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Solid Phase Extraction, a Rapid Method for the Determination of Mercaptobenzothiazole in Water from Soothers and Bottle Teats

Key words: 2-Mercaptobenzothiazole, MBT, HPLC, SPE

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Introduction

2-mercaptobenzothiazole (MBT) is generally manufactured for its application as a corrosion inhibitor in rubber and its sodium salt is used as a vulcanisation accelerator during the production of synthetic rubbers. Unfortunately, excess quantities of unreacted additives which remain in the rubber stock leach into the surrounding fluids and threaten public health. Although these compounds showed low acute general toxicity with laboratory animals treated by oral dermal routes, MBT is a recognized allergen responsible for contact dermatitis reactions to rubberized clothing and footwear. Moreover, comprehensive oral studies provided evidence of carcinogenicity in rats. MBT did not induce mutagenicity in *Ames* bacterial assays, on the other hand, it was mutagenic and induced chromosomal damage in mammalian cells in culture (1, 2).

Several illustrative investigations justify a careful and strict control of MBT leaching into fluids which are confined in rubber enclosures or in contact with rubber container parts. *Gaind* and *Jedrzajezak* (3) studied MBT and mercaptobenzothiazole disulphide (MBTS) release from rubber septums into a iodinated contrast medium (sodium iothalamate). Prior to analysis the contaminants were extracted with dichloromethane, the extract was evaporated to dryness and the residue was redissolved in acetonitrile. The concentrations were determined by reversed phase high performance liquid chromatography (HPLC; RP18 column, acetonitrile-water-acetic acid 300:700:03) in combination with UV detection (MBTS: 272 nm; MBT: 328 nm). *Reepmeyer* and *Juhl* (4) determined MBT leaching from rubber enclosures into injectable solutions. The migration tests were done with NaOH (1N), heated for 3 days at 65 °C. The latter study also combined preliminary extraction of the contaminants, evaporation, redissolution and HPLC separation in combination with UV detection.

In a former paper *Blosczyk* and *Dömling* (5) report on MBT migration from teats into demineralized water during storage at 40 °C for 24 hours. The aquous samples were filtered, directly injected on a Waters Bondapak Alkyl Phenyl column, separated either by isocratic or gradient elution and measured at 320 nm. Additionally, *Blosczyk* (6) improved the analytical procedure. Teats and soothers were then kept in boiling water for 10 minutes, cooled down to room temperature and stored in a dessicator for 1 hour. Afterwards, 1 dm^2 of the pretreated samples was cut into small pieces and stored for 24 hours in 200 ml distilled water at 40 °C. This aqueous migration liquid was extracted twice with 50 ml of dichloromethane, evaporated to dryness and dissolved in 5 ml acetonitrile. Chemical analyses were conducted with 20 µl volumes of extract injected on a C_8 column and chromatographed with a mixture of water, containing 1% of acetonitrile, (A) and pure acetonitrile (B).

This research resulted in the draft manuscript of the European Standard prEN 1400-3 (September 1996) «Child care articles, Soothers for babies and young children, Part 3, Chemical requirements and tests», which is considered as a recommended method for the determination of MBT. Although not at all elaborated, it is mentionned in annex that the application of preconcentration columns can be used as a substitute for the laborious extraction with dichloromethane. In this paper we describe a rapid and save alternative solid phase extraction (SPE) procedure for the extraction step preliminary to the determination of MBT in water.

Experimental

Reagents and materials

Vulcanization agent
- 2 mercaptobenzothiazole: Merck 5996

Other reagents
- Acetonitrile: Labscan 2502
- Dichloromethane: Labscan C2510L
- Bond Elut C_8: 500 mg/6 ml (Varian 1210-2053)
- Bond Elut C_{18}: 500 mg/6 ml (Varian 1210-2052)

Apparatus

The used HPLC system consisted of a Varian 9010 pump, equipped with an injection loop of 10 µl, a GILSON 232XL automatic injection system, a variable wavelenght UV detector (Applied BIO System) working at 320 nm and a PC workstation. Separations were performed with an ALLTIMA C8 column from ALLTECH (n° 88076, 250 × 4,6 mm, 5 µm), using acetonitrile:water:acetic acid (700:300:0.3) as mobile phase. All runs were done at 1 ml min^{-1}; the retention time of MBT was 4 ± 5% min.

