Analysis of Residual Solvents in Co-amoxiclav Coated Tablets Using Solid Phase Microextraction–Gas Chromatography

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Abstract. Co-Amoxiclav tablets are coated tablets. The tablet coating process requires an organic solvent in the sealing stage. Several processes are carried out to remove organic solvents in the finished drug product, such as heating and pressure reduction, which still leaves organic solvents in the finished drug preparation. The purpose of this study was to optimize the solid phase micro-extraction method–gas chromatography for the analysis of organic solvent residues in co-amoxiclav tablets and the determination of the residual solvent content. The extraction method used was the headspace-SPME method, sample extraction by heating so that the solvent residue evaporated and absorbed by the PDMS-DVB (Polydimethylsiloxane-divinylbenzene) fiber. The extraction process was carried out by optimizing the temperature and time. The gas chromatography system used was the detector temperature of 250 °C, and the injected port temperature was 210 °C. The oven temperature was programmed, ranging from 38 °C to 210 °C with a temperature increase of 10 °C/min with a holding time of five minutes. The helium carrier gas flow rate was 0.71 mL/min. Analysis of residual solvent was conducted by looking at the retention time and peak area then comparing them with the standard solution. The optimal temperature and time of the extraction using SPME were 50 °C and 30 min. The residue analysis results showed that the co-amoxiclav coated tablets contained 2.88 ± 0.58 ppm dichloromethane residues.

Keywords: SPME · Co-Amoxiclav · Coated Tablets · Gas Chromatography

1 Introduction

Amoxicillin is combined with clavulanic acid to protect the beta-lactam ring from the beta-lactamase enzyme, and this combination is available in the form of film-coated tablets. Tablets are coated for various reasons, including protecting the active ingredients from air, humidity, or light, masking unpleasant tastes and odors, improving the appearance, and controlling drug release in the gastrointestinal tract [1].

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The coating process at the sealing stage often involves several organic solvents, such as methanol, ethanol, dichloromethane, and toluene. Several processes are then carried out to remove organic solvents in the finished drug product, such as heating and pressure reduction, but those still leave organic solvents in the finished drug preparation; the remaining organic solvents trapped in the drug formulation are called residual solvents. These residual solvents tend to cause toxic effects on patients and change drug characteristics, including changes in the release and permeability of the drug [2]; therefore, it is necessary to limit the number of organic solvent residues in tablet preparations. Limitation of the amount of solvent residue in tablets is carried out by measuring the residual solvent level to be compared with the ICH (International Conference on Harmonization) guidelines, issued by the harmonization conference of technical requirements to determine the limit of solvent residues that remain in active ingredients, excipients, and finished drug products [3]. In the previous study, amoxicillin tablet contained dichloromethane and toluene as residual solvents. According to the ICH guidelines, the detection limit for dichloromethane is around 600 ppm; if it exceeds this limit, dichloromethane toxicity will occur [4]. Dichloromethane is neurotoxic, carcinogenic, and easily soluble in fat (lipophilic). In addition, dichloromethane can also penetrate the blood-axon barrier to cause disturbances in the central nervous system and peripheral nerves [5].

The gas chromatography -flame ionization detector method has been developed for routine residual solvent analysis in pharmaceutical products. This technique was chosen because it has high sensitivity, good separation ability, low detection limit, and simplicity of instrumentation [4]. In residual solvent analysis, sample preparation has an important role since this process is carried out to remove interference or contaminants and convert the analyte into a form suitable for separation and detection. If the sample preparation method is unsuitable, the analyte may be wasted before analysis, so an appropriate extraction technique must be selected.

Liquid-liquid extraction, solid-phase extraction, and Solid-Phase Microextraction (SPME) are extraction processes for drug analysis preparations. In liquid-liquid extraction, repeated extraction is required to obtain high recovery so that it tends to require a lot of organic solvents, while in solid-phase extraction techniques, the separation process with high recovery ($> 99\%$) is easier to achieve without repeated extraction so that the analyte is separated from the matrix. The sample then becomes more efficient and reduces the organic solvent used. Currently, the solid extraction method has been developed into the SPME technique of polymer-coated fiber used as an extraction device, and the extracted analyte can be directly analyzed by gas chromatography [6, 7].

The working principle of SPME and SPE is similar, namely the adsorption of the analyte to the stationary phase, but in the SPE technique, the stationary phase used is a cartridge (absorbent) such as Florisil, while fiber is used in the SPME as the stationary phase. However, disadvantages of the SPE technique are the use of pure organic solvents and the presence of an irreversible adsorption process of analyte and cartridge reproducibility that varies. Thus, the SPME method is preferred, which is fast, simple, solvent-free, and sensitive for analyte extraction. The extraction technique using solid-phase microextraction (SPME) is influenced by the extraction time and temperature to obtain optimal extract results. The increase in temperature will accelerate the release of

analyte from the sample matrix and increase the diffusion coefficient of the analyte so that the analyte can reach the fiber quickly, resulting in an equilibrium partition between the analyte and the fast fiber; also, the analyte is rapidly adsorbed while extraction time encourages greater absorption of volatile compounds to the fiber by increasing the analyte partition equilibrium between SPME fiber and sample [8].

