Determination of Residual Solvents in Drug Substances by Gas Chromatography with Thermal Desorption Cold Trap Injection

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A simple and reliable method has been developed for the determination of residual solvents in drug substances by gas chromatography with thermal desorption cold trap injection. Residual solvents in a sample were desorbed by heating at a temperature higher than the melting point of the sample, then trapped in a cold trap cooled at −130 °C. After the cold trap was heated, solvents were injected into an analytical column. These operations were performed automatically by the control unit of the injector. This method is useful for the determination of residual solvents in drug substances because of its high sensitivity without the interference of drug substances. As an example of the application of the method, residual solvents in five drug substances were determined.

Key words residual solvent; drug substance; thermal desorption cold trap injection; gas chromatography

Because of the toxicity of residual solvents in drug substances and drug products, the need for a rapid and reliable method for the determination of residual solvents has become greater. The United States Pharmacopoeia (USP) proposed limits of six solvents and standard procedures employing two injection methods (dissolution method and headspace method) for gas chromatography (GC).[1] In the dissolution method, the sample solution is directly injected after dissolving of the sample in an appropriate solvent.[2-7] The dissolution method has the disadvantages of rapidly reducing the column efficiency by non-volatile compounds and of interference by the solvent used for the dissolution. In the case of the headspace method, it is possible to inject only volatile compounds,[8,9] but it is difficult to detect low concentrations of residual solvents.

On the other hand, GC with thermal desorption injection is an effective method for the determination of low concentrations of volatile compounds in solid samples such as air pollutants absorbed on an absorbent.[10] In the case of capillary GC, volatile compounds are condensed in a cold trap before injection to an analytical column. Since only volatile compounds are automatically injected into a column in the thermal desorption cold trap (TCT) method, non-volatile compounds in the sample do not interfere. Therefore, we applied TCT-GC to determine residual solvents in drug substances and studied optimal conditions for TCT-GC.

Experimental

Materials All reagents were obtained from Wako Pure Chemical Industries, Ltd. Water was purified with MILLI-Q II (Millipore, Ltd.). Sample A, B, C, D and E were organic drug substances that were prepared in our laboratory.

Sample A: 2-[3-[4-(*m*-chlorophenyl)-1-piperazinyl]propyl]-*s*-trazolo-[4,3-*a*]pyridin-3(2*H*)-one hydrochloride ($C_{19}H_{22}ClN_5O \cdot HCl$, M.W. = 408.33).

Sample B: 7-fluoro-8-(2-methoxymethylmorpholino)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid ($C_{20}H_{20}N_3O_5SF$, M.W. = 433.46).

Sample C: 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)benzimidazole difumarate ($C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$, M.W. = 534.57).

Sample D: ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate ($C_{25}H_{24}N_2O_4S$, M.W. = 448.54).

Sample E: 2-(4-methylphenyl)-4-benzothiazolyl acetate ($C_{16}H_{13}NO_2S$, M.W. = 283.35).

Equipment The TCT injector system (GL Sciences, Inc.) used in this work is diagramed in Fig. 1. This system consists of a desorption oven compartment, a cold trap compartment, an injector control unit and a TCT flow controller. The cold trap was a fused silica capillary tubing (0.32 mm i.d.) coated with a methyl polysiloxane phase (film thickness 5 μm).

All analyses were performed on a Hewlett-Packard HP5890A gas chromatograph equipped with a TCT injector system and a flame ionization detector. The analytical column was a 60 m × 0.75 mm i.d. wide bore capillary column coated with a 1-μm chemically bonded methyl polysiloxane phase (Supelco, Inc., SPB-1). The carrier gas was helium (99.9999%) with 30-cm/s linear velocity. The temperatures of the injector and the detector were maintained at 200 °C and the column temperature was maintained at 35 °C. Chromatograms were recorded on a Shimadzu Chromatopac C-R6A data processor.

