

Extraction of mercaptobenzothiazole compounds from rubber products

C. HANSSON¹, O. BERGENDORFF¹, M. EZZELARAB¹ AND O. STERNER²

Department of ¹Dermatology and

²Organic Chemistry 2, University of Lund, Sweden

At evaluation of contact dermatitis caused by solid material, patch testing is usually performed with the material as such and with extracts of it. In this study, optimization of the extraction technique monitored by quantitative HPLC analysis of the extracted haptens is described for mercaptobenzothiazole derivatives. Several solvents with different properties are included. Acetone has traditionally been a solvent widely used for the extraction of organic haptens from solid products. However, acetone and other ketones are not inert solvents. The rubber accelerators 2-(4-morpholinyl mercapto) benzothiazole (MMBT) and N-cyclohexyl-2-benzothiazyl sulfenamide (CBS) react with acetone, yielding 2 new compounds, which were isolated and characterised by NMR and MS. For the extraction of solid rubber products, methyl tert-butyl ether (MTBE) was found to be a more suitable solvent which is unreactive to most common haptens.

Key words: hapten; extraction; patch testing technique; rubber; mercaptobenzothiazole; allergic contact dermatitis; acetone; tetrahydrofuran; methyl tert-butyl ether. © Munksgaard, 1997.

Accepted for publication 22 December 1996

The extraction of allergens from solid material is a common procedure in the evaluation of contact dermatitis, serving mainly as a complement to patch tests with the solid materials as such. Some suggestions have been made about appropriate extraction solvents, but no quantitative chemical analyses of the extract procedure have been described (1, 2). The compounds reported to cause contact dermatitis are numerous and differ considerably from each other in their chemical properties. Since the extraction conditions for various compounds must be reliable and efficient, a thorough investigation of the extraction procedures used for the haptens most commonly present in solid material seemed to be called for. We recently published an HPLC method for the analysis of mercaptobenzothiazole derivatives (3) (Fig.1). In the present investigation the method is used for the quantitative analysis of the amounts of mercaptobenzothiazoles found in extracts obtained from rubber products by using different solvents.

In the extraction procedure, the haptens are transferred at low concentration into a large volume of extraction medium. These solutions must be concentrated before samples can be applied to the skin, and a nontoxic, volatile solvent is preferable for this purpose. Here we describe some of

the chemical events occurring during the concentration of the extracts to a small volume prior to performing patch tests.

Acetone is probably the most common extraction solvent used today. However, in our analyses of mercaptobenzothiazole solutions we found that acetone reacted with several of the rubber haptens. New compounds were formed and these were isolated and their molecular structures determined by NMR and mass spectrometry (MS). The compounds were also synthesised on a preparative scale, yielding pure crystalline substances.

Materials and Methods

Chemicals

The mercaptobenzothiazole compounds 2-mercaptobenzothiazole (2-MBT), 2,2'-dibenzothiazyl disulfide (MBTS), 2-(4-morpholinyl mercapto) benzothiazole (MMBT), and N-cyclohexyl-2-benzothiazyl sulfenamide (CBS) were obtained from Bayer (Antwerp, Belgium). Acetone, methylene chloride, sodium acetate and acetic acid were purchased from Merck (Darmstadt, GFR). Tetrahydrofuran (THF) was procured from Fisons (Loughborough, England) and methyl tert-butyl ether from LabScan (Dublin, Ireland). Rubber

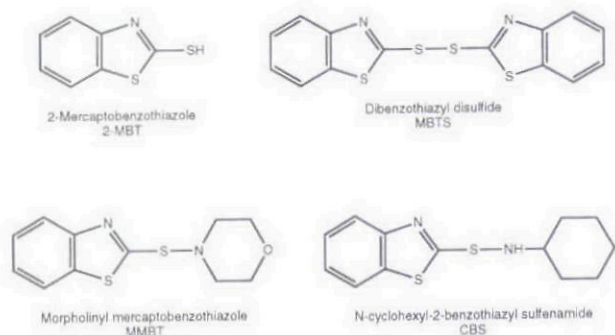


Fig. 1. Molecular structures of mercaptobenzothiazole derivatives.

products were obtained from a local rubber factory.

5 mM stock solutions of each of the mercaptobenzothiazole derivatives were freshly prepared daily in THF.

^1H and ^{13}C NMR were recorded in CDCl_3 , using a Varian XL 300 and a Bruker ARX 500 spectrometer. Mass spectra (EI ionisation, positive ions) were recorded with a Jeol SX 102 spectrometer.

