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HPLC analysis of alkyl thioureas in an orthopaedic brace and patch testing with pure ethylbutylthiourea

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Ethylbutylthiourea (EBTU) is an accelerator used in the production of chloroprene (neoprene) rubber. EBTU occurs in a mixture with diethylthiourea (DETU) and dibutylthiourea (DBTU) in the accelerator. An analytical method originally developed for analysis of zinc dithiocarbamates in rubber has been used to analyse EBTU, DETU and DBTU in a knee brace responsible for an allergic contact dermatitis in a gardener suffering from arthrosis. EBTU was isolated and gave positive reactions when tested as a pure compound. The test reaction was accompanied by positive reactions to DETU and DBTU.

Key words: allergic contact dermatitis; chloroprene rubber; high-performance liquid chromatography; HPLC; knee brace; neoprene; thiourea. © Blackwell Munksgaard, 2004.

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Thiourea (TU) derivatives are used as accelerators in the manufacture of chloroprene rubber (also called neoprene) and known to cause allergic reactions to a number of different products, such as diving suits, protective goggles, athletic shoe insoles, knee braces, keyboard wrist supports and continuous positive airway pressure masks (1). The TU accelerators (Fig. 1) that have been reported to be allergenic are ethylenethiourea (ETU), dimethylthiourea (DMTU), diethylthiourea (DETU), dibutylthiourea (DBTU) diphenylthiourea (DPTU) and ethylbutylthiourea (EBTU). Underivatized TU is also used as an accelerator in production of synthetic rubbers but is perhaps better known as an allergen in, for example, diazo copy paper and silver polish.

Allergic contact dermatitis from EBTU has previously been established from contacts with athletic shoe insoles (2) and divers' spectacle bands (3). In this article, we present the case of a 59-year-old man with allergic contact dermatitis from a knee brace made of chloroprene rubber. We have also isolated, characterized and performed patch testing with the different components in the accelerator that was responsible for the allergic reaction. The chemical analysis of the knee brace was based on an HPLC method differing from earlier publications on chloroprene rubber analysis (4–6). At our laboratory, we are

currently using a method developed by Mathieu and coworkers (7) for analysis of dithiocarbamates in rubber products, and we have applied this method to analyse dialkyl TUs in the chloroprene rubber material that our patient is allergic to.

Case Report

A 59-year-old gardener presented with a severely itchy dermatitis on his right knee going back more than 1 year. He also suffered from arthrosis in this knee and for that reason he was wearing a knee brace. The exacerbation of his dermatitis coincided with the use of the brace. The dermatitis started on the right knee but continued to spread itchily all over the body. He had tried other brands of braces but with the same reaction to them. He had therefore stopped using any brace and on our examination only hyperkeratosis on the right knee and excoriations around the knee were seen.

He was initially patch tested with our standard series, an extended rubber series and with his own brace as is. All tests were read according to international guidelines and were evaluated at D3 and D7. He showed a positive reaction to thiuram mix in the standard series but no reaction to any of the individual thiuram compounds in the mix. In the rubber series, strong reactions to DETU and

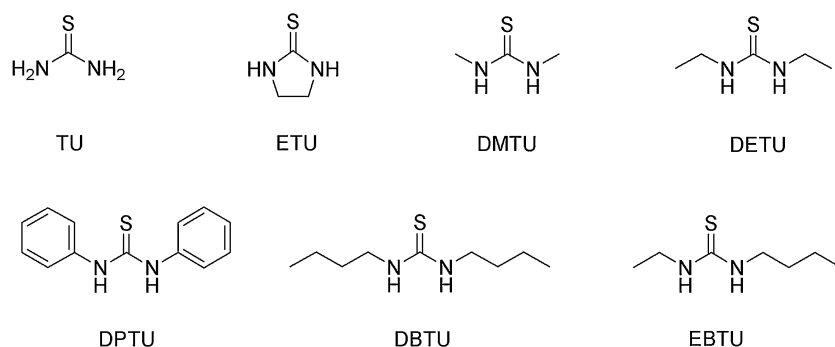


Fig. 1. Chemical structures of thiourea (TU), ethylenethiourea (ETU), dimethylthiourea (DMTU), diethylthiourea (DETU), diphenylthiourea (DPTU), dibutylthiourea (DBTU) and ethylbutylthiourea (EBTU).