Procedure of TCT-GC A gas sampling bottle (1000 ml) with a silicone

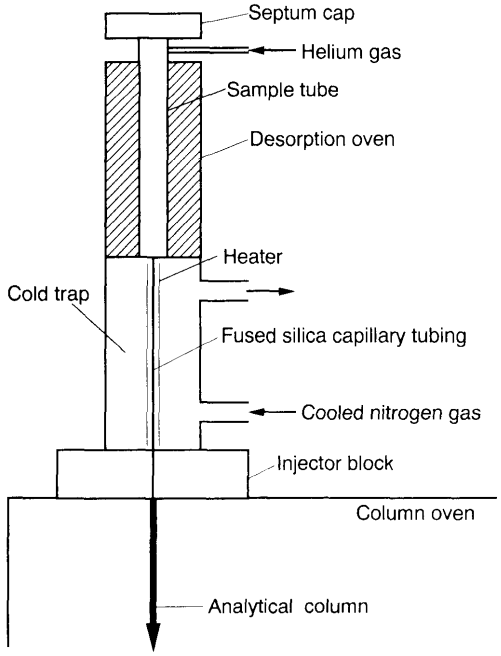

Fig. 1. Thermal Desorption Cold Trap (TCT) Injector

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septum (GL Sciences, Inc.) was used for preparation of the standard gas. The air in the bottle was displaced by nitrogen, and 2 μl of a solvent was transferred accurately in the bottle. The gas in the bottle was allowed to stand for 2 h to vaporize and diffuse uniformly, and this gas was used as the standard gas. The sample to be analyzed was put in a sample tube and the sample tube was installed in the desorption oven. The residual solvents in the sample were desorbed by heating the sample tube at an appropriate temperature for 10 min and were then transferred to the cold trap under a carrier gas stream (helium; flow rate 15 ml/min). The desorbed solvents were trapped in the cold trap cooled at −130 °C by passing a stream of nitrogen cooled by liquid nitrogen. After the complete transfer of the desorbed solvents from the sample to the cold trap, the cold trap was heated at 200 °C for 3 min, and the solvents were injected into the analytical column with the carrier gas. When heating of the cold trap began, recording of the chromatogram was started. These procedures were controlled automatically by the injector control unit. Standard gas in the range of volume from 50 to 1000 μl was introduced by a gas-tight syringe into a empty sample tube installed in the desorption oven with the cold trap cooled at −130 °C.

Reference Method (Dissolution Method) Solutions of samples A or C in water and the solutions of samples B, D or E in dimethyl sulfoxide were prepared (samples A, C, D and E: 10 mg/ml, sample B: 5 mg/ml). Analyses of the solutions were performed by injecting 1 μl onto the analytical column. Samples A and E were analyzed under the same GC condition as TCT-GC. For samples B, C and D, the same size column coated with a 1-μm chemically bonded polyethylene glycol phase (Supelco, Inc., Supelcowax-10) was used because the ethanol in sample B, C and D could not be detected by SPB-1 column due to its strong polarity.

Results and Discussion

Optimization of Trapping Temperature of the Cold Trap The effect of trapping temperature on trapping ability was investigated for 11 solvents which has been generally used for the manufacturing of pharmaceuticals. A constant volume (400 μl for methanol and 200 μl for the others) of the standard gas was transferred to the cooled cold trap maintained at various temperatures (−130—0 °C) and the trapped solvent was injected into the analytical column by heating the cold trap at 200 °C for 3 min. Plots of the peak areas of solvents trapped in the cold trap are shown in Fig. 2 as a function of trapping temperature. Because all solvents tested were trapped at −130 °C, the trapping temperature of the cold trap was kept at −130 °C for trapping.

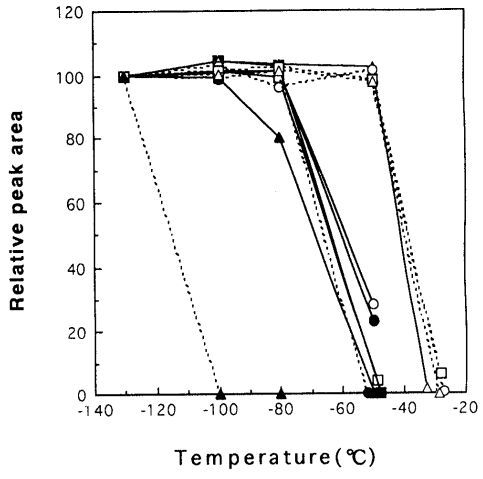

Fig. 2. Effect of Trapping Temperature of the Cold Trap on Peak Area

—○—, ethanol; —●—, dichloromethane; —□—, chloroform; —■—, isopropanol; —△—, 1,4-dioxane; —▲—, acetone; ---○---, isobutanol; ---●---, methanol; ---□---, methyl ethyl ketone; ---△---, benzene; ---▲---, diethyl ether.

Optimization of Heating Temperature of the Cold Trap for Injection The effect of heating temperature of the cold trap on the amount of injected solvents into the analytical column was investigated. As an example, plots of the peak area of acetone, isobutanol and ethanol are given in Fig. 3 as a function of heating temperature. For all the solvents tested, the peak areas of the solvents were not affected by the heating temperature in the range from 100 °C to 200 °C. From these results, the heating temperature of the cold trap for injection was set at 200 °C, which was equal to the detector temperature. Figure 4 shows a typical chromatogram of 11 solvents. All of these solvents were baseline-resolved.

