Extraction of Volatile Anticancer Drugs in Air Using a Solid-Phase Extraction Type Device Followed by Gas Chromatography–Mass Spectrometric Analysis

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Objectives

Ifosfamide (IF), cyclophosphamide (CP), and bendamustine (BD) are widely used anticancer drugs. These drugs have slight volatility; therefore, medical-staff exposure is of concern in the medical field. However, an accurate and quantitative detection method of these volatile drugs in air has not been reported. In this study, we developed the quantitative extraction and detection method of these volatile anticancer drugs in air. For the extraction of analytes, a solid-phase extraction-type collection device packed with styrene-divinylbenzene polymer particles was used. The extracted analytes were quantitatively eluted with 5 mL of ethanol, and the solution was concentrated to 100 μL with nitrogen purging. The analytes were analyzed using gas chromatography–mass spectrometry (GC-MS). The limit of detection of the proposed method for IF and CP was 0.017 and 0.033 ng L⁻¹, respectively in air at an air sampling volume of 300 L. IF and CP showed slight volatility, whereas BD was not detected in GC-MS due to its lower volatility. The spiked recoveries of IF and CP in the proposed method were within the range of 95.5 to 101%. Finally, the proposed method was applied to determine the exposure of IF and CP during the dispensing of CP within a hospital dispensary room. The investigated volatile anticancer drugs were not detected in real air samples, indicating that the protection measures employed are sufficient.

Keywords

Anticancer drug, ifosfamide, cyclophosphamide, volatile drug

Introduction

Ifosfamide (IF) and cyclophosphamide (CP) are widely used alkylating agents to treat cancers.¹² These anticancer drugs are volatile and, therefore, present a danger for medical staff. In addition, CP is carcinogenic to humans and is classified as group 1 by the International Agency for Research on Cancer.¹ The detection of IF and CP in the urine of medical staff has been reported as a result of exposure to anticancer drugs.⁴ The National Institute for Occupational Safety and Health announced an alert to prevent medical staff exposure to the volatile anticancer drugs.⁵ The alert recommends using a closed-system transfer device and safety cabinets. However, the detection of CP in the urine of pharmacists has still been reported.⁶⁷ In Japan, bendamustine (BD) is also a known volatile anticancer drug. In order to evaluate the risk of exposure to volatile anticancer drugs by medical staff, wipe tests have been performed.⁸⁹ Wipe tests evaluate the risk of the drugs to medical staff, although it does not evaluate the exposure level. In addition, this method cannot discriminate whether the drugs detected are from the adsorption of gaseous drugs or the scattering of drugs. The detection of anticancer drugs in urine samples is also an effective method to evaluate exposure, although the exposure route cannot be known. Therefore, to sufficiently evaluate the risk of exposure to volatile anticancer drugs from air, the detection of these drugs in air samples is required.

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) is typically used for the detection and analysis of IF, CP, and BD.⁶,⁷,¹⁰⁻¹² IF and CP, owing to their volatility, can be detected using gas chromatography–mass spectrometry (GC-MS).⁹¹¹⁻¹⁴ A previous study suggested evaporating CP into the air, where spiked CP was evaporated from polyvinyl chloride and PTFE filters during continuous air sampling.¹⁵ This study also reported that a better recovery of IF and CP by XAD-8 (methacrylic acid polymer) than Porapak R (N-vinyl pyrrolidone polymer) and activated carbon. However, there are very few reports for extracting and detecting volatile anticancer drugs from air. Therefore, quantitative and sensitive detection method of anticancer drugs in air is needed to confirm
the suitability of measures to prevent medical staff exposure in dispensing anticancer drugs. In addition, reports regarding the volatility of IF, CP, and BD are very limited.

Our research group has developed a solid-phase extraction (SPE)-type collection device for the extraction of gaseous semi-volatile organic compounds, such as polycyclic aromatic hydrocarbons\(^1\)-\(^3\) and phthalate esters\(^2\),\(^3\) in air samples. The SPE-type collection device was prepared by packing a styrene-divinylbenzene polymer particle of Sunpak-H into a glass cartridge. The air sample containing the target analytes was collected using a gas sampling pump through the collection device, typically at 10 L min\(^{-1}\). After collection, the analytes were quantitatively eluted from the adsorbent using a small amount of organic solvent, and the analytes were quantitatively determined by HPLC or GC-MS. The collection device can be reused after drying of adsorbent under a flow of nitrogen.

In this study, an analytical method to determine the volatility of anticancer drugs in air samples using an SPE-type collection device followed by GC-MS analysis was developed. The volatility of the anticancer drugs and several analytical performances, including sensitivity, recovery, and repeatability, were investigated in the manuscript.

**Experimental**

**Chemicals**

IF and CP were purchased from Shionogi and Co., Ltd. (Osaka, Japan), and BD was obtained from SymBio Pharmaceuticals Ltd. (Tokyo, Japan). Methanol, ethanol, 1-propanol, and acetone were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). The chemical structures of IF, CP and BD are shown in Fig. 1.