DBTU were registered. His brace showed a positive test when tested as is, and chemical analysis of the brace, as described below, showed, besides the presence of DETU and DBTU, a still higher concentration of a third, unknown TU derivative. After contact with the producer and purification of material obtained from the producer, pure EBTU was achieved. Patch testing with the isolated EBTU showed a strong reaction to this compound as well (Table 1). Local treatment with a corticosteroid cream and a new brace of polyamide cloth healed the patient.

Methods and Materials

Spectroscopy

The mass spectra (FAB ionization, positive mode) were recorded with a Jeol SX102 (Tokyo, Japan) spectrometer. ¹H-NMR spectra were recorded with a BRUKER DRX-400 (Rheinstetten, Germany) spectrometer at 400 MHz.

Chemicals

Acetone used for the extractions was of analytical grade and acetonitrile, ethyl acetate and heptane were of HPLC grade. Zinc sulfate (analytical grade), ethylenethiourea (ETU), DETU, diphenylthiourea (DPTU) and DBTU were obtained from Merck (Darmstadt, Germany). DMTU was obtained from Janssen (Beerse, Belgium). All TUs were of synthesis grade. EBTU was obtained as a blend of dialkyl TUs (Ekaland

DATU LI) from MLPC international, Rion des Landes, France. Purification of EBTU is described below.

HPLC

Chromatographic analyses were performed by HPLC on a reversed phase column (Alltima C18, 4 μm, 150 × 4.6 mm, PEEK lined, Alltech Associates, Deerfield, IL, USA), eluted with acetonitrile-aqueous zinc sulfate (10⁻⁵ mol/l) 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 240 nm with a 1100 Series diode array detector (Hewlett-Packard Co., Palo Alto, CA, USA). Peak area was used to determine the concentration, and the TUs were identified by comparison of retention times and UV spectra recorded by the diode array detector.

Isolation of EBTU

300 mg of the Ekaland DATU accelerator was dissolved in 1 ml ethyl acetate and chromatographed on a 2 mm preparative SiO₂-TLC plate (Merck Si 60, F₂₅₄, 20 × 20 mm) eluted with heptane-ethyl acetate (1:1). The bands were visualized by UV light at 254 nm, and the central band was scraped off with a knife. The substance was then extracted with ethyl acetate, filtered and evaporated under vacuum. MS and NMR of this material confirmed the structure of EBTU. The isolated material was used as reference sample during analysis of the knee brace and as test substance at patch test.

Analysis of the knee brace

A sample (0.5 g) of the knee brace was cut by a pair of scissors into small pieces and placed in a 10-ml test tube with a Teflon-lined screw cap containing 5 ml acetone. The test tube was placed on a shaker and after 10 min extraction at room

Table 1. Patch test results

Allergen	Concentration	D3	D7
Thiuram mix*	0.025 mg/cm ²	++	—
DETU†	1% in pet.	+++	++
DBTU†	1% in pet.	+++	++
EBTU†	1% in pet.	+++	++

pet. = petrolatum.

*TRUE Test™.

†Finn Chamber™.

temperature the extract was pipetted into a round-bottomed flask and evaporated under vacuum. The extract was then dissolved in 5 ml of acetonitrile, then 10-fold diluted in acetonitrile and filtered before injection on the HPLC column. The chromatogram was compared with analyses of the Ekaland DATU accelerator and pure samples of DETU, DBTU and EBTU.