Based on the above background, the authors performed extraction optimization, method validation, and analysis of residual solvent levels on Co-Amoxiclav tablets using gas chromatography–flame ionization detector with a solid-phase microextraction (SPME) preparation technique.

2 Materials and Methods

2.1 Instruments and Materials

The instruments used in this research process consisted of gas chromatography (Shimadzu GCMS-QP 2010s) with flame ionization detector, Stabilwax–DA (Crossbond® Carbowax® Polyethylene glycol) chromatography column with a length of 30 meters with 0.25 mm of diameter ID and 0.25 μm film thickness (Restek-USA), Hot plate Stirrer Thermo Scientific (Cimarec), fiber polydimethylsiloxane–Divinylbenzene (PDMS–DVB), and 65μm needle 25 Ga (Supelco). The materials used in this study were Co-Amoxiclav tablets from Indofarma, acetone pro analysis (Emsure–Germany), dichloromethane pro analysis (Emsure–Germany), dimethyl sulfoxide pro analysis (Emsure–Germany), toluene pro analysis (Emsure–Germany), and dimethylformamide pro analysis (Emsure–Germany).

2.2 Methods

2.2.1 Gas Chromatography Conditioning

Shimadzu GCMS–QP 2010s gas chromatography with flame ionization detector was adjusted. The gas chromatography system used was the detector temperature of 250 °C and the injection port temperature of 210 °C. Elution was then carried out with a temperature program ranging from 38 °C to 210 °C with a temperature increase of 10 °C/min with a hold time of five minutes. The helium carrier flow rate gas was 0.71 mL/min.

2.2.2 Selection of Sample Injection Method

The injection method was chosen by evaporating the residual solvent using PDMS-DVB SPME fiber. Approximately 625 mg powder tablets were placed in a 10 ml vial, heated with a hot plate stirrer at a temperature of 50 °C for 30 min and then injected into gas chromatography using the split and splitless injection methods. Then, the peak of the chromatogram was observed.

2.2.3 Sample Extraction and Qualitative Analysis of Residual Solvent

Sample extraction was carried out by placing Co-Amoxiclav-coated tablets in a 10 ml vial, and then the residual solvent evaporation used PDMS-DVB SPME fiber by heating

at 50 °C for 30 min and injected into GC-FID. The retention time of the suspected analytes compared to the standard.

2.2.4 SPME Extraction Optimization

Extraction optimization was carried out in the following way. Approximately 625 mg powder of co-amoxiclav tablet sample was put in a 10 ml vial container and then evaporated the residual solvent with PDMS-DVB SPME using heating at a temperature of 50° C for 15 and 30 min and a temperature of 30 °C for 15 and 30 min with a hot plate stirrer. The optimization results would be used for sample extraction.

2.2.5 Preparation of Standard Solution

The stock solution was prepared with 1 μL of dichloromethane added with 10 ml of dimethyl sulfoxide to obtain a concentration of 100 ppm. Then, it was extracted with SPME and injected into gas chromatography

2.2.6 Determination of Residual Solvent in Co-amoxiclav Tablets

Tablets were powdered and weighed and then placed in a tightly closed 10 mL vial, then extracted with SPME using PDMS-DVB fiber and heated at 50 °C for 30 min, replicated four times. Then, the levels were determined by entering the response area of the sample into the equation standard curve and then determining the ppm and mg/tablet levels.

3 Results and Discussion

The sample preparation process of Solid-Phase Microextraction (SPME) is based on the partition of the analyte between the sample matrix and the stationary phase coated by silica or fiber. The extraction can be directly injected into the gas chromatography injector. The SPME extraction method used in this research was headspace. There are 2 types of SPME extraction techniques: headspace and direct immersion. In the headspace technique, the analyte from the sample does not have direct contact with the SPME fiber. Direct contact occurs between the fiber and the analyte in direct immersion because the fiber is directly immersed into the analyte, extracted with a small volume [9]. In addition, the method used in this study was headspace because direct extraction can only be carried out on high clarity samples, and samples with complex matrices can cause flogging and SPME damage.

Moreover, the choice of fiber is also influential on the extraction process. The basis for selecting fiber for extraction depends on the polarity of the substance to be analyzed. The fiber used in this study was polydimethylsiloxane–divinylbenzene (PDMS-DVB) since this fiber is bipolar, meaning that the fiber can extract polar and non-polar analytes. This fiber is a combination of PDMS (polydimethylsiloxane) fiber, which is non-polar due to the Si-CH_3 bond, and DVB (divinylbenzene), which is polar due to the CH_2-CH bond [10].