Calibration curves of 11 solvents were found to be linear (correlation coefficients > 0.99). The range of linearity was 200—1000 ng for methanol and 100—1000 ng for the other.

The limits of detection (signal-to-noise ratio of three) are shown in Table 1. It was possible to detect solvents at the 10-ppm level when 10 mg of sample was analyzed. Lower limits can be achieved, if necessary, by using larger

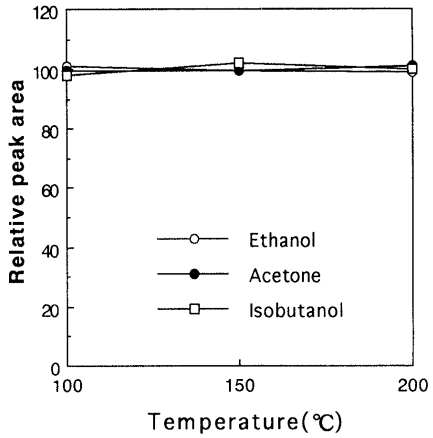

Fig. 3. Effect of Heating Temperature of the Cold Trap for Injection on Peak Area

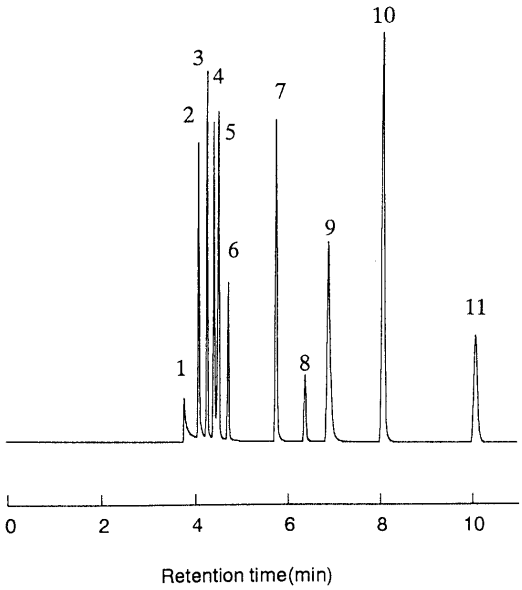

Fig. 4. Typical Chromatogram of Standard Gas

1, methanol; 2, ethanol; 3, acetone; 4, isopropanol; 5, diethyl ether; 6, dichloromethane; 7, methyl ethyl ketone; 8, chloroform; 9, isobutanol; 10, benzene; 11, 1,4-dioxane.

amounts of sample.

Determination of Residual Solvents in Drug Substances The desorption of solvents from a sample is the most important step in the whole procedure. In order to establish the optimal desorption condition, we studied the optimal conditions for analyses of residual solvents in two drug substances (samples A and B). Acetone and isobutanol were used in the manufacturing process of sample A and ethanol was used in sample B. The effect of desorption temperature on the amount of solvent desorbed from a sample for 10 min is shown in Fig. 5. The amount of desorbed solvent from sample A increased with an increase in desorption temperature below 230 °C. In the case of sample B, the amount of desorbed solvent also increased with an increase in desorption temperature below 230 °C. Since the melting points of sample A and B are 230 °C and 220 °C, it is considered that residual solvents would be desorbed (vaporized) thoroughly when the solid sample melts. These results show that heating a sample at a temperature higher than its melting point is necessary to desorb residual solvents completely. No desorption time dependency was found in the range of 5—15 min at the desorption temperature of 250 °C on determination of residual solvents in samples A and B. Therefore, the desorption temperature and desorption time were set at 250 °C and 10 min, respectively, for both sample A and B. The desorption temperatures for samples C, D and E were set 20—30 °C higher than the melting points of each sample.

The results of the determination of residual solvents in drug substances are shown in Table 2. The dissolution method was compared with this method to estimate recoveries.

Table 1. Limit of Detection ($S/N=3$)

Solvent	Limit of detection (ng)
Ethanol	6
Dichloromethane	10
Chloroform	12
Isopropanol	2
1,4-Dioxane	7
Acetone	2
Isobutanol	3
Methanol	90
Methyl ethyl ketone	2
Benzene	2
Diethyl ether	5

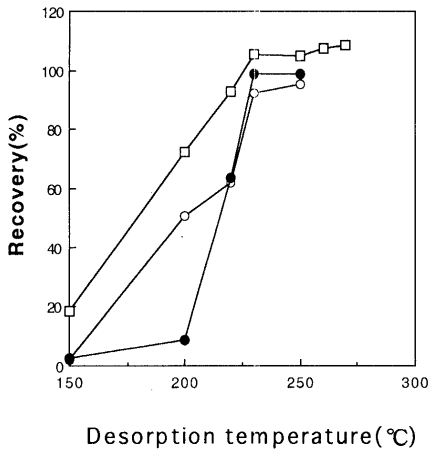

Fig. 5. Effect of Desorption Temperature on Analysis of Residual Solvents in Samples A and B

○, acetone in sample A; ●, isobutanol in sample A; □, ethanol in sample B.

