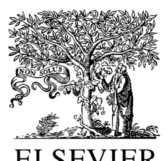

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Gas chromatography–negative ion chemical ionization mass spectrometry as a powerful tool for the detection of mercapturic acids and DNA and protein adducts as biomarkers of exposure to halogenated olefins

Wolfgang Völkel, Axel Pähler, Wolfgang Dekant*

Department of Toxicology, University of Würzburg, Versbacherstrasse 9, 97078 Würzburg, Germany

Abstract

The studies on metabolism of halogenated olefins presented here outline the advantages of modern mass spectrometry. The perchloroethene (PER) metabolite *N*-acetyl-*S*-(trichlorovinyl)-L-cysteine (*N*-ac-TCVC) is an important biomarker for the glutathione dependent biotransformation of PER. In urine of rats and humans exposed to PER, *N*-ac-TCVC was quantified as methyl ester after BF_3–MeOH derivatization by gas chromatography with chemical ionization and negative ion detection mass spectrometry (GC–NCI-MS). The detection limit was 10 fmol/μl injected solution using [$^2H_3$]*N*-ac-TCVC methyl ester as the stable isotope internal standard. Cleavage of *S*-(trichlorovinyl)-L-cysteine by β-lyase enzymes results in an electrophilic and highly reactive thioketene which reacts with nucleophilic groups in DNA and proteins. Protein adduct formation was shown in kidney mitochondria by identification of dichloroacetylated lysine after derivatization with 1,1,3,3-tetrafluoro-1,3-dichloroacetone by GC–NCI-MS. In addition, chlorothioketene was generated in organic solvents and reacted with cytosine to give N^4-chlorothioacetyl cytosine. After derivatization with pentafluorobenzyl bromide this compound exhibited good gas chromatographic properties and was detectable with a limit of detection of 50 fmol/injected volume. The detection of chemically induced protein modifications in the target organ of toxic metabolite formation and the study of DNA modifications with chemically generated metabolites provide important information on organ toxicity and possible tumorigenicity of halogenated olefins. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Mercapturic acids; DNA; Proteins; Olefins; Chloroethenes

1. Introduction

The determination of biomarkers such as urinary metabolites of particular xenobiotics or DNA and protein adducts are very important for the classification of chemicals in human risk assessment [1]. A main emphasis for these studies is therefore the development of simple and sensitive methods for the detection and quantitation of these biomarkers [2,3].

Halogenated olefins (tetrachloroethene, PER; trichloroethene, TRI) which are extensively used in industry as metal degreasing solvents and as dry cleaning agents are good examples to demonstrate applications of modern mass spectrometry (MS) for such problems. Long-term exposure of rodents to both PER and TRI has been shown to increase the incidence of liver tumors in male mice and of renal tumors in male rats [4,5]. The chronic toxicity of

*Corresponding author. Tel: +49-931-201-3449; fax: +49-931-201-3865.

E-mail address: dekant@toxi.uni-wuerzburg.de (W. Dekant)

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TRI and PER is most likely mediated by bioactivation reactions. Halogenated olefins such as PER and TRI are metabolized by both cytochrome P450 and glutathione dependent biotransformation pathways leading to the generation of reactive metabolites which may covalently bind to cellular macromolecules (Fig. 1). Cytochrome P450 oxidation of both PER and TRI results in formation of the corresponding chlorinated acetyl chlorides which react with amino groups in macromolecules or with water to give dichloro- or trichloroacetate [6,7]. In addition, the bioactivation by glutathione conjugation of PER and TRI and the cleavage of the gluathione conjugates to the cysteine conjugates *S*-(trichlorovinyl)-L-cysteine (TCVC) and *S*-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVC) by transpeptidases is likely responsible for the nephrotoxicity and possible renal tumorgenicity of these halogenated olefins [8]. The corresponding thioketenes formed by the β-lyase mediated cleavage of TCVC respectively 1,2-DCVC are presumed to be the ultimate metabolites responsible for the mutagenic and nephrotoxic effects [9–11].

The cysteine conjugates are transformed by *N*-acetyltransferases to mercapturic acids which are excreted with urine. Therefore, mercapturic acids and the thioketene adducts of proteins and DNA may represent important biomarkers of exposure, which have to be detected and quantified in biological samples. Nevertheless, both mercapturic acids and thioketene adducts of DNA and protein represent only minor metabolites and after exposure to workplace relevant concentrations of PER or TRI very low amounts of these biomarkers have to be detected.

Several methods may be used to quantify biomarkers of exposure ranging from high-performance liquid chromatography–UV absorbance detection (HPLC–UV) for urine metabolites to ^{32}P-postlabelling methods determining DNA adducts. However, these methods often have several disadvantages. HPLC–UV is not sufficiently sensitive for mercapturic acids without an efficient chromophore. ^{32}P-postlabelling methods to quantify DNA adducts are difficult to standardize because of many possible variations for optimal use [1].

