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Determination of acrolein by headspace solid-phase microextraction gas chromatography and mass spectrometry

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Abstract

We developed a headspace solid-phase microextraction (headspace SPME) method to measure acrolein in human urine. This new technique resolves some problems with the headspace gas chromatography and mass spectrometry (GC–MS) method which we developed previously. With the original method, a column and a filament were damaged by the injection of air. A 0.5-ml urine (or phosphate-buffered saline) sample in a glass vial containing propionaldehyde as an internal standard was heated for 5 min. The SPME fiber (65 μm carbonwax–divinylbenzene fiber) was exposed to the headspace and then inserted into a GC–MS instrument in which a DB-WAX capillary column (30 m×0.32 mm, film thickness 0.5 μm) was installed. The total analysis time was 15 min. The inter-assay and intra-assay coefficients of variation were 10.07 and 5.79%, respectively. The calibration curve demonstrated good linearity throughout concentrations ranging from 1 to 10 000 n*M*. The headspace SPME method exhibits high sensitivity and requires a short analysis time as well as the previous method. We conclude that this method is useful to measure urinary acrolein. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Acrolein

1. Introduction

Acrolein (2-propenal, CH_2=CHCHO) is an irritant of mucous membranes and seems to play an important role in the urotoxicity of alkylating agents such as cyclophosphamide and ifosphamide [1]. The prevention of acrolein toxicity has been attempted with scavengers (MESNA) and by large volume lavage when these agents are administered in large doses. When these drugs are given as prophylaxis, the incidence of hemorrhagic cystitis is decreased [2–4]. However, the pharmacokinetics of acrolein have not been clarified, and therefore preventive methods are not well established.

We previously devised a rapid and sensitive method for the measurement of acrolein using the headspace technique for GC–MS [5], but there were difficulties with this technique: columns and filaments are damaged by the injection of air and the sealing of the SPME fiber was disrupted by the high pressure of the column. Recently, a novel technique, solid-phase microextraction (SPME) has been developed and applied to the analysis of various compounds [6–10]. We have now established a headspace SPME method to measure acrolein.

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2. Experimental

2.1. Reagents

Acrolein and propionaldehyde were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA) and Nacalai Tesque (Kyoto, Japan), respectively. All other reagents used were of the highest grade.

2.2. Preparation of standard acrolein solutions

A volume of 0.5 ml phosphate-buffered saline (0.5 *M*, pH 4) or human urine was spiked with 50 μl of 10 n*M* propionaldehyde (internal standard) and 50 μl of various concentrations (1–10 000 n*M*) of acrolein solutions. Prior to assay, the urine was stored at 4°C, and was acidified (pH 2–4) with 2 *N* H_2SO_4. The standard solutions of aldehydes were unstable, so they were freshly prepared. The stock urine sample was stored in a freezer until it was spiked to use for the inter-assay.

2.3. Instrumentation

A Shimadzu GC17A-QP5000 gas chromatograph-mass spectrometer (Kyoto, Japan) with an electron-impact ionization detector was used. A DB-WAX capillary column (30 m×0.32 mm, film thickness 0.5 μm, J&W Scientific, Folsom, CA, USA) was installed. Helium was used as the carrier gas at a flow-rate of 2.0 ml/min and a pressure of 40 kPa.

For quantitative analysis by selective ion monitoring (SIM) for acrolein and its saturated form, propionaldehyde (internal standard), the mass spectrometers were set to monitor molecular ions at m/z 56.05 and 58.05.

The SPME holder for manual sampling, a 65-μm carbonwax–divinylbenzene fiber was purchased from Supelco (Bellefonte, PA, USA).

2.4. SPME method

The spiked phosphate-buffered saline or human urine was transferred to a glass vial (10-ml volume) and tightly sealed with a butyl rubber septum and an aluminum cap. The vial was heated in order to vaporize acrolein and propionaldehyde. The SPME fiber was exposed to the headspace and then inserted into the GC injector port for thermal desorption of the extracted analytes in splitless mode (0.5-min splitless time). Thereafter we changed to the split mode. The splitting ratio was 1:10.

3. Results

Acrolein was eluted on a gas chromatogram for 1.4–1.45 min and propionaldehyde was eluted for 1.25–1.3 min (column temperature at 70°C) (Fig. 1 10 n*M* acrolein and 10 n*M* propionaldehyde). These two aldehydes were clearly differentiated by their molecular ions at m/z 56.05 and 58.05.

