column (30.0 m × 0.25 mm × 0.33 μm) and its column flow rate was set at 1.14 mL/min with a split ratio of 20. High-purity nitrogen (99.999 %) was used as carrier gas with hydrogen as burn gas, air as aid-burn gas. The injection port and detection temperature was setting at 250 and 270 ºC, respectively.

**Preparation of sample and standard solution:** 0.25 g *n*-propanol was accurately weighed and dissolved using DMF to 250 mL volumetric flask as internal standard solution. Each stock standard solution of acetone and isopropanol standard was prepared individually by dissolving each standard compound in internal standard solution to get the concentration of 1.0 mg/mL. Aliquots of these stock standard solutions were combined to get the mixed stock solution and then diluted using internal standard solution by step-by-step dilution method to get nine working standard solutions with different concentrations (200-0.4 μg/mL).

Cefotaxime products were grinded into fine powder and 0.1 g of it was dissolved into 10.0 mL volumetric flask using internal standard solution to get a final concentration of 10.0 mg/mL for gas chromatography analysis.

## RESULTS AND DISCUSSION

**Effect of chromatographic column:** The column is one of the key factors of gas chromatographic separation. Though non-polar and weak-polar gas chromatographic column had good separation efficiency for most of organic solvents, capillary column of different polarity had different separation effect on some target reagents. some of the solvents such as ether and isopropyl alcohol cannot be separated on non-polar column, at the same time ethyl ether, isopropyl alcohol and acetone cannot be completely separated on weakly polar column[13]. The polarity and boiling point of the components were both contributed to its retention behaviours on column. This work also show acetone, isopropanol, *n*-propanol cannot be completely separated using KB-1 weak-polar capillary column as the analysis column, while a good separation result of the three analytes was obtained on strong polar PEG-20M column (Fig. 1).

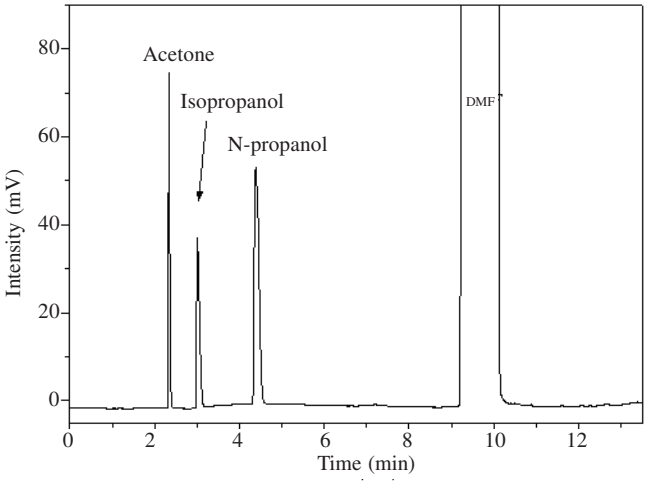


Fig. 1. Chromatogram of the three analytes solution on PEG-20M column

**Effect of sample solvent:** Sample solvents have great effect on chromatographic retention behaviours. Water was used firstly as the sample solvent, but a peak broadening of the analytes and its retention time shorten was observed with the increasing injection times, which was due to water solvent have strong inter-action with stationary phase of strong polar PEG-20M column. More importantly, due to water having higher surface energy than station phase, resulting in poor wettability and water drops formation, thus a deterioration of column performance is producing, which caused band broadening, even splitted peaks.

Different solvents including dimethylformamide, dimethyl sulfoxide, carbon tetrachloride, dimethyl acetamide, ethanol, *n*-hexane, dioxane, methoxy-ethanol were investigated in this work. The results show than DMF and DMSO solvent could provide better separation. This was due to DMF and DMSO have a good ability to dissolve and extract most organic solvents. Furthermore, due to its high boiling point and long retention time in column, it will enable the volatile components was wash out firstly to avoid their interference. Though DMSO (b.p. 189 ºC) had more wider detection range than DMF (b.p. 153 ºC), considering the boiling point of the target organic solvents, acetone, isopropanol and *n*-propanol, is 57, 82 and

98 ºC, DMF as solvent is enough to keep them test-first-out with no interferences.

**Optimization of chromatographic conditions:** The sample-input temperature was mainly influenced by the boiling point scope of samples and the temperature of the column. The sample-input temperature must ensure that all samples completely in vaporization state and all the evaporation components could flow out the column thoroughly. In principle, a higher sample-input temperature is favourable, there is generally close to the sample components boiling point that of the highest boiling point, but must be less than the decomposition temperature of easy decomposition components. Compromise with the boiling points of DMF and target organic solvents, 250 ºC was employed as the sample-input temperature for further research.

A suitable column temperature is not only help all target analytes be separated completely, but also ensure that all components can flow out column thoroughly. Considering the analysis time and resolution, temperature-programmed steps were employed in this work. The initial column temperature was set to 60 ºC for 3 min to obtain a good separation for acetone, then increasing at 10 ºC/min to 170 ºC for 10 min to wash out DMF and other interferences as much as possible.

The detector temperature is referred to the heating block temperature, rather than the actual detection of points. The detector temperature has two actions: one is ensuring that all components flow out from column without condensing. The other is meeting the requirements of detection sensitivity. Due to the sensitivity of FID detector is affected slightly by temperature, so the effect of detector temperature was investigated and 270 ºC was selected for further work.

