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A sensitive gas chromatographic–tandem mass spectrometric method for detection of alkylating agents in water: Application to acrylamide in drinking water, coffee and snuff

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A sensitive analytical method for the analysis of acrylamide and other electrophilic agents in water has been developed. The amino acid L-valine served as a nucleophilic trapping agent. The method was applied to the analysis of acrylamide in 0.2–1 mL samples of drinking water or Millipore-filtered water, brewed coffee, or water extracts of snuff. The reaction product, *N*-(2-carbamoylethyl)valine, was incubated with pentafluorophenyl isothiocyanate to give a pentafluorophenylthiohydantoin (PFPTH) derivative. This derivative was extracted with diethyl ether, separated from excess reagent and impurities by a simple extraction procedure, and analyzed by gas chromatography-tandem mass spectrometry. ($^2H_3$)Acrylamide, added before the reaction with L-valine, was used as internal standard. Acrylamide and the related compound, *N*-methylolacrylamide, gave the same PFPTH derivative. The concentrations of acrylamides were $\leq$0.4 nmol L^{-1} ($\leq$0.03 μg acrylamide L^{-1}) in water, 200 to 350 nmol L^{-1} in brewed coffee, and 10 to 34 nmol g^{-1} snuff in portion bags, respectively. The precision (the coefficient of variation was 5%) and accuracy of the method were good. The detection limit was considerably lower than that of previously published methods for the analysis of acrylamide.

1 Introduction

Acrylamide is an important industrial chemical. It is produced from acrylonitrile and used mainly for the production of polyacrylamides.[1] The polymers are used for purification of waste water and drinking water and in the petroleum and paper industry. Polyacrylamides are formulated in cosmetics and soap preparations as thickeners and in dental fixtures, hair grooming preparations, and preshave lotions. The acrylamide monomer is used in research laboratories for the preparation of polyacrylamide gels for electrophoresis. Acrylamide is also a component of tobacco smoke[2] and was recently shown to be formed during heating of carbohydrate-rich foods, such as potato and potato products, beetroot, crispbread, and tea.[3–5]

Acrylamide is neurotoxic in animals and man[1] and causes carcinogenic, genotoxic, and reproductive toxicity effects in experimental animals.[6,7] Based on evidence from animal studies, acrylamide has been classified by the IARC as a probable human carcinogen.[6]

The WHO guideline value for acrylamide in drinking-water is 0.5 μg L^{-1} corresponding to a daily intake of about 1 μg of the compound.[8] The National Food Administration in Sweden has estimated that the average intake of acrylamide through food is about 40 μg per day, far exceeding the WHO guideline value for drinking water.

Acrylamide in water extracts has been measured directly by HPLC (high pressure liquid chromatography) with detection by UV[9,10] or mass spectrometry.[3,4,11,12] Alternatively, acrylamide has been assessed after conversion to its 2,3-dibromopropionamide derivative followed by HPLC with UV detection[13] or gas chromatography (GC) with either electron capture[11,14,15] or tandem mass spectrometry detection (MS-MS).[3] Detection limits of 0.2 μg L^{-1} and 12 μg L^{-1}, respectively, have been reported for LC-MS-MS analysis of standard solutions of acrylamide in water and for GC-MS analysis of the 2,3-dibromopropionamide derivative.[11] Sörgel *et al.*[12] have developed an LC-MS-MS assay suitable for analyzing acrylamide concentrations of 1 μg L^{-1} in urine, 5 μg L^{-1} in breast milk and 2 μg L^{-1} in placental perfusate.

The aim of the present study was to develop a more sensitive analytical method that could be used for the analysis of acrylamide in water, food, other consumer goods, and in tissue samples. The method was based on the use of the amino acid L-valine as a nucleophilic trapping agent followed by derivatization to give a pentafluorophenylthiohydantoin derivative. This derivative was extracted, separated from excess reagent and impurities by a simple extraction procedure, and analyzed by GC-MS-MS.