For protein adduct detection immunochemical methods are often used, however, problems with

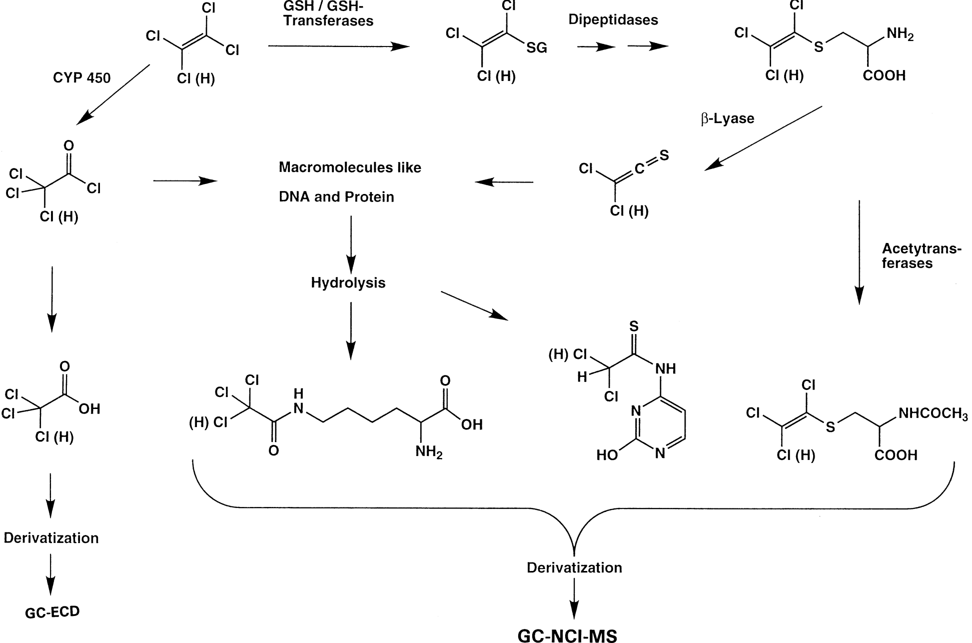


Fig. 1. Pathways for the formation of mercapturic acids, DNA and protein adducts in the metabolism of perchloroethene and trichloroethene. ECD=Electron-capture detection.

cross-reactivity and quantitation of adduct concentrations are limiting their usefulness [12,13].

The work presented here shows that three similar analytical methods using only one tool, gas chromatography–negative chemical ionization mass spectrometry (GC–NCI-MS), may be applied for detection and quantitation of the biomarkers.

2. Experimental

2.1. Reagents

Chlorodifluoroacetic acid was obtained from Fluorochem, Old Glossop, UK. Chloroacetic chloride, diazabicyclo[2.2.2.]octane, DNA bases, lysine, methanolic BF_3 (14% in MeOH), pentafluorobenzyl bromide, PER, TRI and all other chemicals were obtained from Sigma–Aldrich (Deisenhofen, Germany) in the highest purity available.

2.2. Synthesis

N^6-Chloroacetyl adenine, N^4-chloroacetyl cytosine and N^2-chloroacetyl guanine, potential DNA adducts formed from chloroketene were synthesized by the method of Müller et al. [14] and characterized by HPLC and their electronic spectra. N^4-Chlorothioacetyl cytosine was synthesized by the reaction of cytosine (36 μmol) with a mixture of both isomers of *S*-(1,2-dichlorovinyl)thioacetate (23 μmol) in dimethylformamide (DMF)–ethyl acetate [15]. Chlorothioacetyl cytosine was purified by semipreparative HPLC to yield a purity >95%. *N*-Acetyl-*S*-(trichlorovinyl)-L-cysteine (*N*-Ac-TCVC) and [$^2H_3$]*N*-ac-TCVC as internal standard were synthesized as described [16,17]. Purity of both compounds was >98% as checked by HPLC. N^ϵ-(Dichloroacetyl)-L-lysine and N^ϵ-(trifluoroacetyl)-L-lysine were synthesized by haloacetylation of the ε-amino group of L-lysine using the corresponding thioesters dichloroacetyl thioacetate and trifluoroacetyl thioacetate as described previously [7]. Characterization of the compounds was performed by GC–NCI-MS confirming the modification of the ε-amino group.

All reference materials were stored as desiccated solid samples at −20°C and purity was checked by HPLC–UV prior to use.

