

High-performance liquid chromatography analysis of rubber allergens in protective gloves used in health care

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A high-performance liquid chromatography (HPLC) method developed for analysis of zinc dithiocarbamates was validated and used to perform a survey of disposable medical gloves used in southern Sweden. The gloves were extracted with acetone at room temperature for 10 min by shaking. The extracts were injected into a polyether ether ketone lined HPLC column, and peaks were analysed by a diode-array detector. The survey shows that of 19 gloves analysed, 10 contained zinc diethyldithiocarbamate (0.070–3.5 mg/g), 3 contained zinc pentamethylenedithiocarbamate (1.0–4.3 mg/g), 4 contained zinc dibutyldithiocarbamate (0.9–1.1 mg/g), and 2 contained 2-mercaptobenzothiazole (0.005–0.008 mg/g). None of them contained thiurams.

Key words: allergic contact dermatitis; high-performance liquid chromatography; medical gloves; mercaptobenzothiazoles; rubber; thiurams; zinc dithiocarbamates. © Blackwell Munksgaard, 2006.

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Disposable protective gloves made of rubber or plastic materials are used in large numbers by health-care workers for protection against infectious materials. A side-effect of this use is the development of allergic contact dermatitis to some of the chemicals added during the manufacturing of the gloves, such as thiurams, dithiocarbamates, and mercaptobenzothiazole derivatives. Studies on the frequency of positive patch test reactions to individual rubber allergens show that thiurams are the most registered of these allergens. In recent years, rubber glove producers have replaced thiurams with dithiocarbamates and mercaptobenzothiazole compounds, but as yet, there are no indications of a change in sensitizations due to this replacement (1).

Proper handling of rubber-related occupational injuries is often very cost-efficient for the employer and beneficial to the patient. However, this requires a successful determination by epicutaneous tests of the allergen or the allergens responsible for the dermatitis. Also, the patient must be offered rubber products, for example gloves devoid of this allergen. We believe that this latter issue is 1 main field of application for a chemical method of analysis of rubber gloves, mainly for 2 reasons: complete declarations of the chemicals used in the production of disposable

gloves are often hard to obtain from manufacturers and the additives undergo chemical changes during vulcanization, leading to an unknown composition. For these reasons, it is advantageous to have access to a chemical method for analysis of rubber additives.

In our ongoing research on rubber allergy, we have used several different methods for both quantitative and qualitative analysis (2, 3), and in this study, we present our results from analysis of dithiocarbamates using a high-performance liquid chromatography (HPLC) method developed by Mathieu et al. (4). This method is based on the principle that the potential for the dithiocarbamates to come into contact with metal surfaces in the chromatographic system should be eliminated. Therefore, the injector loop, capillaries to the column, and the column itself are made of metal-free polyether ether ketone (PEEK). Furthermore, the mobile phase is supplemented with zinc sulfate to outnumber possible free metal ions other than zinc ions. The sample is then subjected to a gradient elution and peaks are detected by ultraviolet (UV) detection. Because of the high number of UV-absorbing substances present in rubber extracts and eluting from the column, it is necessary to use a diode-array detector that presents a UV spectrum for the compounds appearing on

the chromatogram. This method makes it possible to simultaneously analyse mercaptobenzothiazoles, zinc dithiocarbamates, and thiurams in rubber glove extracts. In a recent study, we have also extended this method to the analysis of dialkylthiouras in neoprene rubber (5).

The purpose of this study was to validate this method with respect to the analysis of zinc dithiocarbamates in disposable gloves and to make a survey of the content of different rubber allergens in disposable gloves used in health care in our community.