2 Materials and methods

2.1 Chemicals

Pentafluorophenyl isothiocyanate (PFPITC, 97 % purum) was purchased from Fluka (Buchs, Switzerland) and acrylamide (99%), *N*-methylolacrylamide and L-valine (99%) were from Aldrich-Chemie (Steinheim, Germany). ($^2H_3$)Acrylamide (95% ^2H) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, USA). Other chemicals used were of analytical grade. PFPITC was purified on a Sep-Pak silica cartridge (Waters Co, Milford, MA) according to Törnqvist *et al.*[16] All glassware was silanized with dichlorodimethylsilane (Fluka, Buchs, Switzerland) as described by Hindsø Landin *et al.*[17]

Due to toxicity of several of the chemicals used for this study, all work was carried out in a hood.

2.2 Reaction rate study

Two hundred μL of a solution of acrylamide in water (0.053 M) was added to 3 mL of L-valine (0.2 M) (final concentration of L-

valine was 0.19 M), adjusted with sodium hydroxide to pH ~ 11. The solution was transferred to a 1 cm quartz cuvette covered with a cap. The UV absorption at 255 nm was recorded at intervals during 5 h. The temperature was maintained at approximately 35 °C (experiment 1) or 37 °C (experiment 2) by a heating coil.

2.3 Preparation of samples

Calibration samples with acrylamide concentrations in the range 0–1400 nmol L^{-1} (0–100 μg L^{-1}) were prepared using ordinary tap water and Millipore filtered water, respectively. Brewed coffee was prepared in a drip coffee maker with a paper filter (6.4 g of coffee grinds, average roast, per 150 mL of tap water). One portion paper bags with snuff (0.5 g or 1 g) were extracted with 10 mL of water. Five coded samples were obtained from the SCC Miljölaboratoriet (Malmö, Sweden) containing acrylamide and *N*-metylolacrylamide in the concentration range 0–104 nmol L^{-1} (0–7.43 μg L^{-1}) acrylamide and 0–68.6 nmol L^{-1} (0–6.94 μg L^{-1}) *N*-metylolacrylamide, respectively, in tap water.

2.4 Derivatization

One mL samples of water and 200 μL samples of brewed coffee or extracts of snuff were used for the analyses. ($^2H_3$)Acrylamide, 15 pmol (1.07 ng) or 60 pmol (4.26 ng) per sample, respectively, was added as internal standard. A solution containing 0.5 M L-valine and 0.5 M sodium hydroxide (or 0.5 M triethylamine) in water (pH about 11) was added to the samples to give a final concentration of 0.1 M valine (samples of pure water) or 0.25 M valine (samples of coffee or snuff). The samples were incubated at 37 °C for 30 h in teflon capped silanized 10 ml glass tubes. n-Propanol, 0.5 mL per mL water solution, and PFPITC, 33 μL (150 μmol), were added. The samples were shaken at room temperature over night followed by incubation at 45 °C for 90 min. The pentafluorophenylthiohydantoin (PFPTH) derivatives were extracted with diethyl ether (3 × 3 mL). The ether was evaporated under a gentle stream of nitrogen and the samples were dissolved in 2 mL of methanol/water (1:1) and extracted with 2 mL of hexane. Hexane was discarded and the PFPTH derivatives were extracted with 2 mL of toluene. The toluene phase was washed with 0.1 M sodium carbonate (2 × 1 mL) and water (2 × 1 mL) and then evaporated. The samples were reconstituted in 50 μL of toluene and stored at −20 °C until analysis. *N*-methylolacrylamide in water, 32 or 106 nmol L^{-1} (3.24 or 10.5 μg L^{-1}), was processed in the same way.