3.1. Optimization of the headspace SPME procedure in phosphate-buffered saline

In order to develop a headspace SPME method for analysis of acrolein, several parameters such as extraction temperature, extraction time, desorption time, GC injector temperature, and column temperature were optimized in repeated assays.

3.2. Extraction temperature

An extraction temperature of 35°C showed the highest acrolein concentration. Temperature >35°C did not have higher concentrations (Fig. 2).

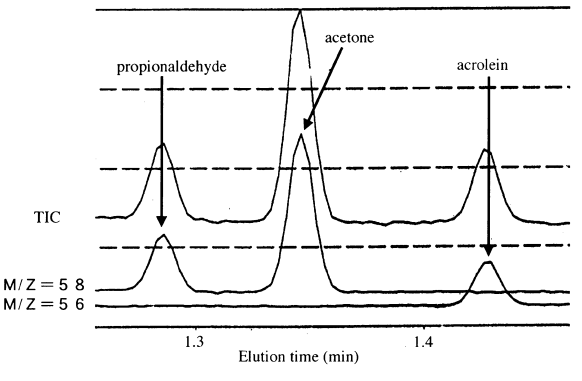

Fig. 1. SIM chromatogram for acrolein (10 n*M*) and propionaldehyde (10 n*M*).

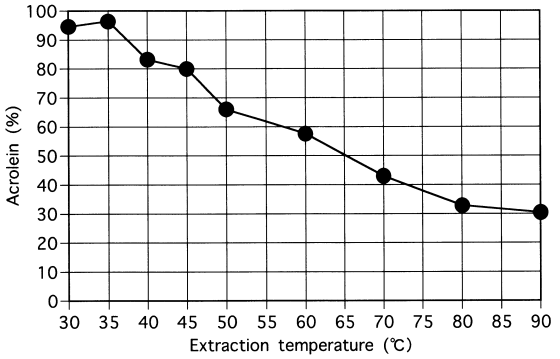

Fig. 2. Effect of extraction temperature.

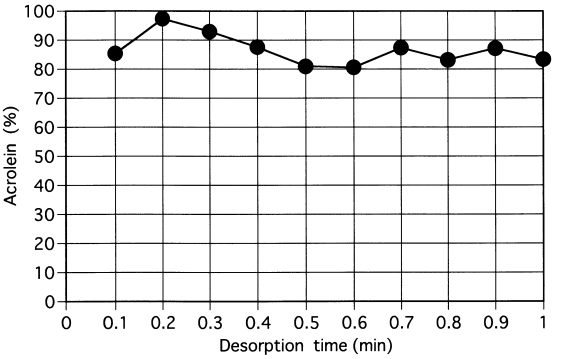

Fig. 4. Effect of desorption time.

3.3. Extraction time

It was found that 15 and 30 s were too short to extract acrolein sufficiently and longer extraction times above 45 s did not have any additional effect (Fig. 3).

3.4. Desorption time

Desorption times (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 min) were tested. The most appropriate was 0.2 min (Fig. 4).

3.5. GC injector temperature

Higher acrolein concentrations were obtained as the temperature rose (Fig. 5), but the peak became wider above 200°C. Therefore, we judged that the most appropriate temperature was 150°C.

3.6. Column temperature

A column temperature of 120°C had a short elution time (Fig. 6), but acrolein and propionaldehyde eluted very closely, and the acetone peak interfered with the peaks of the analytes. A column temperature of 50°C had a very long elution time. Therefore, we chose 70°C.

3.7. Quantitation of acrolein in phosphate-buffered saline

The precision of the method was calculated in intra- and inter-day studies. The relative standard deviation (RSD) values at two different concentrations are shown in Table 1. The intra-day RSDs were 5.79% (10 n*M*) and 6.60% (1000 n*M*). The inter-day RSDs were 10.07% (10 n*M*) and 4.92%

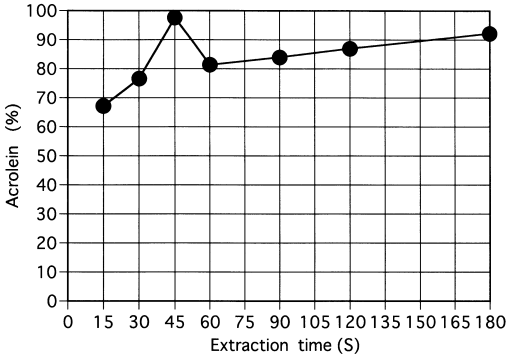

Fig. 3. Effect of extraction time.