2.3. Quantification of N-ac-TCVC in urine of PER-exposed rodents or human volunteers[1]

A total of 1.18 nmol [$^2H_3$]*N*-ac-TCVC in methanol (100 μl) as internal standard was added to either 3 ml of human urine samples or to 0.3–1.5 ml of rat urine samples. The obtained solutions were then adjusted to pH 1–2 with 1 *M* hydrochloric acid and extracted three times with 2 ml of ethyl acetate each. The ethyl acetate extracts were taken to dryness and the obtained residues derivatized with 200 μl methanolic BF_3 for 30 min at 60°C. Excess BF_3 was decomposed with 1 ml of water and the solutions were extracted with 1 ml of chloroform. After drying over sodium sulphate and evaporation of the chloroform, the obtained residues were dissolved in 0.5 ml of chloroform. Two μl of the derivatized sample were analyzed by GC–electron-capture MS. For separations, a DB-1 (J&W Scientific, Folsom, CA, USA) fused-silica capillary column (30 m×0.25 mm I.D., 1 μm film thickness) was used. Quantitation of *N*-ac-TCVC using characteristic fragments (m/z 178, m/z 180, m/z 181 and m/z 183) was performed relative to the content of the internal standard ([$^2H_3$]*N*-ac-TCVC) and referenced to calibration curves with authentic material. For GC separation, a linear temperature program (20°C/min) from 55°C to 300°C was applied; injector temperature was 280°C and transfer line temperature was 280°C. The ion source temperature was adjusted to 150°C.

2.4. DNA base adducts preparation for GC–MS

The method is based on a publication of Fedtke et al. [18] with the following modifications: dry residues of reaction mixtures (≈1 mg) were dissolved in 2 ml dimethylformamide and 3 mg of K_2CO_3 and 6 μl pentafluorobenzyl bromide (caution: pentafluorbenzyl bromide is a potent lachrymator. Use an efficient hood). The mixtures were then stirred for 12

[1] The study was carried out according to the Declaration of Helsinki, after approval by the Regional Ethical Committee of the University of Würzburg, Germany, and after written consent by the volunteers.

h at room temperature and concentrated in vacuo. The obtained residues were dissolved in 200 μl of dichloromethane and 2 μl of the obtained solution were injected into the GC system.

For all separations, a DB-5 (J & W Scientific) fused silica capillary GC column (30 m×0.25 mm I.D., 0.1 μm film thickness) was used. A temperature gradient starting at an oven temperature of 60°C and a heating rate of 10°C/min to 290°C were used for separation. The transfer line was kept at a temperature of 280°C. Injector temperature was 250°C and the electron source of the mass spectrometer was adjusted to 200°C in the electron impact ionization mode and in the chemical ionization mode. Solvent delay was 8 min. Mass spectra (*m*/*z* 100–600) were recorded from 8 to 17 min with a scan time of 0.5 s and an inter channel delay of 0.05 s. The detection limit was measured in the single ion monitoring (SIM) mode using characteristic fragments of the relevant compound. Dwell times were 30 ms.

2.5. Analysis of protein adducts

Polyclonal anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was raised against a dichloroacteyl groups carrying hemocyanin-conjugate in rabbits and immunoaffinity purified on N^{ϵ}-(dichloroacetyl)-L-lysine as recently described [19]. This antibody is highly specific to dichloroacetyl groups and was coupled to *N*-hydroxysuccinimide (NHS)-activated agarose columns (Pharmacia, Freiburg, Germany). Columns were rinsed with 6 ml icecold 1 m*M* HCl, then the antibody (10 mg in coupling buffer containing 0.2 *M* $NaHCO_3$ at pH 8.3) was circulated through the column with a peristaltic pump for 1 h. After the coupling of the ligand, the column was rinsed with 0.5 *M* ethanolamine at pH 8.3 to deactivate any residual hydroxysuccinimide groups. For binding of antibody-reactive proteins, the columns were equilibrated by rinsing with 10 ml of 20 m*M* Tris–HCl at pH 8.0. Rat and human plasma samples were desalted by gel filtration chromatography on Sephadex PD10 columns (Pharmacia) and recirculated on the immunoaffinity-columns at 4°C for 12 h in 20 m*M* Tris–HCl at pH 8.0. Specifically and non-specifically bound proteins were eluted from the columns with 10 ml of the same buffer. Specifically bound proteins were then eluted with 10 ml of an acidic buffer (20 m*M* glycine, 0.5 *M* NaCl adjusted to pH 2.7 with concentrated HCl) [20]. After elution of immunoreactive proteins, the columns were immediately rinsed with Tris–HCl buffer containing 0.1% sodium azide for storage at 4°C. Isolated protein fractions were lyophilized and hydrolyzed with 500 μl of 6 *M* HCl at 60°C for 16 h after addition of the internal standard N^{ϵ}-(trifluoroacetyl)-L-lysine. After addition of 500 μl water, samples were lyophilized and redissolved in 200–500 μl acetonitrile–pyridine (99:1, v/v). Derivatization was performed after adding 30 μl of 1,3-dichloro-1,1,3,3,-tetrafluoroacetone (DCTFA) at 60°C for 30 min in a closed reaction vial. Reaction products generated for GC–MS analysis by reaction with DCTFA were 2,2-bis(chlorodifluoromethyl)-4-*N*-dichloroacetyl-4-aminobutyl-1,3-oxazolidine-5-one as derivate of N^{ϵ}-(dichloroacetyl)-L-lysine and 2,2-bis(chlorodifluoromethyl)-4-*N*-trifluoroacetyl-4-aminobutyl-1,3-oxazolidine-5-one as derivate of N^{ϵ}-(trifluoroacetyl)-L-lysine.