2.5 GC-MS-MS analyses

Analyses of the PFPTH derivatives were carried out using a Finnigan TSQ-700 instrument in the negative ion chemical ionization mode (NCI). One μL samples were injected on a 30 m DB5-MS (0.32 mm id, 1 μm phase thickness) fused silica capillary column (J & W Scientific, Folsom, CA, USA); retention gap, methyl deactivated fused silica (2 m, 0.53 mm id; Chrompack Inc., Raritan, NJ, USA); injector temperature programming 100–320 °C, 157 °C min^{-1} for 1.4 min, and then isothermal at 320 °C for 16 min; GC temperature programming, 100 °C for 1 min, 20 °C min^{-1} to 240 °C, 10 °C min^{-1} to 320 °C and finally isothermal at 320 °C for 5 min. Helium was used as carrier gas at a constant pressure of 5.6 psi (head pressure). The operating procedures for the mass spectrometer were: methane as reagent gas at 4.8 torr (640 Pa); ion source temperature, 120 °C and ionization energy, 70 eV. Argon was used as collision gas at a pressure of 1 mtorr. The retention times were about 13 min 54 s for the analyte and 13 min 53 s for the deuterated internal standard, respectively. The following fragment ions were selected for monitoring (precursor ions given in parenthesis): *m*/*z* 303, 304 and 319 (*m*/*z* 375) for the analyte and *m*/*z* 303, 304 and 319 (*m*/*z* 378) for the internal standard. The precursor ions *m*/*z* 375 and *m*/*z* 378 represent loss of HF from the PFPTH derivatives ($[M - 20]^-$). The quantifications were based on the peak area of the analyte compared to that of the deuterated internal standard.

The possible contribution of the internal standard precursor ion *m*/*z* 378 to the daughter ions of *m*/*z* 375 was studied. No trace of daughter ions of *m*/*z* 375 was observed in samples of the PFPTH derivative of synthetic *N*-(2-carbamoyl-($^2H_3$)ethyl)-valine (12 pmol of the derivative was used in these studies[18]).

3 Results

The reaction of acrylamide with L-valine at pH 11 was followed by measurements of the UV absorbance at 255 nm. The concentration of acrylamide decreased exponentially with time. The half-lives ($t_{1/2}$) in 0.19 M L-valine, the pseudo-first order and the second order rate constants, k' and k_{Val}, for the reaction were determined as 2.2 h, 0.31 h^{-1} and 1.7 $M^{-1} \times h^{-1}$, respectively, at 35 °C, and 1.5 h, 0.46 h^{-1} and 2.4 $M^{-1} \times h^{-1}$ at 37 °C.

The derivatization of acrylamide, including the reaction with valine to form *N*-(2-carbamoylethyl)valine and the subsequent reaction with PFPITC to form the corresponding PFPTH derivative is shown in Fig. 1. ($^2H_3$)Acrylamide, which corrects for yield in all reaction steps, was added as an internal standard. The choice of conditions for the reaction of acrylamide with L-valine in water (30 h, 37 °C and 0.1–0.25 M valine) was based on the reaction kinetic data (30 h corresponds to about 10 half-lives in 0.1 M valine). After completed reaction with L-valine, n-propanol and PFPITC were added for preparation of the PFPTH derivatives of normal and deuterated *N*-(2-carbamoylethyl)valine. The characterization and quantification by GC-MS-MS of these derivatives have been described previously.[2,18]

The calibration curves were linear in the range of concentrations tested and the regression coefficients (R^2) were > 0.999.

The method did not distinguish between acrylamide and *N*-methylolacrylamide. Both compounds gave the same PFPTH derivative. The conversion, through loss of formaldehyde, from methylolacrylamide itself, its adducts with L-valine or the PFPTH derivative, was complete (34 and 107 pmol *N*-(2-carbamoylethyl)valine were recorded in samples spiked with 32 and 106 pmol *N*-methylolacrylamide, respectively). Application of the method to the 5 coded water samples containing acrylamide and *N*-methylolacrylamide gave the following result [measured (theoretical) concentration]: 1.4 (0); 47 (46); 41 (39); 145 (138) and 174 (161) nmol L^{-1} water, respectively.