Table 2. Analysis of Residual Solvent in Drug Substances

	mp	Solvent	Concentration of residual solvents (ppm, $n=3$)		
			TCT-GC		Reference method
			DT	Mean±S.D.	Mean±S.D.
Sample A	230 °C	Acetone	250 °C	93.2±2.3	97.7±1.2
		Isobutanol		39.2±2.0	36.5±1.0
Sample B	220 °C	Ethanol	250 °C	332± 5	316±17
Sample C	150 °C	Ethanol	170 °C	100±11	100± 5
Sample D	135 °C	Ethanol	160 °C	247±11	237±25
		Ethyl acetate		368±17	340±14
Sample E	114 °C	Ethyl acetate	140 °C	430± 9	381± 9

DT, desorption temperature.

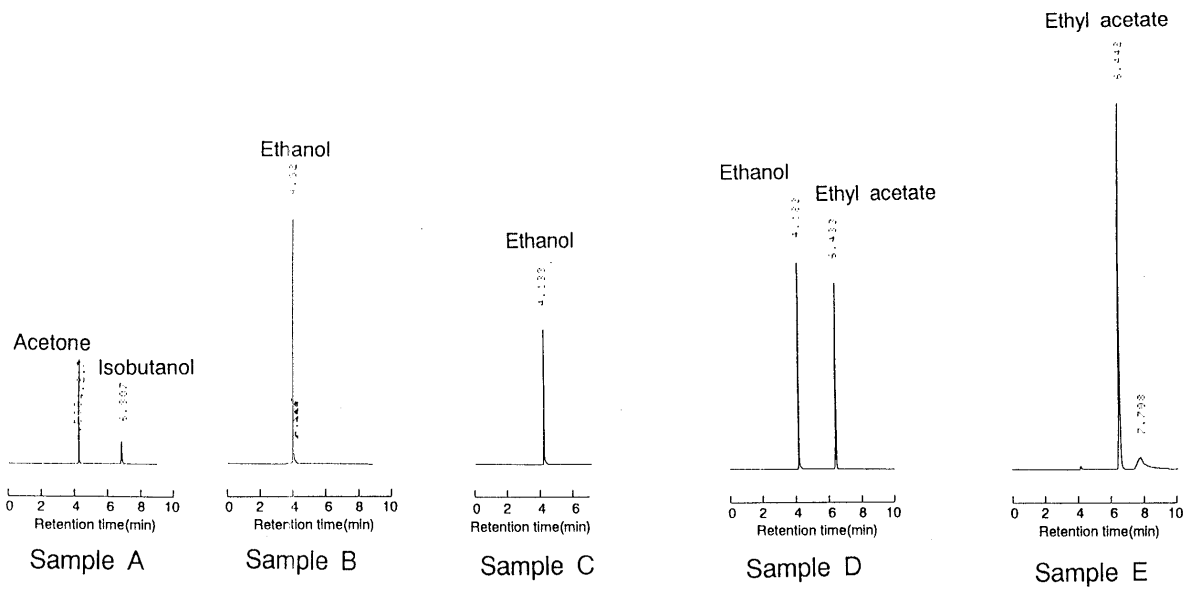

Fig. 6. Chromatograms of Residual Solvents in Five Drug Substances by TCT-GC

The reasonably good agreement between the two methods demonstrates that residual solvents are recovered sufficiently by the TCT-GC method. The relative standard deviations of TCT-GC analyses of residual solvents in samples A, B, C, D and E were 1.5—11%. TCT-GC is able to precisely determine of residual solvents in drug substances. The present results indicate that the TCT-GC can be applied to analyze residual solvents in drug substances. Samples A, B, C and D were decomposed by heating, but chromatograms of the TCT-GC for these samples show no interference, and an unknown organic volatile impurity peak appeared in the chromatogram of sample E (Fig. 6).

In conclusion, the TCT-GC was applicable to the determination of residual solvents in drug substances and sufficient results were obtained. Residual solvents should be measured not only as they are absorbed on the surface of a sample but also in the interior of a sample. For this reason, the desorption temperature should be set higher than the melting point of the sample to completely recover residual solvents in drug substances. This method has high sensitivity because a whole amount of residual solvent in a sample is introduced into an analytical column without interference of the solid sample matrix. This method is useful for the determination of residual solvents in many pharmaceuticals.

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