Materials and Methods

Chemicals

2-Mercaptobenzothiazole (MBT); 2,2'-dibenzothiazyl disulfide (MBTS); 2-(4-morpholinylmercapto)-benzothiazole (MMBT); and N-cyclohexyl-2-benzothiazyl sulfenamide (CBS) were obtained from Bayer (Antwerp, Belgium). Tetramethylthiuram monosulfide (TMTM) 97%, tetramethylthiuram disulfide (TMTD), tetraethylthiuram disulfide 97%, and dipentamethylenethiuram disulfide 99% were obtained from Janssen Chimica (Geel, Belgium). Zinc dimethyldithiocarbamate (ZDMC) was obtained from Fluka (Buchs, Switzerland). Zinc diethyldithiocarbamate (ZDEC) was obtained from TCI (Tokyo, Japan). Zinc pentamethylenedithiocarbamate (ZPD) was obtained from Robinson Brothers Ltd (West Bromwich, UK), zinc dibenzylthiocarbamate 95% from Acros Organics (Geel, Belgium), and zinc dibutylthiocarbamate (ZDBC) >97% from TCI Europe (Zwijndrecht, Belgium). Acetone used for the extractions of analytical grade and acetonitrile of HPLC grade were obtained from Labscan Ltd (Dublin, Ireland). Zinc sulfate (analytical grade) was obtained from Merck (Darmstadt, Germany). MBT, MBTS, MMBT, CBS, TMTD, ZDMC, ZDEC, and ZPD were all of technical grade.

Gloves

Both sterile and non-sterile latex and non-latex disposable gloves were selected for this investigation. The selection was made from the brands used by public health and medical and dental care services in the public health service in southern Sweden.

High-performance liquid chromatography

Chromatographic analyses were performed using HPLC on a reversed phase column (Alltima C18, 4 μ m, 150 $\times$ 4.6 mm, PEEK lined; Alltech Associates, Deerfield, IL, USA), eluted with acetoni-

trile aqueous zinc sulfate (10^{-5} mol/l) at ratio of 50:50 for 5 min, then a linear gradient to 100% acetonitrile for 35 min. Eluent was pumped with a Waters 600 pump (Waters Chromatography Division, Milford, MA, USA) at a flow rate of 1 ml/min and monitored at 280 and 325 nm with a 1100 Series diode-array detector (Hewlett-Packard Co., Palo Alto, CA, USA). All devices in contact with the mobile phase after the injector were made of PEEK. Peak area was used to determine the concentration, and the identifications were made by comparison of retention times and UV spectra recorded by the diode-array detector.

Analysis of the gloves

1 sample (0.5 g) of each glove was cut into small pieces with a pair of scissors and placed in a 10-ml test-tube with a Teflon-lined screw cap containing 5 ml acetone (ScherfChroma, Germany) (6). The test-tube was placed on a shaker, and after 10-min extraction at room temperature, the extract was pipetted into a round-bottomed flask and evaporated under vacuum. The extract was then dissolved in 5 ml of acetonitrile and filtered before injection on the HPLC column. The chromatogram was compared with analyses of reference compounds for quantification. Duplicate analyses were performed for each glove.

Method validation

Linearity was determined in the range of 10–200 μ g/ml, with triplicate standard preparations of ZDEC at 5 levels. Recovery was determined by analysis of 4 samples with added amounts of ZDEC to a glove that itself was deficient of ZDEC. 100 μ l of 5 mg/ml ZDEC in acetone was added to the glove samples and left to dry. When the acetone evaporated, the glove samples were processed and analysed in the usual procedure.

The precision of the standard solution was determined after analysis of 5 standard solutions of ZDEC at 100 μ g/ml. The precision of glove extractions was determined after analysis of 5 samples of a glove containing ZDEC and 5 samples of a glove containing ZPD.

