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Journal of Chromatography B, 719 (1998) 209–212

JOURNAL OF CHROMATOGRAPHY B

Short communication

Determination of acrolein in human urine by headspace gas chromatography and mass spectrometry

Nobuo Sakura[a,*], Shin-ichiro Nishimura[a], Naoto Fujita[a], Akira Namera[b], Mikio Yashiki[b], Tohru Kojima[b]

[a]*Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima, 734-8551 Japan*
[b]*Department of Legal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima, 734-8551 Japan*

Received 15 June 1998; received in revised form 8 September 1998; accepted 8 September 1998

Abstract

A rapid and sensitive headspace gas chromatographic and mass spectrometric (GC–MS) method was developed for the determination of acrolein in human urine. A 0.5-ml urine sample in a glass vial containing propionaldehyde as an internal standard was heated at 80°C for 5 min. A 0.1-ml volume of headspace vapor was injected into a GC–MS instrument. Acrolein and propionaldehyde were coeluted at 3.1 min using a DB-1 capillary column, and well separated by selective ion monitoring (SIM) mode using ions m/z 56.05 and m/z 58.05. The interassay and intraassay coefficient of variation were 0.99% and 3.3%. The calibration curve demonstrated a good linearity throughout concentrations ranging from 1 to 1000 n*M*. However, due to a wide variation of acrolein evaporation rates from human urine, a calibration curve must be established for each urine specimen using a standard addition method and detection limit varied from 1 to 5 n*M*. The total analysis time for two samples from one urine specimen required about 15 min. Therefore, this method is convenient for the urgent monitoring of urinary acrolein in patients to whom alkylating agents are administered.

Keywords: Acrolein

1. Introduction

Acrolein (pro-1-en-3-one, CH_2=CHCHO) is an irritant of the mucous membrane 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 tried by scavenger (MESNA) or by large volume of fluid when these agents are administered in large doses [2]. However, the pharmacokinetics of acrolein have not been clarified and therefore the current preventive methods are insufficient for acrolein toxicity.

Acrolein in biological samples has generally been measured by using colorimetric [3,4], fluorometric [5], high-performance liquid chromatographic [6,7] or gas chromatographic [8] methods. These methods are so time-consuming that it is not possible to

*Corresponding author. Tel.: +81-82-257-5212; fax: +81-82-257-5214.

0378-4347/98/$19.00
PII: S0378-4347(98)00422-8

prevent urotoxicity of acrolein because emergent prophylaxis must be required.

We have developed a clinical useful method to assay the urinary concentration of acrolein. This paper deals with a sensitive, selective and rapid methods using the headspace technique for gas chromatography–mass spectrometry (GC–MS).

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 high purity grade.

2.2. Preparation of standard acrolein solutions

Normal human urine, 0.4 ml, which was acidified (pH 2–4) with 2 *N* H_2SO_4, was spiked with 50 μl of 1000 n*M* propionaldehyde (internal standard) and 50 μl of various concentrations (1–1000 n*M*) of acrolein solution. Prior to assay, the urine was stored at 4°C. Standard solutions of aldehydes were so unstable that they were freshly prepared. In order to obtain the interassay coefficient of variation, the stock urine sample was stored in a freezer until it was spiked.

Acrolein standard, used to analyze the recovery rate, was prepared in phosphate-buffered saline (pH 4).

2.3. Headspace method

The spiked urine or phosphate-buffered saline was transferred to a glass vial (10-ml volume) and tightly sealed with a butyl rubber septum and an aluminium cap. The vial was heated at 80°C for 5 min in order to vaporize acrolein and propionaldehyde maximally. A 100-μl aliquot of the headspace was aspirated with a gas-tight syringe and injected into the GC–MS.

2.4. GC–MS

A Shimadzu QP5000 gas chromatograph–mass spectrometer (Kyoto, Japan) with electron-impact ionization detector was used. The gas chromatograph was set up as follows: a DB-1 capillary column (60 m×0.25 mm, film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA), column temperature, 70°C; injector and interface temperatures, 200°C and 250°C; carrier gas (helium) flow-rate, 1.7 ml/min; split rate, 1:10.