Results

The HPLC chromatogram from the knee-brace extract (Fig. 2a) showed 2 known peaks identified as DETU and DBTU after comparison of retention times and UV spectra obtained from the diode-array detector with reference substances. The chromatogram also showed a strong peak at 3.4 min between the signals from DETU (2.0 min) and DBTU (6.8 min) with an almost identical UV spectrum. These 3 peaks were match-

ing the peaks from a chromatogram of Ekaland DATU (Fig. 2b), the accelerator used in the knee brace. The dominant peak was suspected to be EBTU, based on the information from the supplier that stated Ekaland DATU to contain 25% DETU, 30% DBTU and 45% EBTU by weight percentage. Besides these 3 peaks, zinc dimethyldithiocarbamate, zinc diethyldithiocarbamate and *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6PPD) were identified in the extract. A sample of the accelerator Ekaland DATU was subjected to preparative thin-layer chromatography, and 3 distinct bands appeared on the plate. The middle band with *R_f* at 0.4 was extracted from the plate and, after filtration and evaporation, HPLC analysis showed that the isolated substance was identical to the major component of the extract from the knee brace (Fig. 2c), and the concentration of remaining impurities by DETU and DBTU was estimated to be less than 0.5%.

Further identification of the isolated compound was achieved by ¹H-NMR analysis and high-resolution MS. Reference ¹H-NMR spectra were obtained from samples of DETU and DBTU. These spectra were very similar to the spectrum of the isolated compound, and signals from the TU side chains could easily be assigned, although the tautomeric nature of the TU function resulted in broad multiplet signals from protons on the carbons adjacent to the nitrogen atom (8).

The signals assigned to the butyl group were a triplet at δ 0.94 ($-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$), a multiplet at δ 1.40 ($-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$), one multiplet at δ 1.60 ($-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$) and a broad multiplet at δ 3.0–4.0 ($-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$). The signals assigned to the ethyl group were a triplet at δ 1.24 ($-\text{CH}_2-\text{CH}_3$) and a broad multiplet at δ 3.0–4.0 ($-\text{CH}_2-\text{CH}_3$). High-resolution mass spectrum gave a signal at m/z 161.1113. The calculated mass for ($\text{C}_7\text{H}_{16}\text{N}_2\text{S} + \text{H}^+$) is 161.1112. These data together with the UV spectrum obtained from the diode-array detector established the structure of EBTU.

The composition of the knee brace was determined to be 0.9 mg/g DETU, 2.4 mg/g EBTU and 1.4 mg/g DBTU, resulting in a relative composition among the alkylthioureas of 19% DETU, 51% EBTU and 30% DBTU by weight percentage. Analysis of the accelerator Ekaland DATU gave a relative composition of 25% DETU, 49% EBTU and 26% DBTU by weight percentage.

A reference solution containing 9.1 $\mu\text{g}/\text{ml}$ each of ETU, DMTU, DETU, EBTU, DPTU and DBTU was analysed by HPLC and the resulting chromatogram is shown in Fig. 2d. The

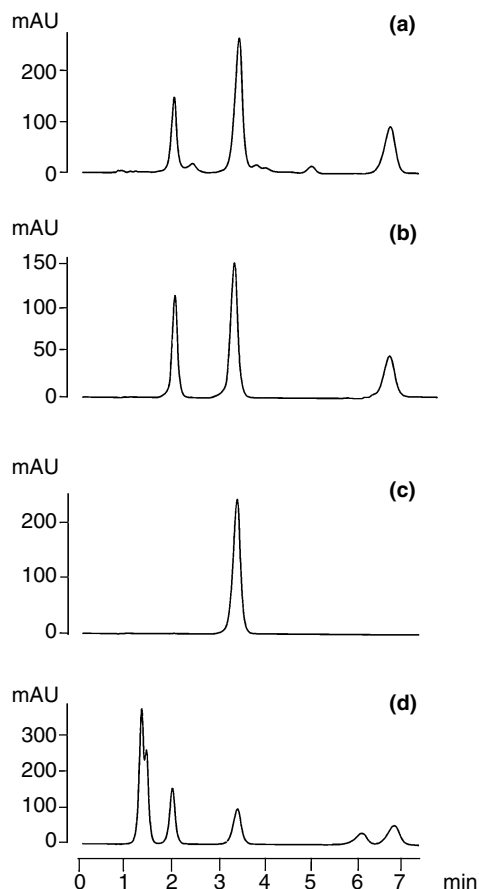


Fig. 2. HPLC chromatograms of dialkylthioureas. (a) Extract of knee brace. (b) Accelerator Ekaland DATU. (c) Purified EBTU. (d) A reference solution containing 9.1 $\mu\text{g}/\text{ml}$ each of ETU, DMTU, DETU, EBTU, DPTU and DBTU (mentioned in order of increasing retention time).