HPLC analysis

A Spectra Physics Isochrom liquid chromatographic pump equipped with a Rheodyne Model 7125 loop injector (20 μl) and an Applied Biosystem Model 757 UV-detector working at 300 nm were used. All separations were performed on a Lichrospher RP 100 C_{18} column (5 μm , 125 $\times$ 4 mm, Merck) using tetrahydrofuran (35–45%, usually 40%) in 0.1 M aqueous sodium acetate as the mobile phase. The pH of the mobile phase was adjusted to 6.0 with acetic acid. All runs were performed at room temperature at a flow rate of 1.5 ml/min.

Extraction studies

Extractions of solid rubber samples, 0.5 g cut into small pieces, were performed in 5 ml extraction solvent in test-tubes provided with screw caps, using a magnetic stirrer or an ultrasonication bath. The extracts were usually filtered through a Millipore filter before injection into the HPLC system.

Analysis of the stability of the mercaptobenzothiazole compounds upon concentration to a small volume involved dissolving 1 mmol each of 2-MBT, MMBT, MBTS, and CBS in 5 ml solvent, followed by evaporation to a small volume (about 100 μl) using a stream of nitrogen or hot air (from a hair dryer fan) and then adding THF to obtain

the original total volume of 5 ml before the actual analysis was performed.

Preparation of compound 1

10 mmol (2.52 g) 2-(4-morpholinyl mercapto) benzothiazole (MMBT) was dissolved in 25 ml acetone, 25 mg 2-mercaptobenzothiazole (2-MBT) then being added. The solution was stirred for 24 h at room temperature. No MMBT was left in the reaction mixture at that time, but there was a high concentration of 2-MBT. At fractionated crystallisation in a refrigerator for approximately two days 2-MBT was first crystallised and separated. The crystals of compound 1 (Fig. 6) thus obtained were then recrystallised in methanol. The chromatographic yield in the reaction mixture was 12%.

Results

The peaks for the mercaptobenzothiazole compounds were well separated in the HPLC-chromatograms of the most rubber products analysed. For some of the rubber products it was necessary to change the mobile phase of the chromatographic system in order to obtain well separated chromatographic peaks and for peak identification. We therefore studied the effect of the THF concentration and of pH on the capacity factors of the mercaptobenzothiazoles (Figs. 2, 3).

Each of the rubber products was investigated with the same series of solvents used for extraction and the same range of extraction conditions. The results of the different extraction procedures for a typical cured rubber product, in which, according to the manufacturer, 2-MBT and CBS had been used as accelerators, are presented in Table 1. The

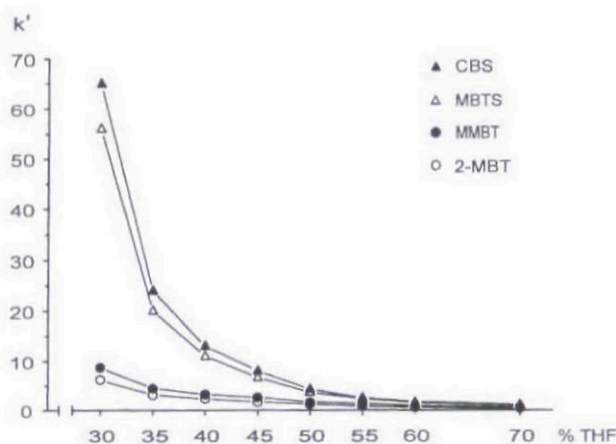


Fig. 2. Relationship between capacity factor k' and the concentration of THF in the mobile phase. Column: Lichrospher RP 100 C_{18} (5 μm , 125 $\times$ 4 mm). Eluent: 0.1 M sodium acetate at pH 6.0 with THF added.

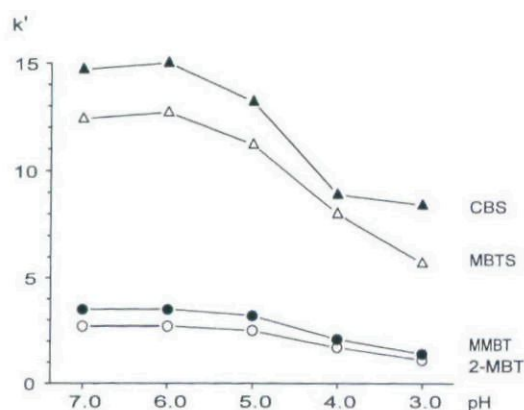


Fig. 3. Relationship between the capacity factor k' and pH for 2-MBT, MBTS, MMBT, and CBS. Column: Lichrospher RP 100 C₁₈. Eluent: 0.1 M sodium acetate with 40% THF added.