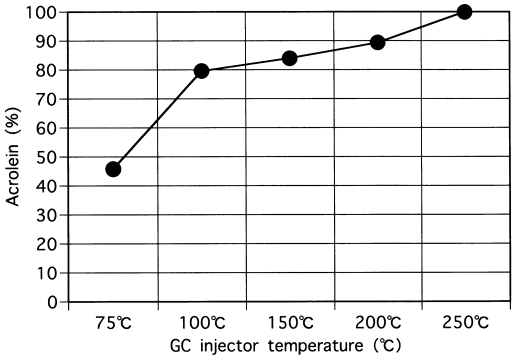

Fig. 5. Effect of GC injector temperature.

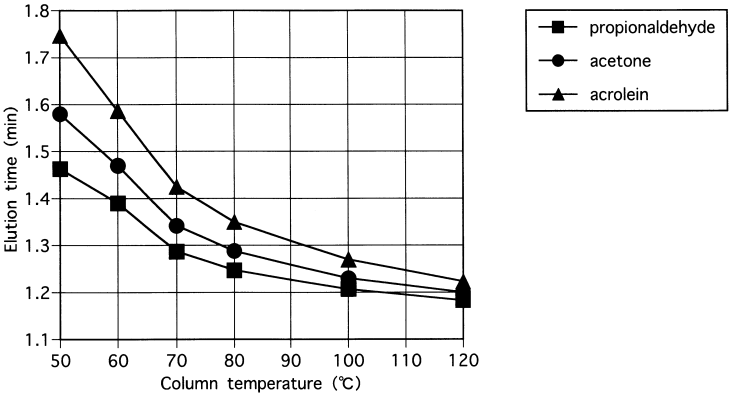

Fig. 6. Effect of column temperature on elution time.

(1000 n*M*). The linearity was evaluated by plotting the calibration curves of the area relative to the propionaldehyde ($A_{acr.}/A_{p.a.}$) versus the concentration of each analyte. The calibration curve demonstrated good linearity throughout concentrations ranging from 1 to 10 000 n*M* (correlation coefficient=0.9995) (Fig. 7). The limit of detection was defined as the concentration of an analyte that produced a signal three times greater than the baseline noise. The limit of detection was 1 nM.

3.8. Quantitation of acrolein in human urine

The precision of the method was calculated in intra- and inter-day studies from the same urine specimen. The RSD values at two different concentrations are shown in Table 1. The intra-day RSDs were 9.44% (10 n*M*) and 9.92% (1000 n*M*). The inter-day RSDs were 14.89% (10 n*M*) and 7.10% (1000 n*M*). Table 2 shows quantitation of acrolein in human urine from different ten healthy children. The calibration curve demonstrated good linearity throughout concentrations ranging from 1 to 10 000 n*M* in all cases. The limit of detection was 1 n*M* in all cases. A typical SIM chromatogram of 1 n*M* acrolein is shown in Fig. 8. The extraction recoveries of acrolein from human urine were calcu-

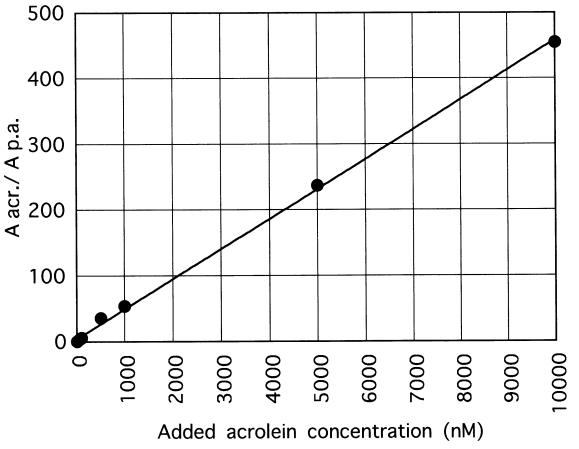

Fig. 7. Quantitation of acrolein in phosphate-buffered saline.