Results

Identification of rubber additives

Table 1 presents the retention times of the rubber additives chosen to study in this investigation. Solutions of pure compounds dissolved in acetonitrile were injected into the column, the retention times

Table 1. The retention times in minutes for the rubber allergens investigated in this study

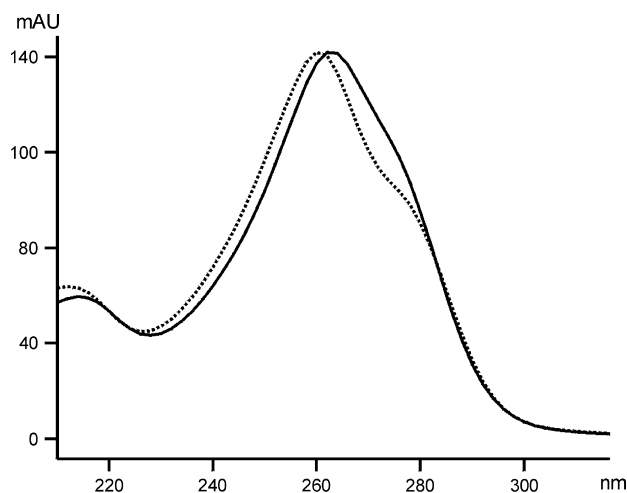
Substance	Abbreviation	Retention time (min)
2-Mercaptobenzothiazole	MBT	4.5
Tetramethylthiuram monosulfide	TMTM	4.5
Tetramethylthiuram disulfide	TMTD	6.3
2-(4-Morpholinylmercapto)benzothiazole	MMBT	11.5
Zinc dimethyldithiocarbamate	ZDMC	12.1
Tetraethylthiuram disulfide	TETD	20.1
Dipentamethylenethiuram disulfide	PTD	21.4
N-Cyclohexyl-2-benzothiazyl sulfenamide	CBS	26.3
Zinc diethyldithiocarbamate	ZDEC	27.7
Zinc pentamethylenedithiocarbamate	ZPD	29.4
2,2'-Dibenzothiazyl disulfide	MBTS	31.1
Zinc dibenzylthiocarbamate	ZBEC	42.2
Zinc dibutylthiocarbamate	ZDBC	48.0

were recorded, and the UV spectra were compared with reference spectra.

All the substances studied were separated by this chromatographic procedure, with the exception of MBT and TMTM. They had identical retention times and could therefore only be identified using the spectrum analysis of the diode-array detector or using a completely different HPLC system (2). ZDEC and ZPD were close in retention times but sufficiently separated to make correct identification and quantification possible. Furthermore, identification was also accomplished by the comparison of UV spectra obtained from the diode-array detector (Fig. 1).

Analysis of glove extracts

The concentrations of rubber additives in the examined gloves are shown in Table 2. In 6 of

**Fig. 1.** Comparison of ultraviolet spectra obtained by diode-array detector from high-performance liquid chromatography peaks of zinc diethyldithiocarbamate (dotted line) and zinc pentamethylenedithiocarbamate (solid line).**Table 2.** The content of rubber allergens (mg/g) in the gloves analysed in this study

No.	Material	MBT	ZDEC	ZPD	ZDBC
1	Nitrile	nd	3.5	nd	nd
2	Latex	nd	nd	4.3	nd
3	Latex	nd	1.4	6.2	nd
4	Latex	nd	nd	nd	nd
5	Latex	nd	nd	nd	nd
6	Latex	nd	0.07	nd	nd
7	Latex	0.008	nd	1.05	1.1
8	Latex	nd	nd	nd	nd
9	Neoprene	nd	nd	nd	nd
10	Latex	0.005	0.7	nd	1.0
11	Latex	nd	1.2	nd	nd
12	Latex	nd	nd	nd	nd
13	Latex	nd	nd	nd	nd
14	Latex	nd	1.5	nd	Traces
15	Latex	nd	2.2	nd	nd
16	Latex	nd	nd	nd	nd
17	Latex	nd	0.7	nd	nd
18	Latex	nd	1.0	nd	0.9
19	Nitrile	nd	3.0	nd	nd

MBT, 2-mercaptobenzothiazole; nd, not detected; ZDBC, zinc dibutylthiocarbamate; ZDEC, zinc diethyldithiocarbamate; ZPD, zinc pentamethylenedithiocarbamate.

the 16 latex gloves, no common additives could be found. All other latex gloves contained zinc dithiocarbamates, and a few of them also contained MBT. A typical chromatogram is shown in Fig. 2. The 2 nitrile gloves contained ZDEC, but in the neoprene glove, no rubber additive could be identified. In none of the gloves could any thiuram be found. The potential of the analytical method to analyse thiurams has been verified by analyses of thiuram-containing rubber not included in this study.