The split ratio from 1:10-1:30 was investigated in this work. When the split ratio was higher than 1:15, several broaden chromatographic peaks were observed, which perhaps caused by column overloading. But when the split ratio was too lower, the sensitivity would be declined. Compromise the sensitivity and separation efficiency, the split ratio of 1:20 was employed in this work.

**Validation of the proposed method:** Calibration curves were constructed using the rate of peak areas of analytes with the internal standard measured at seven increasing sample content, in a range of 0.4-200 mg/mL. Good linearity was obtained for all analytes throughout the concentration range and the regression equations were Y = 0.851X – 0.059 for acetone and Y = 0.802X – 0.046 for isopropanol with regression coefficient ($r^2$) of 0.9991 and 0.9993, respectively. Intra-assay and inter-assay precision expressed as the relative standard deviation (RSD) of concentrations calculated from the quality control samples on same day (n = 7) and five different days were less than 1.7 and 2.9 %. Based on a signal-to-noise ratio of 3, the limits of detection (LOD) for acetone and isopropanol were 0.05 and 0.09 μg/mL, respectively.

**Residues analysis of cefotaxime products:** Cefotaxime products purchased from local market were grinded into fine powder and 0.1 g of it was dissolved into 10 mL volumetric flask using internal standard solution for gas chromatography analysis (Fig. 2). No interfering peaks from the drugs matrix were observed at the retention time of compounds of interest, which demonstrates the good selectivity of the developed separation strategy. The experimental results shown in Table-1 indicate the content of the two volatile organic solvents determined in cefotaxime products is lower than that marked by the manufacturer. Recovery was tested to investigate the effect of the actual sample matrix by spiking three different concentrations of analytes into the actual sample and the results were shown in Table-2. The means recoveries for all analytes were in a range of 92.7-103.2 % with RSD less than 3.5 %, which indicated the method was reliable and can be used for the multi-residues analysis in cephalosporin antibiotics products.

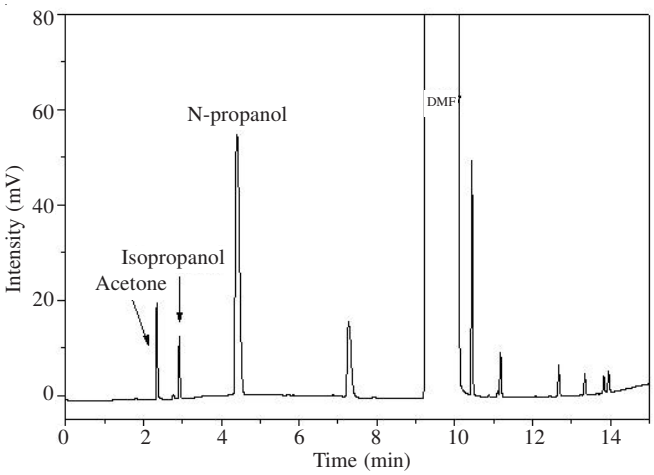


Fig. 2. Chromatogram of volatile organic solvents in cefotaxime products

TABLE-1
RESULTS OF ORGANIC SOLVENTS RESIDUES IN CEFOTAXIME PRODUCTS

| Acetone (mg/g) | RSD (%, n = 5) | Isopropanol (mg/g) | RSD (%, n = 5) |
|---|---|---|---|
| 3.87 | 1.22 | 0.187 | 1.59 |
| 3.92 | 0.93 | 0.192 | 1.04 |
| 4.01 | 0.95 | 0.209 | 0.93 |
| 3.86 | 1.07 | 0.185 | 1.70 |
| 3.94 | 1.33 | 0.194 | 1.52 |

TABLE-2
RECOVERIES OF ACETONE AND ISOPROPANOL IN CEFOTAXIME PRODUCTS (n = 5)

| Analytes | 0.20 (mg/g) | | 1.0 (mg/g) | | 5.0 (mg/g) | |
|---|---|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Acetone | 95.7 | 3.1 | 96.1 | 2.7 | 101.2 | 1.9 |
| Isopropanol | 96.1 | 2.9 | 93.8 | 2.5 | 94.4 | 2.3 |
| Acetone | 93.5 | 3.4 | 97.1 | 3.0 | 94.2 | 2.9 |
| Isopropanol | 92.7 | 2.6 | 94.8 | 2.5 | 97.3 | 2.0 |
| Acetone | 96.4 | 2.5 | 95.2 | 2.6 | 98.0 | 2.1 |
| Isopropanol | 93.7 | 3.5 | 95.7 | 2.3 | 95.1 | 1.9 |

## Conclusion

This work developed a simple and sensitive method for the multi-residues analysis of two volatile organic solvents, acetone and isopropanol, in cefotaxime products by capillary gas chromatography with *n*-propanol as the internal standard material. Varieties of influencing factors for separation and detection of these volatile organic solvents were investigated and optimized. Good linearity were obtained in a range of 0.4-200 μg/mL with the correlation coefficients ($r^2$) of 0.9991 for acetone, 0.9993 for isopropanol alcohol, respectively. The average recoveries of acetone and isopropanol in three different spiked concentrations were ranged in 92.7-103.2 % with the relative standard deviation less than 3.5 % (n = 5). The proposed method offers a good alternative for routine analysis due to its simplicity and at the same time reliability.

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