The concentrations of acrylamide (acrylamide equivalents) in drinking water, coffee and snuff are shown in Table 1. Fig. 2a and b show representative chromatograms of the GC-MS-MS analyses of acrylamide in tap water and in coffee, respectively.

4 Discussion

Acrylamide is an α,β-unsaturated carbonyl compound. Nucleophilic groups, such as thiols and amines, react with acrylamide through addition to the double bond. Our method for the analysis of acrylamide was based on the use of L-valine, at pH around 11, as a nucleophilic trapping agent. The solubility in water of this amino acid and its reactivity towards acrylamide

are sufficiently high. Valylglycylglycine was also useful as a trapping agent (data not shown). Because of the low pK_a of N-terminal valine of the peptide, the reaction was carried out in neutral solution.

The method then takes advantage of the favorable features of the analytical method previously used for the analysis of adducts of alkylating agents with N-terminal valine in hemoglobin.[2,16,18] The PFPTH derivatives of *N*-(2-carbamoylethyl)-valine were prepared in water/n-propanol and were then separated from excess reagents and impurities by a simple extraction procedure.

4.1 Precision, accuracy and specificity

The precision of the method was estimated to be 5% using an ANOVA single factor test of the result of duplicate assays of standards (n = 5) and triplicate assays of samples of coffee (n = 3) and extracts of snuff (n = 2). The accuracy of the method was demonstrated by deviations of less than ±7% from the theoretical concentrations in the analysis of 5 coded samples containing mixtures of acrylamide and *N*-methylolacrylamide in water.

The practical limit of detection of acrylamide in water extracts was set by the concentration in the water used for extraction (in the present experiments ≤0.4 nmol (≤0.03 μg L^{-1}). As shown by the chromatogram of water (Fig. 2a) the limit of detection may be estimated as about 0.04 nmol acrylamide L^{-1} (~0.003 μg L^{-1}). Thus, the detection limit is at least 10 times lower than that of previously published methods for the analysis of acrylamide in food or in body fluids.

Recently, a similar approach was used to measure styrene 7,8-oxide in pentane extracts of blood from reinforced plastics workers exposed to styrene.[19] The detection limit of the assay was 0.2 nmol (0.025 μg) styrene oxide L^{-1} blood.

The present study is of pilot character. Although validation work is required for specific applications of the method, its high sensitivity would certainly be useful, for example, in studies of acrylamide in body fluids. Acrylamide and the less toxic compound, *N*-methylolacrylamide, gave the same PFPTH derivative. This may be a drawback of the method.

4.2 Contribution of water, coffee and snuff to acrylamide exposure (Table 1)

In the Stockholm area, polyacrylamide flocculants are used in the treatment of municipal and industrial effluents but not in the treatment of potable water. Our results indicated trace amounts of the compound in samples of tap water and Millipore-filtered water of our laboratory −0.4 nmol (0.03 μg) per L being an upper estimate of the concentration. The possible contribution of the reagents used for derivatization to the amount of acrylamide found in water samples was not studied. An intake of about 0.06 μg acrylamide day^{-1} from drinking-water is acceptable according to the WHO guideline value (1 μg day^{-1}) and negligible compared to other sources of acrylamide exposure. Two types of Swedish snuff and three brands of average roast coffee were used for the measurements of acrylamide (possibly including *N*-methylolacrylamide) in snuff and coffee. Assuming that the concentrations of acrylamide(s) found in the samples are representative, the amount of acrylamide(s) in 20 g of snuff would be comparable to the average daily intake of acrylamide from food (40 μg). Also, a daily consumption of several cups of coffee would give a significant contribution to the intake of acrylamide(s).