**GC-MS measurement**

A JEOL JMS-Q1000 Mk-II GC-MS (JEOL, Tokyo, Japan) was used for all GC measurements. Helium (>99.999% purity) was used as the carrier gas with a head pressure of 100 kPa. A fused silica capillary column (HP-5, 30 m length × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) was used for separation. The column temperature was initially held at 190 C for 2 min, and then increased to 220°C at a rate of 5°C/min. The injector and interface temperatures were set at 300°C. The sample solution (2 μL) was injected into the GC-MS with a split mode injection (ratio of 10:1). The mass spectrometer was operated in the selected ion monitoring mode based on the preliminary measurements of IF (m/z: 134 and 211) and CP (m/z: 120 and 211).

**SPE-type collection device**

A styrene-divinylbenzene polymer particle of Sunpak-H (particle size 50/80 mesh, Shinwa Chemical Industries, Ltd., Kyoto, Japan) was used as the adsorbent. The specific surface area of the adsorbent was 100 - 150 m\(^2\) g\(^{-1}\). The SPE-type collection device was prepared as follows. A stainless steel wire mesh (14.9 mm diameter, HORIBA STEC Co., Ltd., Kyoto Japan) and a glass fiber filter (GA-200, 15 mm diameter, Advantec Tokyo Kaisha, Ltd., Tokyo, Japan) were inserted into a specially designed glass cartridge (14.9 mm diameter, HORIBA STEC). Then, Sunpak-H (0.3 g) was packed into the cartridge, fixed by a glass fiber filter and PTFE O-ring. A PTFE adapter (HORIBA STEC) was attached to connect the cartridge to a gas sampling pump. Figure 2 shows a schematic illustration of the SPE-type collection device. This device was used to collect analytes from the air sample, both real and standard.

**Analytical method**

The collection and elution performances of the collection device for volatile anticancer drugs were evaluated using a standard solution (100 μg mL\(^{-1}\) of each analyte in ethanol). In order to evaluate the standard samples, a standard solution (100 μL) was spiked onto the collection device. For evaluating the collection recovery of the analytes, another collection device was tandemly connected to the first device, and clean air was collected using a gas sampling pump (HORIBA STEC) through the two collection devices at a sampling speed of 10 L min\(^{-1}\). The analytes were eluted from the collection device by loading a few milliliters of an organic solvent (methanol, ethanol, 1-propanol, or acetone). The eluted solvent was concentrated to a few milliliters of an organic solvent (methanol, ethanol, 1-propanol, or acetone). The remaining concentration was measured by GC-MS. The recovery for the standard analytes was calculated by the ratio of the detection of the respective analyte in the former collection device to the total peak area (obtained as the sum of the peak area of the former and latter collection devices). When any analytes were detected on the latter collection device, the collection recovery could be calculated to 100%. The elution recovery of the spiked analytes was calculated by the ratio of the detection on the 1st elution to the total of the 1st and 2nd elutions.

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**Fig. 1** Chemical structure of (A) IF, (B) CP, and (C) BD.

**Fig. 2** Schematic illustration of the SPE-type collection device.
When any analytes were detected on the 2nd elution, the elution recovery could be calculated to 100%.

The recovery of IF and CP during the concentration process was investigated as follows: 5 mL of the elution solvent (ethanol) was used to elute the analytes, and then concentrated to 100 μL with nitrogen purging. First, a standard solution (10 mL) of IF and CP (1.0 μg mL⁻¹) dissolved in ethanol was prepared and divided into two vials (5 mL each). One solution was injected directly into the GC-MS, and the peak areas of the analytes were measured. Another solution was concentrated to 100 μL using a nitrogen purging in a water bath, followed by redissolving in ethanol (5 mL), and finally measured by GC-MS. The recovery during the concentration process was determined by comparing the peak areas obtained from the concentrated solution to the standard solution.

Results and Discussion

Optimization of elution and extraction methods

First, the retention times of the analytes in the GC-MS measurement were investigated by injecting a standard solution. However, BD was not eluted from the column even at the maximum column temperature. This could be due to the significantly low estimated vapor pressure of BD (1.3 × 10⁻⁵ mmHg at 25°C, according to PubChem database). Therefore, IF and CP were the focus in the following studies as the volatile anticancer drugs. The estimated vapor pressures of IF and CP are 3.0 × 10⁻⁵ and 4.4 × 10⁻³ mmHg at 25°C, respectively, according to PubChem database. After spiking the standard solution (IF and CP dissolved in ethanol) and air collection (600 L, 60 min), the standard analytes were eluted using an organic solvent. Methanol, ethanol, 1-propanol, and acetone were investigated as solvents for elution, with methanol and ethanol showing a better elution recovery than 1-propanol and acetone. In addition, ethanol showed a significantly higher sensitivity than methanol, which could be the result of differences in evaporation behavior in the GC injection port. Owing to these results, ethanol was chosen as the elution solvent. The elution recoveries of IF and CP in ethanol are shown in Table 1. In this study, the volume of the elution solvent is the volume obtained in the vial. Notably, this is not the same as the solvent volume loaded into the collection device owing to the absorption of the solvent onto the adsorbent. IF and CP were completely eluted from the collection device using 5 mL of ethanol; therefore, the desorption solvent was determined to be 5 mL of ethanol. The desorption time was approximately 3 min. A further decrease in solvent volume might be possible by decreasing the amount of the adsorbent and the optimizing cartridge shapes.