For all separations, a DB-5 (J & W Scientific) fused silica capillary GC column (20 m×0.18 mm I.D., 0.1 μm film thickness) was used. A temperature gradient starting at an oven temperature of 60°C and a heating rate of 10°C/min to 300°C were used for separation. The transfer line was kept at a temperature of 280°C. Injector temperature was 250°C and the electron source of the mass spectrometer was adjusted to 150°C.

2.6. Gas chromatography–mass spectrometry

GC–MS analyses were performed either on a Fisons Trio 2000 or a Fisons MD 800 mass spectrometer coupled to a Carlo Erba 8000 series GC system and equipped with an AS 800 autosampler (Fisons Instruments, Mainz, Germany). All injections were made splitless (valve time 1.0 min). Helium was used as carrier gas (average linear velocity: 25 cm/s) and methane was used as reactant gas for chemical ionization in all separations.

3. Results and discussion

3.1. Quantitation of mercapturic acids by electron-capture MS

After derivatization of mercapturic acids to corre-

sponding volatile esters these esters may be to quantified by GC–MS. A method based on GC–NCI-MS has been described for the quantitation of *N*-ac-TCVC in the urine of PER or *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine [*N*-ac-*S*-(1,2-DCVC)] in the urine of TRI-exposed rodents and humans [21,22]. The method used in this study is based on these procedures, but uses a simplified clean-up, a derivatization procedure with higher yields and a GC column with a higher load capacity to be able to analyze large numbers of samples with an increased sensitivity. In electron-capture MS, quantitation is based on the relative response to an internal standard which is structurally very similar to the tested compound, best results are obtained with stable isotope labelled standards such as $[^2H_3]$*N*-ac-TCVC in the case of *N*-ac-TCVC. Standards added to urine sample, compensate for loss after derivatization of both *N*-ac-TCVC and $[^2H_3]$*N*-ac-TCVC with methanolic BF_3 (Fig. 2), the use of the modified method provided mass spectra with the base peak at m/z 178 for *N*-ac-TCVC methyl ester and at m/z 181 for the $[^2H_3]$*N*-ac-TCVC methyl ester, which is consistent with the dichlorothioketene loss described previously [21]. This mechanism may be explained by the abstraction of the acidic proton at the α-C atom by negative ions formed after ionization of methane by electrons (Fig. 3). The resulting trichlorovinyl thiolate forms the thioketene with loss of chloride, which binds to the electron deficient fragment with the mass of m/z 143 generating the fragment with the mass of m/z 178 (Fig. 4). Monitoring m/z (^{35}Cl) 178, m/z 180, m/z 181 and m/z 183 in single ion mode permitted the quantitation of *N*-ac-TCVC methyl ester at concentrations of 10 fmol/2 μl injection volume with a signal-to-noise ratio of 5:1. When individual samples were repeatedly analyzed, deviation of the obtained quantitative results was lower than 5%. Standards were prepared in control urine of non-exposed rats and humans. A typical GC separation of a human urine sample after inhalation to 10 ppm PER is shown in Fig. 5. A well resolved and intensive signal for *N*-ac-TCVC (t_R=11.97 min) was obtained.

3.2. DNA base modification

As shown in Fig. 1 cysteine conjugates like 1,2-DCVC or TCVC are cleaved by β-lyase to vinyl thiolates. The synthesis and characterization of

MeOH / BF_3

K_2CO_3 / DMF

CH_3CN/Pyridine

60°C, 1 h

Fig. 2. Derivatization reactions of mercapturic acids, DNA and protein adducts for quantitation by GC–NCI-MS.

e^-/CH_4
M^-
m/z 126
m/z 161
m/z 143; D_3 146
m/z 178; D_3 181

Fig. 3. Fragmentation of [$^2H_3$]- (D_3) and *N*-acetyl-(trichlorovinyl)-L-cysteine methyl ester by GC–MS using methane as reactant gas in the negative chemical ionization mode.