Linearity

A standard curve with triplicate injections of a standard preparation of ZDEC at 5 different levels showed a linear calibration curve in the range of 10–200 µg/ml. The correlation coefficients were determined by least-square analysis to be 0.998, and the precision was determined to range between 1.4% and 4.4%.

Limit of detection

The lowest concentration of ZDEC in a reference sample where a satisfactory UV spectrum could be achieved for identification was 1 µg/ml. This concentration in the extract corresponded to a concentration of 10 µg/g ZDEC in the glove. Signal to noise ratio in this case was 10:1.

Recovery

The recovery was determined to be 73% ± 1.9% ($n = 4$) by extraction of 0.5 mg of ZDEC added to

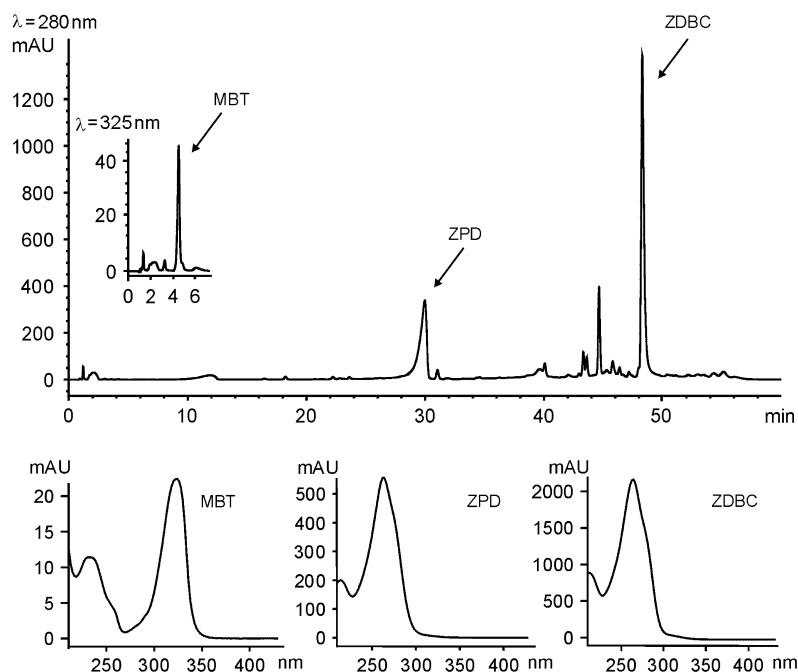


Fig. 2. HPLC chromatogram (top) of an extract of glove no. 7 at 280 nm, showing the occurrence of ZPD and ZDBC. Inserted is a part of the same analysis monitored at 325 nm, showing a peak corresponding to MBT. Ultraviolet spectra (bottom) of the HPLC peaks obtained from the diode-array detector. HPLC, high-performance liquid chromatography; MBT, 2-Mercaptobenzothiazole; ZDBC, zinc diethyldithiocarbamate; ZPD, zinc pentamethylenedithiocarbamate.

a glove that itself was deficient of ZDEC. These data do not display effects from the rubber matrix. Although in another experiment that we have published (6), a recovery of about 77% was obtained. In that case, a glove that itself contained dithiocarbamates was extracted several times with new portions of solvent until the extract was almost devoid of dithiocarbamates.

Precision of the standard

5 injections of standard solutions of ZDEC at 100 μ g/ml resulted in a precision expressed as relative standard deviation of 3.1%.