Table 1. Extraction of a cured rubber product with different solvents

Solvent	Extraction time (min)	Quantity analysed ($\mu\text{mol/g}$ rubber product)	
		2-MBT	MBTS
MTBE	10	0.14	0.04
	30	0.24	0.06
THF	10	0.05	0.05
	30	0.13	0.04
acetone	10	0.02	0.05
	30	0.03	0.05
ethanol	10	0.01	0.00
	30	0.02	0.02

extracts were analysed for the presence of 2-MBT, MBTS, and CBS. The analyses were performed after a predetermined extraction period. Fresh standard solutions of each of the mercaptobenzothiazole derivatives were used as external standards. No CBS was found in the rubber after vulcanisation. Although no MBTS was added during production of the rubber, it was found to be present at low concentrations in both the cured and the uncured products.

Analysis of the components in the standard MBT-mix (1 μmol of each) in THF (5 ml) after concentration to a small volume (about 100 μl) was performed in model experiments. Analysis of the content of mercaptobenzothiazole compounds after concentration from 5 ml to about 100 μl by air (from a hair dryer fan) at room temperature manifested a 40% decrease in 2-MBT, and a 20% increase in MBTS. The content of MMBT and CBS remained constant after concentration (Figs. 4A, B). Upon hot air evaporation of the THF solution, only trace amounts of 2-MBT remained and oxidation to MBTS was noted. Some decrease in

MMBT and CBS concentrations was also noted (Fig. 4C).

Concentration in acetone yielded several new products which were not present in the original rubber compounds. These compounds are described below. After hot air evaporation of acetone, only trace amounts of 2-MBT were found, but a 70 % increase was noted in its oxidation product MBTS. The concentration of MMBT and CBS was decreased by 20–50% when the hot air evaporation procedure was used.

On mixing the 4 components of the MBT-mix in acetone at room temperature and before evaporation of the solvent, we registered the formation of 2 new products as peaks in the HPLC chromatogram. The amounts of these compounds increased at evaporation of the solvent. We have also analysed MBT-mix test preparations produced by first dissolving the four MBT-mix components in a small volume of acetone in order to obtain a more homogeneous petrolatum preparation. However, with this procedure high concentrations of compound 1 and compound 2 are formed (Fig. 5). In comparing the possible reactions between each of the components of the mercapto-mix, we found

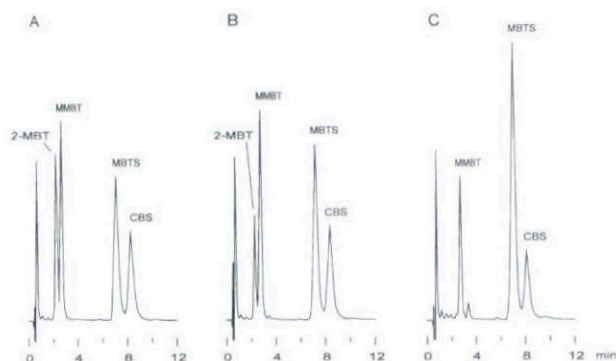


Fig. 4. HPLC chromatogram of a sample containing the MBT-mix components in THF. A) before and B) after concentration at room temperature using a hair dryer fan, and C) after concentration by hot air from a hair dryer fan. Samples B and C (5 ml) were concentrated to 100 μl and then dissolved in 4.9 ml THF.

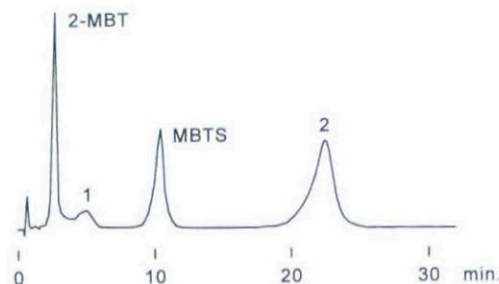


Fig. 5. HPLC chromatogram of a petrolatum preparation of MBT-mix with the components first dissolved in acetone.

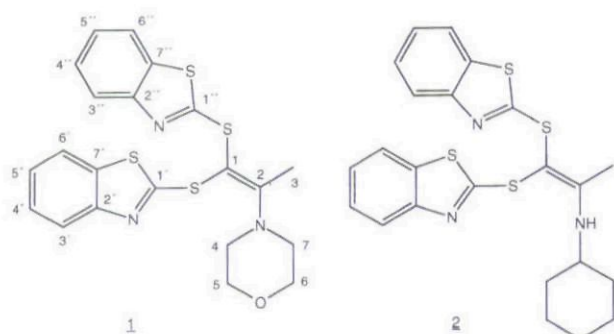


Fig. 6. Molecular structure of compound **1** and of compound **2**.