thioketene and ketene adducts of DNA bases were described previously [14,15,23] and in most cases the exocyclic amino group of the DNA base was (thio)acetylated by the electrophile. The adducts may be detected by HPLC–UV or –fluoresence detection with a limit of detection in the low pmol range. However, this limit was not sufficient for the detection of adducts in biological systems. After derivatization of the adducts with pentafluorobenzyl bromide (Fig. 2) thermally stable and volatile deriva-

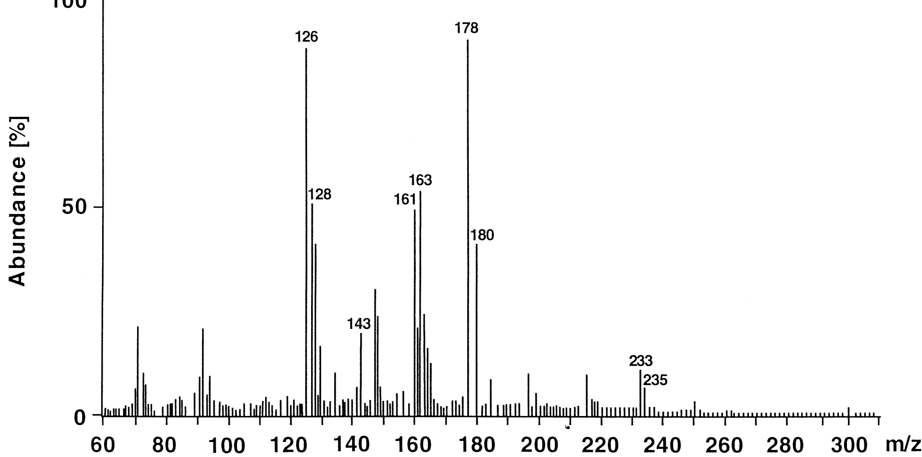


Fig. 4. Mass spectrum of *N*-acetyl-(trichlorovinyl)-L-cysteine methyl ester.

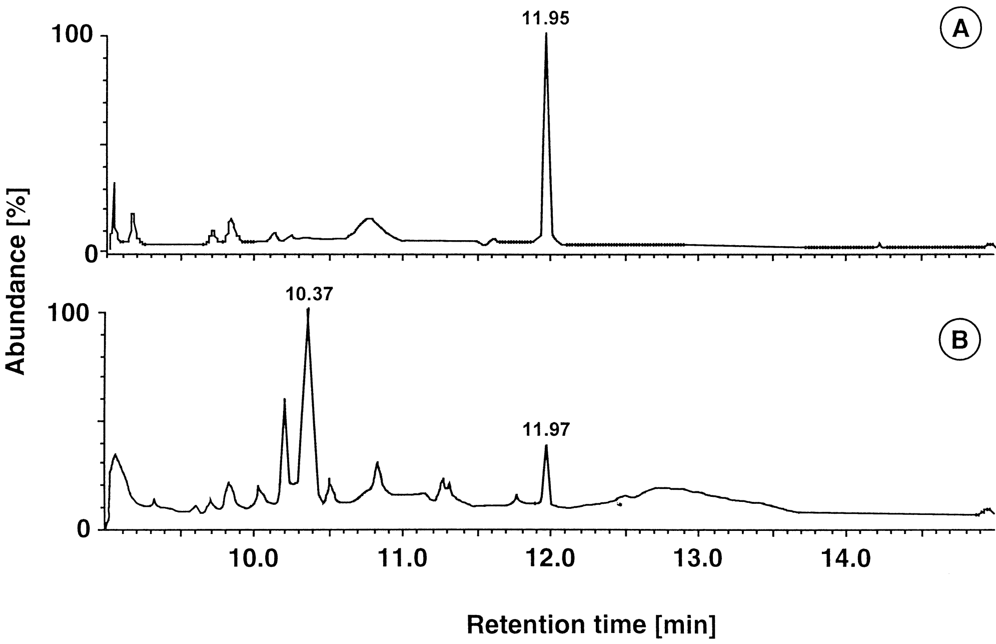


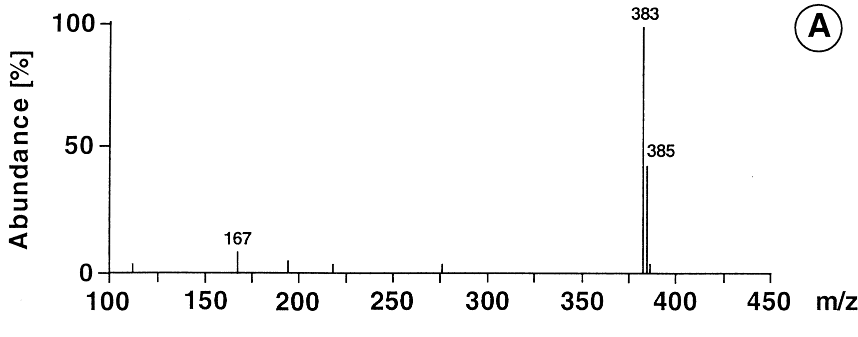
Fig. 5. GC separation of a human urine sample collected between 18 and 23 h after start of exposure to 10 ppm PER for 6 h. (A) m/z 181 for [$^{2}H_{3}$]-N-ac-TCVC methyl ester (t_R 11.95 min); (B) m/z 178 for N-ac-TCVC methyl ester (t_R 11.97 min).