Precision of glove extractions

5 samples each of glove no. 6 and glove no. 7 were analysed to determine the precision of the method. Glove no. 6 was found to contain 0.07 mg/g ZDEC (relative standard deviation = 14.5%), and glove no. 7 contained 1.05 mg/g ZPD (relative standard deviation = 5%).

Discussion

Several hundreds of additives are used in the production of rubber, and especially the accelerators are interesting from a contact dermatitis point of view. This group of additives comprises dithiocarbamates, thiurams, thiazoles, and thioureas. With

the exception of dithiocarbamates, the other compounds are fairly easy to analyse. The reason why dithiocarbamates offer difficulties is because of their high propensity to chelate metal ions from the solvent or from contact surfaces to which the analyte is exposed during analysis. These chelates are more or less strong depending on the metal species, and they differ in their chemical and chromatographic behaviour. These difficulties have been dealt with either by chemically altering the dithiocarbamate by way of a derivatization before analysis or by suppressing the formation of new metal chelates. Derivatization was performed through methylation of dithiocarbamates in 1 step and methoxylation of thiurams in another step (7). The derivatives were then analysed by gas chromatography/mass spectrometry (GC/MS). This procedure did not distinguish between thiuram monosulfides and thiuram disulfides. Another procedure combines a qualitative analysis by high-performance thin layer chromatography with derivatization of thiuram and dithiocarbamate side chains with trifluoroacetic anhydride (8). Derivatives are then analysed by GC, but thiurams and dithiocarbamates cannot be differentiated. A third possibility is to react dithiocarbamates with cobalt chloride to form stable cobalt trisdithiocarbamate complexes (9). These complexes are analysed by HPLC with a UV detector. The problem with this method is that in extracts containing dithiocarbamates

with 2 or more different side chains, the cobalt complexes contain mixtures of side chains, giving complicated chromatograms unsuitable for quantification purposes. In a recent publication on the analysis of ZDEC and ZDBC in condoms, the problem with chelate formation was solved by the addition of a large amount of ZDMC to the extract (10). This added dithiocarbamate is said to protect the analytes from transmetalation reactions. An obvious disadvantage with this method is that it will not allow analysis of ZDMC itself, a frequently found accelerator in rubber products.

When performing chemical analyses of contact allergens in rubber products, it is advantageous to choose a method that is able to give as complete a picture of the composition as possible. The rubber allergens are fairly easy to analyse by HPLC, with the sole exception of dithiocarbamates. This problem has been solved by the technique developed by Mathieu et al. (4), which makes it possible to simultaneously analyse MBT, zinc dithiocarbamates, and thiuram disulphides in rubber gloves. In this study, we have performed a validation of the method published by Mathieu et al. (4) and then used the method to make a limited survey of disposable rubber gloves. Our validation of the method shows that it is suitable for analysis of contact allergens over a concentration range that is relevant for an evaluation of rubber gloves. With a single and simple analytical procedure, several important rubber allergens can be identified, compared with other methods requiring both derivatization and expensive equipment like GC/MS. We used this concept at our laboratory to analyse different rubber products and have found that the technique is also suitable for analysis of dialkylthioureas extracted from a chloroprene rubber material (5). We have also used this method in a study where we optimized the procedure for extracting disposable gloves for patch test purposes (6). That study showed that the amounts of dithiocarbamates in these extracts are in about the same level as recommended for patch testing. The study also presents an evaluation of the extraction technique by comparing different agitation methods, solvents, and extraction times. An observation we have made through these studies is that different batches from the same brand of gloves can display a high variation in the amount of allergens.

Another observation made is that gloves manufactured from nitrile contain considerable amounts of rubber allergens. In this study, the nitrile gloves (nos 1 and 19) contain the highest amounts of dithiocarbamates. It is therefore necessary to be careful when recommending patients allergic to rubber to use gloves made of synthetic rubber.