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

3. Results and discussion

3.1. Assay conditions for headspace method

Maximal evaporation of acrolein was obtained with an incubation temperature of 80°C, and a heating period of 5 min. Prolonged heating markedly decreased acrolein evaporation.

Acrolein and propionaldehyde were coeluted on a gas chromatogram at 3.1 min (Fig. 1), and these two aldehydes were clearly differentiated by their molecular ions at *m/z* 56.05 and 58.05. Propionaldehyde seems to be suitable for an authentic compound.

A headspace-GC–MS method is rapid and sensitive. It is extremely useful when urgent determination of acrolein is required during administration of large doses of alkylating agents. No specific sample manipulations are necessary and total analysis time is only about 10 min.

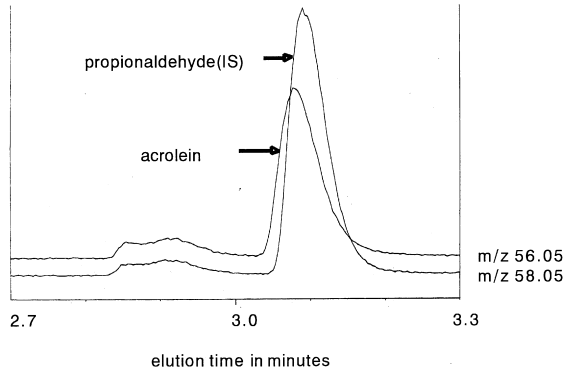

Fig. 1. SIM chromatogram for acrolein (*m/z* 56.05) and propionaldehyde (IS) (*m/z* 58.05).

3.2. Quantitation of acrolein in human urine

The interassay and intraassay coefficients of variation for 100 n*M* acrolein were 0.99% and 3.3%. The same evaporation rates were obtained with varying urine volumes from 0.5 ml to 1.0 ml, although results using 0.25 ml of urine were not consistent.

Evaporation of acrolein from the same urine specimen spiked with aldehydes correlated well over a wide range of concentrations (1~1000 n*M*) (Table 1). However, the evaporation rate varied among different urine specimens, and the rates at 100 n*M* acrolein ranged from 87.9 to 7.1%, compared to the evaporation from phosphate-buffered saline. This variation is suspected to be derived from the differences in the urinary contents of chloride [9] or other substances.

This problem of quantitation was overcome by using a combination of the internal standard technique and the standard addition technique. In addition to the internal standard (50 μl of 1000 n*M* propionaldehyde), 50 μl of 100 n*M* acrolein was added to another vial. The quantitative precision was determined by the difference in acrolein evaporation rates between one specimen spiked with only internal standard and the other containing internal standard and acrolein. Dual assay takes a little more time (about 15 min), since the preheating procedure can be undertaken for the foregoing assay period for GC–MS.

In previous chromatographic methods, however, the derivatization and extraction procedures are necessary and total analysis time is greater than 30 min [4,6,7,8]. Their detection limits vary from 4 μg/l to 1 mg/l [6–8], but our proposed method shows high sensitivity with a detection limit of 1 to 5 n*M* (56 to 280 ng/l).

Table 1
Differences in the characteristics of the quantitation

Urine sample	Range of linearity (n*M*)	Correlation coefficient	Recovery rate (%)	Detection limit (n*M*)
1	1000–5	0.907	54.6	5
2	1000–1	0.968	70.9	1
3	1000–3	0.986	45.0	3
4	1000–1	0.989	7.1	1
5	1000–5	0.942	77.5	5
6	1000–5	0.904	87.9	5
7	1000–1	0.962	84.2	1
8	1000–4	0.935	34.6	4
9	1000–1	0.997	28.3	1
10	1000–5	0.950	53.0	5

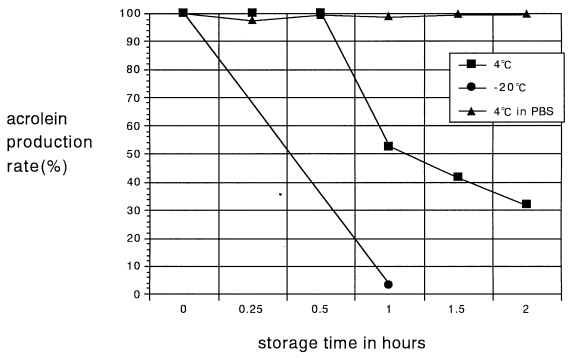

Fig. 2. Stability of acrolein in urine or phosphate-buffered saline.