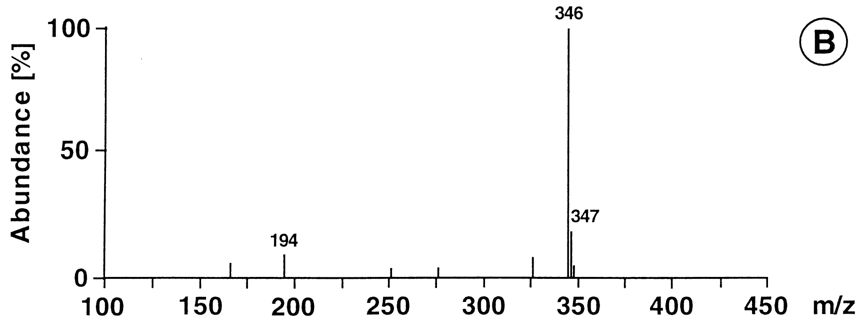
tives with good GC characteristics are formed and electron-capture MS may be used due to the pentafluorobenzyl group as electrophore. Under these conditions only a few fragments of the compound are generated (Fig. 6A shows the mass spectra of N^4-chlorothioacetyl cytosine), the base peak m/z (^{35}Cl) 383 corresponds with the molecular ion. In the electron impact mode the molecule was cleaved to give the pentafluorobenzyl (PFBn) ion m/z 181 and the MH-PFBn-ion m/z (^{35}Cl) 203 as characteristic fragments (data not shown). Using the single ion mode under NCI conditions a detection limit of 50 fmol/injection volume may be reached. Under the basic conditions of derivatization a second product was formed and identified as the ring closed 3,N^4-thioacetyl cytosine (Fig. 6B). The cyclization of N-chloro(thio)acetyl base adducts in basic solutions was described previously [14,23].

Therefore, it was not surprisingly that all N-chloroacetyl base adducts like N^4-chloroacetyl cytosine may be observed only in the ringclosed form after derivatization with pentafluorobenzyl bromide (PFBnBr). As described for N^4-chlorothioacetyl cytosine the ionization of the pentafluorobenzylated adducts in the NCI mode provided only a few ions and the base peak exhibited the molecule ion of the particular adduct (Table 1).

3.3. Protein modification

Protein modifications are important endpoints in biomonitoring since they represent first biochemical endpoints believed to be closely related to events resulting in a toxic response. Therefore, many methods have been developed to quantify protein adducts. These methods include liberation of the protein bound xenobiotic by hydrolysis, quantitation of the modified amino acid after protein cleavage or use of antibodies with specific affinity to the protein adducts of interest [24–26]. All these methods have advantages and disadvantages, the needed sensitivities for monitoring of human populations may often only be reached by a combination of different techniques. For these studies, we used a combination of immunoaffinity chromatography to isolate protein adducts from blood plasma of exposed rats and





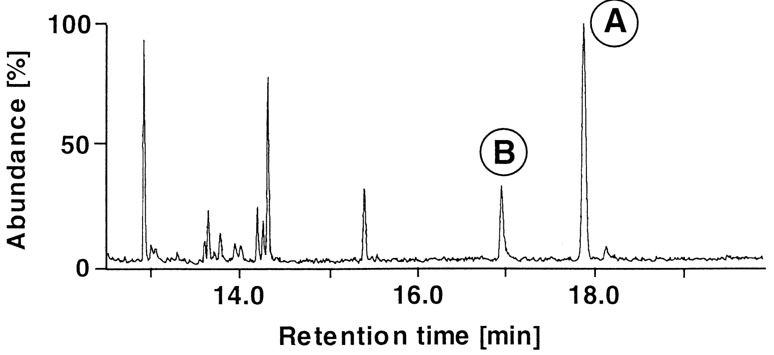


Fig. 6. GC separation and mass spectra of N^1-pentafluorobenzyl-3,N^4-thioacetyl cytosine (A) and N^1-pentafluorobenzyl-N^4-chlorothioacetyl cytosine (B).