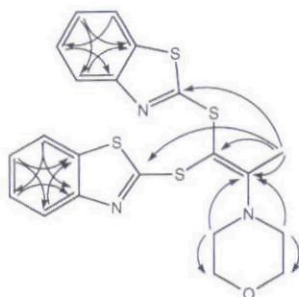


Fig. 7. Significant long-range ^1H – ^{13}C correlations for compound **1**. Spectral data are given in Table 2.

compound **1** to be a product formed from 2-MBT, MMBT and acetone, and compound **2** to be formed from 2-MBT, CBS and acetone. These two compounds were isolated and purified by recrystallisation. The molecular structures of the two compounds were determined by NMR and MS (Fig. 6).

The ^1H and ^{13}C NMR-spectra of compound **1** revealed the presence of a thioketal enamine substituted with morpholinyl, methyl and benzothiazolyl groups. The elemental composition was determined by high resolution mass spectrometry. The exact mass of the molecular ion was 457.0405, a figure in accord with the theoretical mass of 457.0411 derived from the formula $\text{C}_{21}\text{H}_{19}\text{N}_3\text{OS}_4$. In addition to M^+ at m/z 457 (17 %), important fragments were observed at m/z 291, 259, 206 and 167. The peak at 291, which is the base peak, is obtained after the loss of one mercaptobenzothiazole moiety. Determination of the structure was facilitated by extensive 2D NMR analysis, the proposed structure being confirmed with COSY, HMQC and HMBC experiments (Table 2). The significant HMBC correlations are summarised in Fig. 7. From a spectroscopic point of view, the ^{13}C NMR shift for the 1,1-dithio-substituted olefinic carbon is noteworthy, since this is a very unusual

chemical functionality. This substance has never previously been described.

The NMR spectra of compound **2** had some resemblance to those of compound **1**, but in those of compound **2** the morpholinyl moiety was replaced by a cyclohexyl group. This compound has only recently been described and comparison with the published spectroscopic data confirmed its structure (4). These two compounds were then synthesised by reactions between 2-MBT and MMBT in pure acetone and between 2-MBT and CBS in pure acetone. A chromatographically and spectroscopically pure crystalline substance was obtained for both compounds.

The total amounts of the benzothiazole compounds extracted with the standard procedure used at our laboratory for the extraction of patient-supplied solid rubber products were determined by extracting 1.0 g of a rubber sample that contained 2-MBT as an accelerator with 5 ml THF for 10 min. This approach resulted in the extraction of only 1/10 the total amount of 2-MBT used in a standard patch test with 2% 2-MBT per patch.

Discussion

In the selection of an extraction solvent, the properties of both the solvent and the suspected allergens must be considered. In this study, we knew the molecular structure of the mercaptobenzothiazole derivatives and from this were able to predict the superiority of a lipophilic solvent (5). Some of the organic solvents we tested manifested high extraction yields of mercaptobenzothiazoles. We preferred MTBE since it is a powerful extraction medium and its high volatility and low toxicity are further favourable properties. No skin reactions

Table 2. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **1**

	^1H (δ ; multiplicity; J)	^{13}C (δ ; multiplicity)
1	—	85.6; s
2	—	168.2; s
3	2.50 (s)	21.0; q
4	3.71 (dd; 4.4, 4.9)	51.0; t
5	3.59 (dd; 4.4, 4.9)	67.0; t
6	3.59 (dd; 4.4, 4.9)	67.0; t
7	3.71 (dd; 4.4, 4.9)	51.0; t
1'/1''	—	171.5/172.9; s
2'/2''	—	154.6/154.9; s
3'/3''	7.83/7.84 (d; 8.1)	121.8/121.8; d
4'/4''	7.38/7.39 (ddd; 1.0, 8, 8)	126.0/126.1; d
5'/5''	7.26/7.27 (ddd; 0.8, 8, 8)	123.9/124.1; d
6'/6''	7.76/7.76 (d; 8.0)	120.9/120.9; d
7'/7''	—	135.3/135.5; s

The spectra were recorded in CDCl_3 , the solvent signals (7.26 and 77.0 ppm) being used as references. The coupling constants J are given in Hz.

were recorded in patch tests with pure MTBE on 20 healthy persons. Although acetone is probably the solvent most widely used in test laboratories today, it is not inert to all haptens. We found some mercaptobenzothiazoles to be unstable in acetone and to give rise to the formation of new products.