In addition, there are two gas chromatography injection methods: splitless injection and split injection. The splitless injection method is suitable for compounds with high

boiling points with low concentrations, while the split injection method is used for volatile compounds with high concentrations. In the split injection method, only a small part of the sample gas vapor entering the column is removed. In this research, the experimental results selected the split injection method with a split ratio of 1: 5 because the peak of the analyte chromatogram had a good separation index. In the chromatogram results, an optimal peak was formed, while the splitless injection chromatogram method results did not form an optimal peak due to tailings (widening peak band). In the split injection method, only a small part of the sample gas vapor entered the column.

Research by Haque et al. (2015) stated that the tablet of amoxicillin contained residual solvent in the form of dichloromethane and toluene [4]. Qualitative analysis in the current study was carried out by comparing the retention time results obtained with the retention time of standard solutions of dichloromethane and toluene. Retention time is the time required for the analyte to pass through the gas chromatography column; if a sample contains several compounds, each compound in the sample will spend a different amount of time in the column according to its chemical composition and will have different retention times [11]. A compound is said to be the same or similar if it shows the same retention time because the retention time is specific. Thus, the qualitative test was carried out four times, the experimental results in the retention time of the chromatogram are in accordance with the retention time of standard dichloromethane.

Further, it is necessary to optimize the temperature and time to obtain the optimum residual extract because the extraction temperature is a determining factor for extracting volatile compounds. In the SPME process, increasing temperature will accelerate the release of analyte from the sample matrix and increase the analyte diffusion coefficient so that there is an equilibrium partition between the analyte and fiber, and the analyte is rapidly adsorbed [8]. Extraction time is also an important parameter with increasing extraction time that can encourage greater absorption of volatile compounds on the fiber so that the analyte partition equilibrium occurs faster between SPME fiber and the sample [12]. In this study, optimization was carried out for sample extraction of Co-Amoxiclav tablets by extracting the residual solvent at a temperature of 30 °C and 50 °C. Using those temperatures is because it was suspected that the residue contained in the tablet had a boiling point that ranged. From the study, the following results were obtained (Table 1).

Based on these results, it can be concluded that the optimum extraction time for dichloromethane was a temperature of 50 °C with a time of 30 min. It was evidenced by the increasing area of the dichloromethane residue, meaning that at a temperature of 50 °C and an extraction time of 30 min, there is an increase in the diffusion coefficient and partition equilibrium so that the analyte is rapidly adsorbed. According to Chmiel et al. (2017), if the temperature is too high, the absorption of the analyte by the fibers will decrease. It is because the too high temperature can reduce the analyte diffusion coefficient between the SPME fiber and the sample. Also, if the increase in extraction time exceeds the optimum limit, it will affect the desorption of the adsorbed compound on the SPME fiber and causes the adsorption of the analyte back to the stationary phase of the fiber [13].

The system suitability test was carried out to see the column efficiency resolution and ensure that the instrument method's analytical capability functioned properly. This

Table 1. SPME Extraction Optimization Results

Extraction temp.(°C)	Extraction time (min.)	Retention time (min.)	Area
30	15	6.577	10053
	30	6.682	60240
50	15	6.632	78339
	30	6.646	202534

parameter is determined based on % RSD of retention time, tailings factor, and column efficiency (N), obtained from experimental results. The requirements for the acceptance of the system suitability are the % RSD value of retention time <2%, tailings factor <2%, and theoretical plate value (N) >2500 [14]. The test results showed that the % RSD parameter of retention time and the area was 0.003117 % and 0.1340%, respectively, and the value met the requirements.

Then, the assay of residual solvent in tablets was determined by extracting Co-Amoxiclav tablets with the SPME method at a temperature of 50 °C for 30 min. The concentration was determined using regression equation y = 160958x–26551 (correlation coefficient = 0.99). The test results with four replications showed that the average residual level of dichloromethane was 2.88 ± 0.58 ppm. It indicates a dichloromethane solvent residue of 2.88 ± 0.58 ppm in 625 mg film-coated tablet of Co-Amoxiclav. This value did not exceed the threshold set by the ICH guideline of 600 ppm.

Moreover, dichloromethane is a class 2 solvent. This group must be limited. Its solvent use can cause irreversible toxicity, such as neurotoxicity and teratogenicity. In addition to causing teratogenic effects, dichloromethane is also carcinogenic. Even more, its long-term effects can lead to gallbladder cancer and lymphoma cancer [15]. The results of a previous study conducted by Haque et al. (2015) revealed that the dichloromethane content in amoxicillin tablets was 2.5 ppm per tablet [4]. Although the samples and the preparation technique used was different but gave similar result. In previous study, a liquid-liquid extraction preparation technique was used. In addition, the tablets used in this study also differed in content with Haque et al.'s (2015) research, in which the content of the tablets used was 250 mg [4]. It also had an effect since the differences in tablet content differed in the number of excipients used, such as organic solvents used in the coating process.

4 Conclusion

The results showed that the residual solvent analysis on coated tablets using SPME could be carried out at 50 °C and 30 min. The SPME-GC-FID analysis uncovered that the Co-Amoxiclav coated tablets contained 2.88 ± 0.58 ppm of dichloromethane as a residual solvent.

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