human volunteers and GC–electron-capture MS after protein hydrolysis for quantitation. A specific antibody to the chemically induced amino acid modification within proteins, N^ϵ-(dichloroacetyl)-L-lysine, was used to selectively extract modified proteins from plasma samples. After this enrichment step, isolated proteins were hydrolysed in the presence of the structurally related internal standard *N*-(trifluoroacetyl)-L-lysine. The liberated lysine derivatives were then reacted with an electrophore label, 1,3-dichloro-1,1,3,3-tetrafluoroacetone (Fig. 2), to yield volatile cyclic oxazolidinone derivatives and analysed by GC–NCI-MS (Fig. 7). With N^ϵ-(dichloroacetyl)-L-lysine, the method was very sensitive and had an excellent reproducibility including a recovery of greater than 80% after immunoaffinity

Table 1
m/z of fragments of DNA adducts generated with chloroketene and DNA base after derivatization with PFBnBr performed by GC–NCI-MS

DNA-adduct	*m/z* (fragment); NCl: after derivatization with PFBnBr
	331 [M$^-$], 330 [M$^-$-H], 302 [M$^-$F], 181 [PFBn$^-$]
	551 [M^{-1}, 532 [M$^-$-F], 370 [M$^-$PFBn], 181 [PFBn$^-$]
	535 [M$^-$], 514 [M$^-$-F], 354 [M$^-$PFBn], 181 [PFBn$^-$]

chromatography. Using the combination of selective sample enrichment and the very sensitive GC–NCI-MS measurement after electrophore-labelling the detection and characterization of modified rat blood plasma proteins after inhalation of working place concentration of perchloroethene was performed.

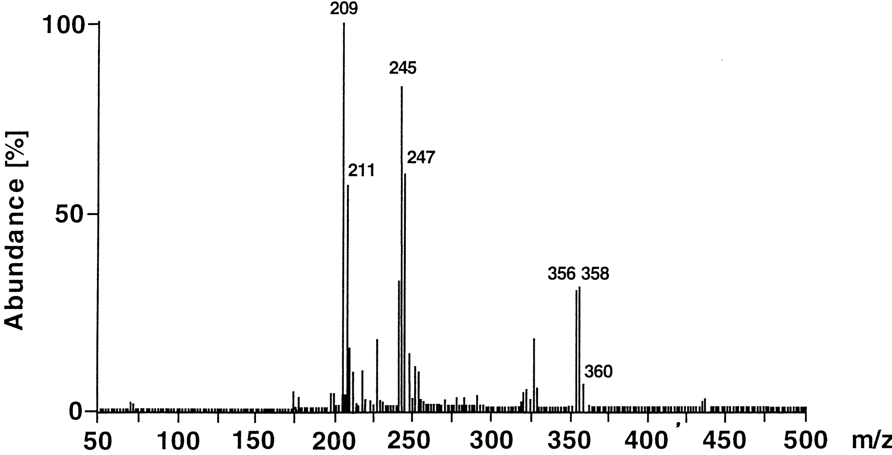
3.4. Discussion of advantages and disadvantages of the used GC–MS methods

The results presented here show that the detection and quantitation of mercapturic acids and adducts of the DNA and proteins by electron-capture MS is very simple and efficient after carefully sample work up and derivatization with specific derivatization agents for each compound. Major advantages of the electron-capture MS method are the specifity, sensitivity, sample validation and efficiency in sample measurement (Table 2).

For the quantitation of mercapturic acids such as *N*-ac-1,2-DCVC and *N*-ac-TCVC, neither HPLC–UV nor GC–electron impact (EI) MS provides to be sufficient sensitive for use in experiments with occupational or environmental relevant doses. In the case of UV detection the lack of a good chromophore restrict the method to a limit of detection in the nmol range. The ionization of mercapturic acids in the electron impact mode provides many fragments with a low signal-to-noise ratio leading to detection limit in the low pmol range [27]. The superiority of GC–MS with chemical ionisation for determination of mercapturic acids to other methods has been reviewed [28].

The formation of negative ions as derivatives of methane in electron-capture mode leads to an abstraction of acidic protons (proton abstraction) providing only few fragments with a high signal-to-noise ratio. One of both cleavage products contains the methyl ester and the *N*-acetyl group of cysteine and is suitable for inserting the stable isotopes on this side of the molecule to generate the internal standard. The other fragment is cleaved to the thioketene and chloride, which bind to the electron deficient part of the molecule containing the amino acid residue.

In comparison to mercapturic acid analysis described above, DNA adducts have to transformed to compounds containing an efficient electrophore like a pentafluorobenzyl group for using electron-capture MS [29,30]. For low-molecular-mass nucleotide adducts GC–NCI-MS is a very sensitive method for quantitation of DNA adducts [31,32].