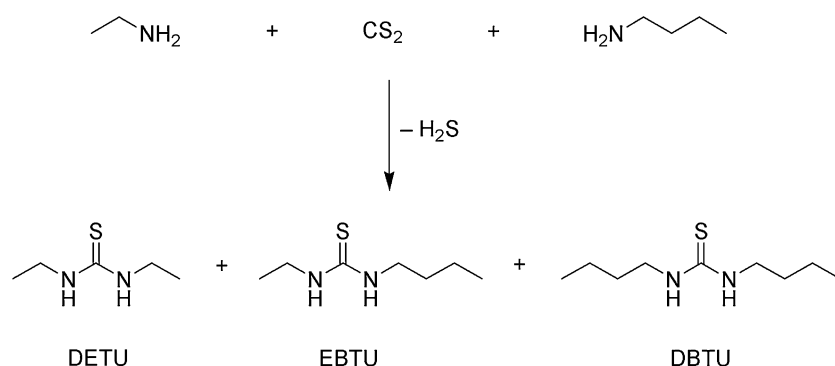


Fig. 3. The chemical reaction leading to a mixture of dialkylthioureas.

first 2 peaks corresponding to ETU and DMTU are poorly resolved and indicate that the chromatographic condition is not capable of separating these 2 compounds.

Discussion

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 a technique developed by Mathieu and coworkers (7) that makes it possible to simultaneously analyse 2-mercaptobenzothiazole, zinc dithiocarbamates and thiuram disulfides in rubber gloves. Their method is based on 2 specific measures that improve the chromatographic performance of the injected dithiocarbamates. First, the analyte will to a minimal degree come into contact with metal surfaces and for this reason only polyether ether ketone (PEEK) capillaries are used between the injector and the column. The column is also PEEK-lined. Secondly, zinc sulfate is added to the mobile phase in order to stabilize the dithiocarbamates. Because of the complex composition of rubber extracts, a diode-array detector is necessary for the characterization of different peaks in the chromatogram. We use this concept at our laboratory to analyse different rubber products and have found that the technique is useful for a wide range of rubber chemicals. In this article, we show that the method is also suitable for analysis of dialkyl TUs extracted from a chloroprene rubber material. This is an important finding because it means that an unknown rubber material can be analysed with respect to all common accelerators (thiurams, dithiocarbamates, mercaptobenzothiazoles and TUs) in a single analysis. However, if both ETU and DMTU are present in the extract, a complementary HPLC analysis with a different mobile phase will be necessary.

A common way of manufacturing disubstituted TUs is to react primary amines with carbon disulfide during loss of hydrogen sulfide (9). In the case of EBTU, an asymmetrically disubstituted TU is formed when 2 different primary amines react with carbon disulfide (Fig. 3). This procedure will result in 3 different TUs and will be used as a mixture during rubber processing. This has been recognized by Emmett and coworkers (5), who also analysed by HPLC extracts of a tennis shoe insole and a scuba mask with respect to EBTU. Mixtures of thiuram disulfides are known to produce asymmetric disulfides spontaneously in mixtures (10). We have not seen any similar reactivity among mixtures of alkyl TUs.

EBTU-containing accelerators like Pennzone from Pennwalt or Ekaland DATU from MLPC international also contain DETU and DBTU. To our knowledge, pure EBTU is not commercially available, nor is any accelerator containing pure EBTU used in chloroprene-rubber manufacturing. As a result, products containing EBTU also contain both DETU and DBTU. This also means that, unless EBTU has been isolated from the raw material or the chloroprene-rubber product, the patient will be patch tested with a mix of DETU, EBTU and DBTU at an approximate ratio of 1:2:1. Studies on cross-reactivity among these dialkylated TUs must therefore include tests with isolated substances. We think that our case is the first presentation of an allergic patch test for pure EBTU.

Acknowledgements

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