The extraction recovery for the IF and CP were investigated using two collection devices. The former device, allowed for the quantitative extraction of the analytes up to an air sampling volume of 5400 L, in which any analyte was detected on the latter collection device. These results indicate the sufficient extraction performance of the Sunpak-H packed collection device for IF and CP and the stability of the analytes in the collection device during air collection.

Then, the recovery of IF and CP during the concentration process with a nitrogen purging was investigated. The results showed that the recovery for IF and CP was lower with a water bath temperature of 50°C, at which the recovery of IF and CP was approximately 60%. At a water bath temperature of 35°C, the recovery of IF and CP was 100 ± 1.9 and 101 ± 3.1%, respectively, and a quantitative recovery was obtained. Therefore, the water bath temperature was determined to be below 35°C.

Evaluation of the volatility of IF and CP

The volatilities of IF and CP were quantitatively evaluated, as shown in Fig. 3. First, 100 μL of the standard solution (1000 μg mL⁻¹ of IF and CP dissolved in ethanol) was spiked on a device packed with a glass fiber filter. The Sunpak-H packed collection device was tandemly connected on the latter side of the filter-packed device using a PTFE adapter, and clean air was collected through the two devices at room temperature (22 ± 2°C). After air sampling, the analytes remained in the former device, adsorbed on the inside of the PTFE adapter, and on the latter collection device were eluted by redissolving in ethanol and measured by GC-MS. Table 2 shows the evaporation ratio (%) of IF and CP from the glass fiber filter for different air sampling volumes (n = 3). The evaporation ratio of both IF and CP increased with increasing the air sampling volume, and approximately 0.2% of IF and CP were evaporated with an air sampling volume of 600 L. The results show that IF and CP have slight volatility at room temperature.

Evaluation of the method

The limit of detection (LOD) and the limit of quantification (LOQ) for IF and CP on the proposed method were determined from the signal-to-noise ratio as 3.3 and 10, respectively. The LOD and LOQ of the proposed method for the determination of IF and CP in a standard solution were 50 and 100 ng mL⁻¹, respectively. Based on these results, the calculated LOD and

| Ethanol volume/mL | Elution recovery, % |
|-------------------|---------------------|
| IF                | CP                  |
| 2                 | 99.0               | 99.3               |
| 3                 | 99.8               | 99.9               |
| 4                 | 99.7               | 100                |
| 5                 | 100                | 100                |

| Air sampling volume/L | Evaporation ratio, % |
|-----------------------|----------------------|
| IF                    | CP                   |
| 600                   | 0.22                 | 0.20               |
| 1800                  | 1.2                  | 0.99               |
| 3600                  | 1.3                  | 2.0                |
| 5400                  | 3.8                  | 3.6                |

Spiked sample: 100 μL of 1000 μg mL⁻¹ IF and CP.
and of a blank sample (not spiked) at an air sampling volume of 300 L are shown in Fig. 4. In both samples, 5 mL of ethanol was loaded onto the device after air sampling, and the desorbed solution was concentrated to 100 μL. From the blank chromatogram, impurities were not detected on the retention times of IF and CP, and baseline separations of IF and CP were achieved in a spiked sample.

Real sample analysis

The proposed method was applied to measuring air samples in a dispensary room at the University of Yamanashi Hospital. The air samples were collected for 30 min (300 L) while preparing the intravenous fluids containing CP (Endoxan) as regular work. CP was dispensed using a safety cabinet. All of the samples were collected outside of the safety cabinet at a height of 1.0 m above the floor and inside the safety cabinet at a height of 1.0 m above the workbench. The samples were collected over a period of 5 days. During air collection, IF was also dispensed within the same safety cabinet several times. The collected samples were eluted and measured within the day. In these real air samples, IF and CP were not detected. At this hospital, CP was not detected (measure by a specialized institution) in urine samples of the medical staff (13 dispensing staffs, 4 nurses, and 4 doctors). These results indicate that the preventive measures employed were sufficient to avoid medical staff exposure to volatile anticancer drugs.

Conclusions

A novel method for the analysis of gaseous volatile anticancer reagents, IF and CP, in air samples was developed using a Sunpak-H packed SPE-type collection device, followed by GC-MS analysis. Gaseous IF and CP were collected using the device, and quantitatively eluted with 5 mL of ethanol. IF and CP showed slight volatility, indicating a risk of medical staff exposure to these volatile anticancer drugs. At the same time, the possibility of preventing exposure to volatile anticancer drugs was confirmed by employing sufficient preventive measures. The proposed method showed sufficient repeatability, sensitivity, and recovery for the determination of IF and CP in air samples. This method could also be suitable for the determination of IF and CP in aerosols. The quantitative detection of IF and CP in air samples is an effective method for evaluating the risk of medical staff exposure to volatile anticancer drugs in the air.

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