Our present survey shows that of the 19 gloves analysed, 10 contained ZDEC, 3 contained ZPD, 4 contained ZDBC, and 2 contained MBT. None contained thiuram disulfides, an observation in agreement with the remark of Geier et al. (1) that most manufacturers have replaced thiurams by dithiocarbamates. In a recent German study, 5 of 12 gloves contained ZPD (11), and Knudsen et al. reported ZPD in 7 of 19 glove brands investigated (8). In the latter study, the authors had information from the manufacturers claiming that ZPD was not used. A highly reasonable explanation was given by the authors that ZPD is a conversion product from the frequently used accelerator dipentamethylthiuram tetrasulfide.

It is interesting to note that no additives could be detected in 6 of the gloves. The manufacturers were contacted for information on the additives used. In 1 of these gloves, ZDEC had been added, and for 2 gloves, the manufacturer claimed that none of the substances (mentioned in Table 1) had been added. No information was received for 3 of the gloves. Any proper assessment of contact allergy from different materials is dependent on the knowledge of the content of allergens in the investigated product. In the case of rubber and plastic materials, the only available information is from what the manufacturers have added before vulcanization/curing. But these chemical processes will undoubtedly change the composition of remaining substances and in some cases, it also produce new chemical species (12). To obtain a better insight into these processes, we will use this method to investigate into the chemical changes during the production of rubber. We are now also studying the use of new additives, in collaboration with rubber industry, to further validate the usefulness of the analysis.

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References

1. Geier J, Lessmann H, Uter W, Schnuch A. Occupational rubber glove allergy: results of the Information Network of Departments of Dermatology (IVDK), 1995–2001. *Contact Dermatitis* 2003; 48: 39–44.
2. Hansson C, Agrup G. Stability of the mercaptobenzothiazole compounds. *Contact Dermatitis* 1993; 28: 29–34.
3. Bergendorff O, Hansson C. The spontaneous formation of thiuram disulfides in solutions of iron (III) dithiocarbamates. *J Agric Food Chem* 2002; 50: 1092–1096.

4. Mathieu C, Herbreteau B, Lafosse M, Morin P, Renaud M, Cardinet C, Dreux M. Liquid chromatography of unstable zinc dithiocarbamates. Application to rubber gloves analysis. *J High Resolut Chromatogr* 2000; 23: 565–566.
5. Bergendorff O, Persson C, Hansson C. HPLC analysis of alkyl thioureas in an orthopaedic brace and patch testing with pure ethylbutylthiourea. *Contact Dermatitis* 2004; 51: 273–277.
6. Bergendorff O, Persson C, Hansson C. Extraction of haptens from solid products and their delivery to the skin, exemplified by dithiocarbamates from rubber gloves. *Contact Dermatitis* 2005; 53: 22–26.
7. Knudsen B B, Larsen E, Egsgaard H, Menne T. Release of thiurams and carbamates from rubber gloves. *Contact Dermatitis* 1993; 28: 63–69.
8. Knudsen B B, Hametner C, Seycek O, Koch H-U, Peters K-P. Allergologically relevant rubber accelerators in single-use medical gloves. *Contact Dermatitis* 2000; 43: 9–15.
9. Kaniwa M, Isama K, Nakamura A et al. Identification of causative chemicals of allergic contact dermatitis using a combination of patch testing in patients and chemical analysis. Application to cases from rubber gloves. *Contact Dermatitis* 1994; 31: 65–71.
10. Depree G J, Bledsoe T A, Siegel P D. Determination of zinc dialkyldithiocarbamates in latex condoms. *J Chromatogr Sci* 2004; 42: 80–84.
11. Brehler R, Rütter A, Kütting B. Allergenicity of natural rubber latex gloves. *Contact Dermatitis* 2002; 46: 65–71.
12. Nieuwenhuizen P J, Reedijk J, Van Duin M, McGill W J. Thiuram- and dithiocarbamate-accelerated sulfur vulcanization from the chemist's perspective: methods, materials and mechanisms reviewed. *Rubber Chem Technol* 1997; 70: 368–429.

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