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Table 1 Concentrations of acrylamide[a] in water, coffee, snuff

	Concentration/ nmol L^{-1} or nmol g^{-1}	Intake from 2 L drinking water, 4 cups of coffee (600 mL) or 20 g of snuff/nmoL (μg)[b]
Water	≤0.4	≤0.8 (≤0.06)
Coffee[c]	260, 280, 350	150–210 (11–15)
Snuff[d]	10, 34	200–680 (14–48)

[a] May include *N*-methylolacrylamide. [b] Recalculation of "nmol"to "μg"was based on the molecular weight of acrylamide. [c] Three brands of average-roast coffee. [d] Two types of moist Swedish snuff in portion bags.

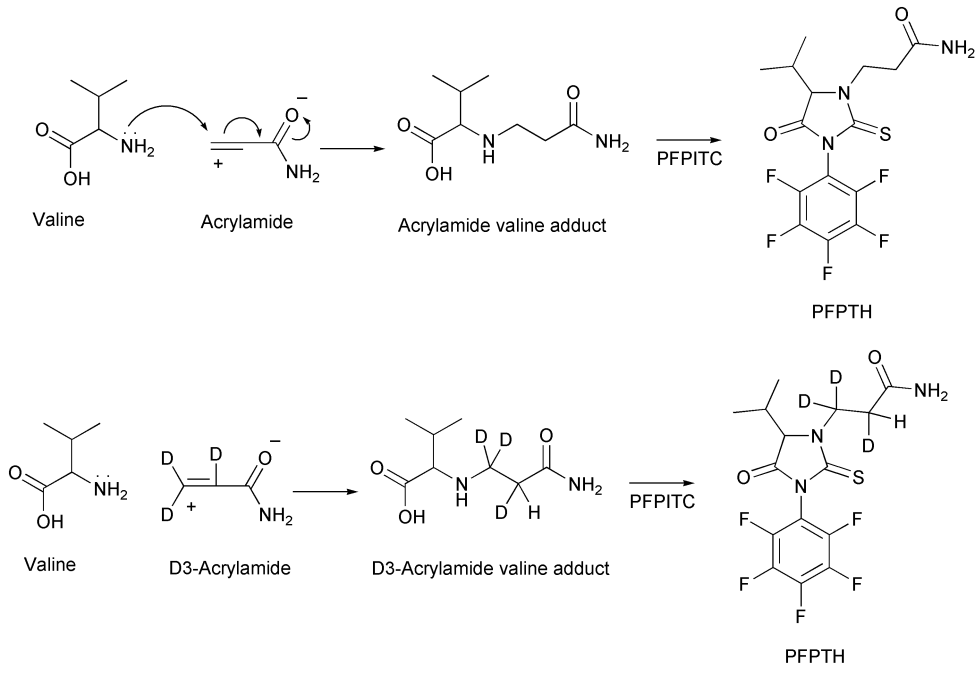

Fig. 1 Reaction scheme of formation of alkylvalines and their pentafluorophenylthiohydantoin derivatives from acrylamide and its deuterium-substituted analogue, respectively.

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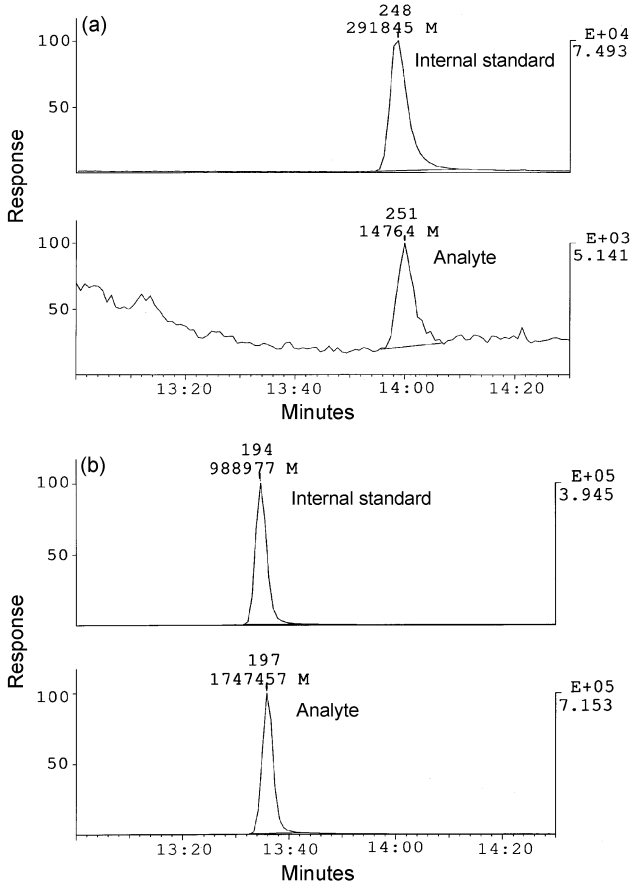