Table 1
Precision data

	Acrolein (n*M*)	Intra-day RDS (%) (*N*=5)	Inter-day RDS (%) (*N*=5)
Phosphate-buffered saline	10	5.79	10.07
	1000	6.60	4.92
Urine	10	9.44	14.89
	1000	9.92	7.10

Table 2
Quantitation of acrolein in human urine

Urine sample	Range of linearity (n*M*)		Correlation coefficient	LOD (n*M*)
1	1–10 000	$y = 0.0387x + 2.280$	0.9998	1
2	1–10 000	$y = 0.0589x + 2.725$	0.9999	1
3	1–10 000	$y = 0.0725x + 4.009$	0.9998	1
4	1–10 000	$y = 0.0650x - 0.927$	0.9999	1
5	1–10 000	$y = 0.0485x + 2.241$	0.9998	1
6	1–10 000	$y = 0.0524x + 3.026$	0.9997	1
7	1–10 000	$y = 0.0397x - 0.366$	0.9999	1
8	1–10 000	$y = 0.0464x + 2.864$	0.9998	1
9	1–10 000	$y = 0.0448x + 3.531$	0.9998	1
10	1–10 000	$y = 0.0490x - 1.456$	0.9998	1

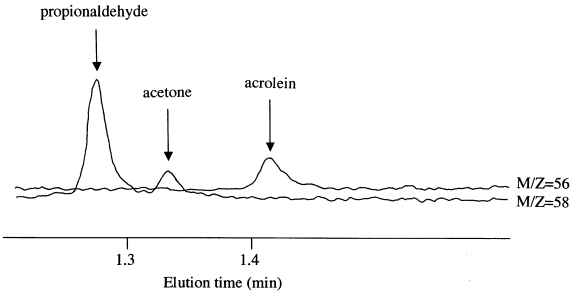

Fig. 8. SIM chromatogram (1 n*M* acrolein and 10 n*M* propionaldehyde).

lated from comparison with the peak area relative to the propionaldehyde and those from phosphate-buffered saline. The recovery rates of 100 n*M* acrolein were from 50.5 to 160.2% and those of 10 000 n*M* acrolein were from 85.6 to 160.2% (Table 3). There were differences in the evaporation rate of acrolein between different urine specimens.

Table 3
Recovery rate from different urine specimens

Urine sample	Recovery rate (%) (acrolein: 100 n*M*)	Recovery rate (%) (acrolein: 10 000 n*M*)
1	88.7	85.6
2	119.9	129.9
3	160.2	160.2
4	119.7	142.9
5	87.8	107.0
6	93.5	115.8
7	50.5	87.2
8	101.3	102.4
9	106.6	99.2
10	80.2	107.5

4. Discussion

The present study has shown that the optimized headspace SPME method is suitable for monitoring acrolein excretion. Our previous headspace method required a 100-μl aliquot of headspace air, which damaged the column coating and filament. However, the SPME method completely eliminated these problems, since the SPME fiber absorbed only the detected substances.

The calibration curve demonstrated good linearity throughout concentrations ranging from 1 to 10 000 n*M*. The headspace SPME method had high sensitivity with a detection limit of 1 n*M*. These advantages are similar to the headspace GC–MS method. There were differences in the evaporation rate of acrolein between different urine specimens. These were considered to be derived from the differences in the urinary contents of chloride or other substances [5].

We previously reported that acrolein in human urine was stable for only 30 min at 4°C and the stability in phosphate-buffered saline at 4°C was at least 2 h [5]. However, this instability of acrolein in human urine does not make no problems, since acrolein must be assayed urgently to prevent urotoxicity.

The optimum conditions of SPME for the determination of acrolein were an extraction temperature at 35°C, 45-s extraction time, 0.2-min desorption time, injector temperature at 150°C, and column temperature at 70°C. The most appropriate incubation temperature was 35°C. The boiling point of acrolein is 52.5°C. It is suggested that at high

temperature, acrolein is desorped from SPME fibers after adsorption [10].

In the previous headspace GC–MS method, we used a DB-1 capillary column which needs a high column pressure (230 kPa) to keep the analysis time short [5]. However, the high column pressure was over the endurance level of the SPME fiber assembly, so we used a DB-WAX capillary column instead of the DB-1 column. With a DB-WAX column which needs a lower column pressure (40 kPa), the elution time was half that with the DB-1 column. Although the SPME method needs an extraction period, the total analysis time is the same due to a shorter elution time.

SPME has several disadvantages. First, the high column pressure is over the endurance level of the SPME fiber. We solved this problem by using another type of column. Second, it is difficult to insert the SPME fiber into the GC injector port since the needle point of the SPME fiber is an HPLC type. Third, the SPME fiber is expensive and damaged easily.

5. Conclusion

Monitoring of acrolein excretion is very important when acrolein toxicity is suspected. A headspace SPME method was used to measure urinary acrolein in place of our original headspace GC–MS method, and the deficiencies of that headspace method were overcome. The total analysis time is only about 15 min, and it demonstrated high sensitivity, similar to the headspace GC–MS method. We conclude that this method is useful for the determination of urinary acrolein.

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