Quantitative extraction studies of rubber products were performed by stirring with a magnetic rod or by ultrasonication. Ultrasonication is a technique long used by chemists in extraction procedures and more recently also in patch testing laboratories (6, 7). However, the technique is somewhat more intricate than the usual stirring procedure. Specifically, the effect of ultrasonication varies with the thickness of the glass wall of the test tube, and with the position of the test tube in the sonication bath. Extractions must be performed at the same position in the bath for reproducible results to be obtained. No major difference in the extraction yields was noted in our experiments between extraction of benzothiazole compounds from rubber products by stirring and by optimised ultrasonication, but more reproducible results were obtained by stirring, the 2-MBT also being extracted faster with this procedure. Extraction in MTBE with 30 min of magnetic stirring is a good technique for the extraction of MBT derivatives from most rubber products, giving high extraction yields and only negligible levels of oxidation.

Since extraction generally results in the allergens being present at low concentration in a large volume, concentration must be carried out before patch testing can be performed upon patients. The concentration needed is easily achieved by means of a stream of nitrogen or air. Although nitrogen is to be preferred as it is an inert gas, hot air from a hair dryer fan is probably the technique most commonly used in small test laboratories and was therefore included in the present study. Only minor degradation was found when extracts of mercaptobenzothiazole compounds in THF were concentrated to a small volume at room temperature. (Peroxide free THF should be employed). In contrast, the solution of mercaptobenzothiazole compounds in acetone gave rise to the formation of several condensation products. These reactions were more pronounced when hot air was used for concentration of the acetone extracts. The concentrations of the MBT mix components changed strikingly during the procedure, mainly through formation of the oxidised product MBTS, but also due to formation of the 2 new compounds **1** and **2** when using acetone. Elucidation of the structures of the compounds formed was essential for obtaining insight into the participation of acetone in the formation of these compounds.

By using THF as the extraction solvent, the formation of irrelevant compounds can be avoided. However, fresh THF has to be used since, on standing, THF slowly forms peroxides which may be irritant to the skin and possibly also reactive to some haptens. Whether peroxides are formed can easily be checked by using commercial peroxide test strips. As the demand for high quality of THF entails a more expensive extraction procedure, we investigated MTBE as an alternative. MTBE was found to be superior to all other solvents tested. It has a powerful extraction capacity, it is cheap and it forms no peroxides on being left to stand.

Quantification of the mercaptobenzothiazole extracted from a rubber product with our standard procedure, using acetone or THF as solvent, showed there to be a smaller amount in the patch test of the extract than we usually apply in our standard patch test with 2-MBT. The low amounts of mercaptobenzothiazole found here may explain the false-negative reactions sometimes noted in testing MBT-sensitive patients with extracts of rubber products containing MBT derivatives. When negative reactions to pure rubber compounds are obtained in the standard test, but positive reactions to extracts of rubber products, the latter may be attributable to the presence of the other compounds seen in the chromatograms of the extracts.

The extraction of allergens of rubber products was studied with regard to the mercaptobenzothiazoles present. The ratio of the extraction capacities of the solvents used to those of the extraction techniques described should be relevant to most types of rubber products, although the figures obtained are probably specific to each individual rubber product. Other rubber haptens may manifest quite different extraction properties. Adequate extraction procedures should be defined by systematic investigations based on observations of the kind reported here.

Acknowledgements

This investigation was supported by grants from the Swedish Council for Work Life Research, the Vårdal Foundation, and the Alfred Österlund Foundation for Scientific Research. Ms. C. Cieloszczyk and C. Fureby are thanked for their technical assistance.

References

1. Schwartz L, Tulipan L, Birmingham D J. *Occupational diseases of the skin*, Philadelphia: Lea & Febiger, 1957.
2. Fregert S. Extraction of allergens for patch testing. *Acta Dermato-venerologica* 1964; 44: 107-109.
3. Hansson C, Agrup G. Stability of the mercaptobenzothiazole compounds. *Contact Dermatitis* 1993; 28: 29-43.

4. Chapman A V. A facile reaction of sulphenamide accelerators with acetone. *J Nat Rubb Res* 1990; 5: 259–265.
5. Jung J H, McLaughlin J L, Stannard J, Guin J D. Isolation, via activity-directed fractionation, of mercaptobenzothiazole and dibenzothiazyl disulfide as 2 allergens responsible for tennis shoe dermatitis. *Contact Dermatitis* 1988; 19: 254–259.
6. Ehrin E, Karlberg A-T. Detection of rosin (colophony) components in technical products using an HPLC technique. *Contact Dermatitis* 1990; 23: 359–366.
7. Bruze M, Trulsson L, Bendsøe N. Patch testing with ultrasonic bath extracts. *Am J Contact Dermatitis* 1992; 3: 133–137.

Address:

Christer Hansson
Department of Dermatology
University Hospital
S-221 85 Lund
Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.