3.3. Stability of acrolein in human urine

Although stability was observed in phosphate-buffered saline for at least 2 h at 4°C, acrolein in human urine was stable for only 30 min at 4°C. The rate of evaporation decreased from the time of storage and markedly decreased at −20°C (Fig. 2). However, this instability of acrolein does not make this method inconvenient, since urinary acrolein must be urgently assayed for the prophylaxis of urotoxicity.

3.4. Clinical application

Urinary concentrations of acrolein were determined in three patients with solid tumors when they were treated by a large dose of cyclophosphamide. Urinary excretion of acrolein reached a maximum point at the end of the transfusion of the agent and then gradually decreased (Fig. 3). The highest level of acrolein was 16.5 n*M* but the patients showed no bladder troubles [10]. The data from the therapeutic monitoring for acrolein will be accumulated and the toxic level of acrolein will be clarified in the near future.

4. Conclusion

A headspace determination of acrolein in human urine was developed. This method is advantageous,

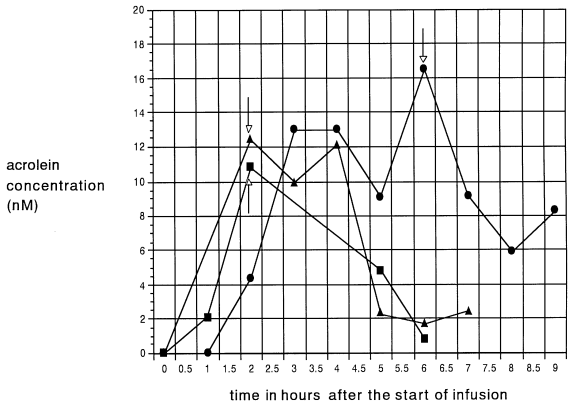

Fig. 3. Acrolein excretion in the urine of patients administered with cyclophosphamide. Patient 1 (■) and 2 (▲) had neuroblastoma and were treated intravenously with cyclophosphamide 1000 mg/2 h and 280 mg/2 h, respectively. Patient 3 (●) had Ewing sarcoma. The patient was intravenously administered with cyclophosphamide, 2700 mg/6 h, and his bladder was syringed with a large volume of saline for the prevention of hemorrhagic cystitis. Arrows indicate the end of infusion of cyclophosphamide.

convenient and time-saving. It is suitable for monitoring patients in which acrolein toxicity is suspected.

References

[1] P.J. Cox, Biochem. Pharmacol. 28 (1979) 2045.
[2] C. Bokemeyer, H.J. Schmoll, E. Ludwigh, A. Harstrick, T. Dunn, J. Casper, Br. J. Cancer. 69 (1994) 863–867.
[3] J.M. Patel, J.C. Wood, K.C. Leibman, Drug Metab. Dispos. 8 (1980) 305–308.
[4] P.J. Boor, G.A.S. Ansari, J. Chromatogr. 375 (1986) 159–164.
[5] Y. Suzuki, S. Imai, A. Hamaguchi, J. Jpn. Soc. Air Pollut. 14 (1979) 151.
[6] S. al Rawithi, A. el Yazigi, P.J. Nicholls, Pharm. Res. 10 (1993) 1587–1590.
[7] F. Bohnenstengel, M. Eichelbaum, E. Golbs, H.K. Kroemer, J. Chromatogr. B 692 (1997) 163–168.
[8] H. Nishikaw, T. Hayakawa, T. Sakai, Analyst 112 (1987) 45–48.
[9] Method 603 for acrolein and acrylonitrile, Federal Register 49 (1984) 49–57.
[10] T. Wagner, D. Heydrich, T. Jork, G. Voelcker, H.J. Hohorst, J. Cancer Res. Clin. Oncol. 100 (1981) 95–104.