Fig. 2 Representative chromatograms of the GC-MS-MS analyses of acrylamide in (a) tap water and (b) coffee. (A time interval of more than one year between the two analyses explain the different retention times.)

6. References

1 E. A. Smith and F. W. Oehme, *Rev. Environ. Health*, 1991, **9**, 215.
2 E. Bergmark, *Chem. Res. Toxicol.*, 1997, **10**, 78.
3 E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson and M. Törnqvist, *Chem. Res. Toxicol.*, 2000, **13**, 517.
4 J. Rosen and K. E. Hellenäs, *Analyst*, 2002, **127**, 880.
5 S. Nemoto, S. Takatsuki, K. Sasaki and T. Maitani, *Shokuhin Eisegaku Zasshi*, 2002, **43**, 371.
6 *IARC Monograph*, 1994, **vol. 60**, p. 389.
7 K. L. Dearfield, G. R. Douglas, U. H. Ehling, M. M. Moore, G. A. Sega and D. J. Brusick, *Mutat. Res.*, 1995, **330**, 71.
8 *Guidelines for Drinking-Water Quality: Health Criteria and Other Supporting Information*, World Health Organization, Geneva, 2nd edn., 1996, **vol. 2.**, pp. 541.
9 E. R. Husser, R. H. Stehl, D. R. Price and R. A. DeLap, *Anal. Chem.*, 1977, **49**, 154.
10 D. S. Barber, J. Hunt, R. M. LoPachin and M. Ehrich, *J. Chromatog., B.*, 2001, **758**, 289.
11 H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaka, H. Kobayashi and M. Yoshida, *Food Addit. Contam.*, 2003, **20**, 215.
12 F. Sörgel, R. Weissenbacher, M. Kinzig-Schippers, A. Hofmann, M. Illauer, A. Skott and C. Landersdorfer, *Chemotherapy*, 2002, **48**, 267.
13 L. Brown and M. Rhead, *Analyst*, 1979, **104**, 391.
14 J. H. Raymer, C. M. Sparacino, G. R. Velez, S. Padilla, R. C. MacPhail and K. M. Crofton, *J. Chromatogr.*, 1993, **619**, 223.
15 C. F. Poole, W.-F. Sye, A. Zlatkis and P. S. Spencer, *J. Chromatogr.*, 1981, **217**, 239.
16 M. Törnqvist, A. Kautiainen, R. N. Gatz and L. Ehrenberg, *J. Appl. Toxicol.*, 1988, **8**, 159.
17 H. Hindsø Landin, S. Osterman-Golkar, V. Zorcec and M. Törnqvist, *Anal. Biochem.*, 1996, **240**, 1.
18 H. Licea Pérez, H.-K. Cheong, J. S. Yang and S. Osterman-Golkar, *Anal. Biochem.*, 1999, **274**, 59.
19 R. Tornero-Velez, S. Waidyanatha, H. Licea Pérez, S. Osterman-Golkar, D. Echeverria and S. M. Rappaport, *J. Chromatogr., B*, 2001, **757**, 59.