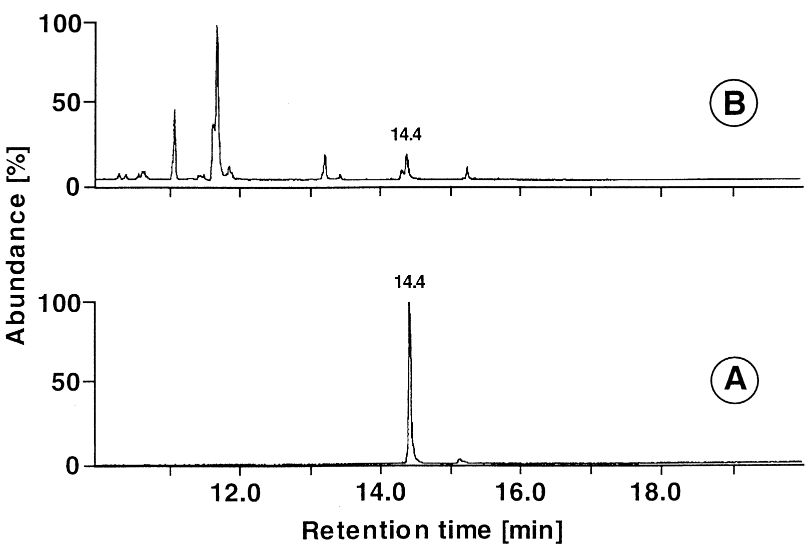


Fig. 7. GC separation of synthetic N^{ϵ}-(dichloroacetyl)-L-lysine (A; t_R 14.44 min) and male rat plasma (24 h after 6 h exposure to 40 ppm PER) proteins after immunoaffinity purification on anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody coupled to NHS-activated agarose columns, protein hydrolysis and derivatization with DCTFA (B) monitoring m/z=209. Mass spectrum of the derivative of N^{ϵ}-(dichloroacetyl)-L-lysine (upper panel).

In addition, labile DNA base derivatives [14,23] may be transformed to stable, volatile and electrophore labelled compounds in organic solvents such as DMF or methanol [30,31]. This is in contrast to the ^{32}P-postlabelling method which requires enzymatically transformation of DNA adducts to the corresponding [5′-^{32}P]deoxyribonucleoside 3′,5′-bisphosphates and chromatographic separations on thin-layer plates in aqueous buffered solutions over the prolonged time of the assay [29,31].

One of greatest disadvantage of ^{32}P-postlabelling assay is the lack of structurally information of the DNA adduct and quantitation. Mass spectrometry provides sufficient data about the chemical structure of adducts and is therefore much more effective as a prescreening method. Furthermore, if the GC–MS

Table 2
Characteristics of the methods of determination for *N*-acetyl-*S*-(trichlorovinyl)-L-cysteine, N^4-dichlorothioacetyl cytosine and N^ϵ-dichloroacetyl-L-lysine

	N-ac-TCVC	N^4-Chlorothioacetyl-cytosine	N^ϵ-Dichloroacetyl-L-lysine
Detection limit (signal-to-noise: 5:1)	10 fmol/μl	50 fmol/μl	10 fmol/μl
Replicate analyses[c]	Repeated analysis (n=4)	Repeated analysis (n=4)	Repeated analysis (n=4)
Accuracy	SD<20%	SD<30%	SD<20%
Calibration curve	R^2=0.999	n.d.[a]	R^2=0.997
Linearity range	10–4000 fmol/μl	n.d.[a]	20–1000 fmol/μl
Absolute recovery[d]	>50%	n.d.[a]	53–72%[b]

[a] Due to the lack of detection of N^4-chlorothioacetyl cytosine in aqueous solutions and biological samples these parameters were not determined.
[b] Including enrichment step, protein hydrolysis and derivatization of a standard sample of dichloroacetylated albumin (n=8).
[c] Intra-assay variability was <5%.
[d] Compensated by addition of isotope labelled standard to sample before workup.

assay is validated, it may be routinely used and may be partly or even completely automated and many samples may be analyzed in a very short time as compared to the use of the ^{32}P-postlabelling assay. The sensitivity of electron-capture MS now reaches that of ^{32}P-postlabelling method or the detection limit is in a range that is sufficient for most experiments [29].

For protein adducts, immunoaffinity enrichment followed by protein hydrolysis and electrophore derivatization has been demonstrated to be able to quantify adducts under relevant exposure situations. The derivatization with DCTFA produces volatile derivatives of amino acids with good GC characteristics and is well suitable for electron-capture MS. The procedure may have broad utility in determining protein adducts in trace levels also in human populations. However, care has to be taken in method development due to the rather harsh conditions of hydrolysis and in standard selection.

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