Sample preparation

The samples (each sample consisted of 2 soothers) were kept in boiling water for 10 minutes. After cooling down for 1 hour in a dessicator the samples (± 2 g) were cut in small pieces and brought into contact with 100 ml water for 24 h at 40 ± 5 °C. Subsequently, the aquous solution was passed through a C_8 Bond Elut preconditioned cartridge: in first instance 2 ml methanol was used as eluens, secondly 10 ml water was used and finally the elution was terminated with 5 ml acetonitrile. Prior to analysis we diluted the solutions if necessary.

Calibration Curve

A stock solution of 100 mg MBT $(100\ ml)^{-1}$ was prepared in acetonitrile. Working standard solutions of 1, 2, 4, 5 and 10 mg l^{-1} were obtained by appropriate dilution of the stock with acetonitrile.

The standard curve, obtained from triplicate injections of each working standard, evidences good linear response (Figure 1, R^2 = 0,9997). The calibration sensitivity, which is the slope of the calibration curve at the concentration range of interest, equals 0.00001 mg l^{-1} (surface unit)$^{-1}$. Since there is a general agreement that the sensitivity of a chemical analysis is limited by both the slope of the

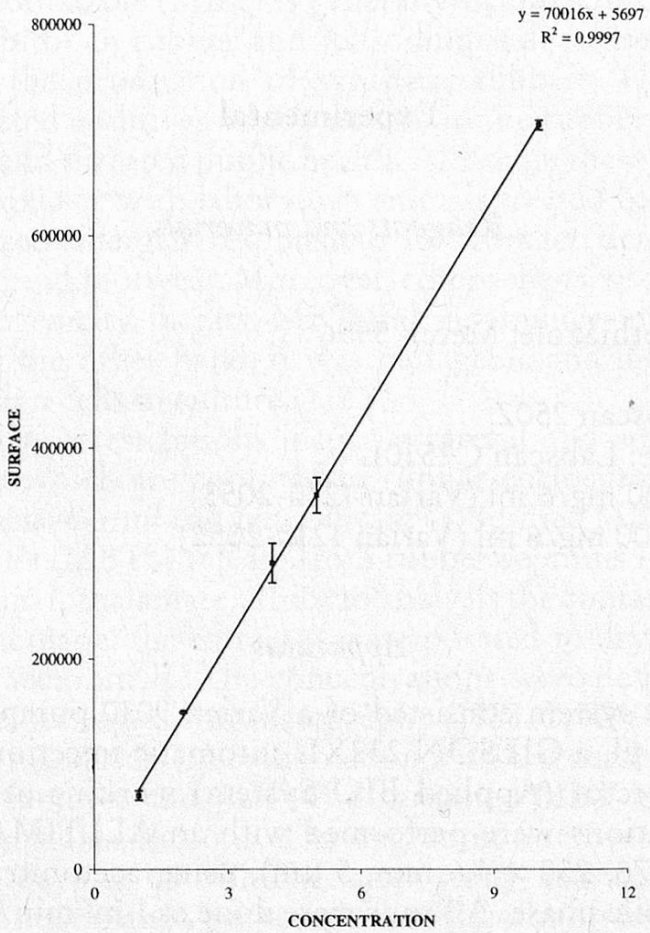

Fig. 1. Calibration curve for triplicate measurements of 5 MBT standard solutions

calibration curve as well as the precision of the measurement, we add here the analytical sensitivity, which is defined as the slope normalised to the standard deviation of the measurement. The value ranges from 5.4 10^{-10} to 1.9 10^{-8} for the calibration concentrations used. The detection limit, defined as 3 times the observed precision of the lowest standard, amounts to 0.056 mg MBT l^{-1}.

Recovery

In order to test the recovery of the proposed cartridge extraction 10 working standards (4 mg MBT l^{-1}) were prepared by appropriate dilution of a stock solution of 100 mg MBT (100 ml)$^{-1}$ in methanol. Therefore, we first prepared an intermediate dilution of the stock solution (1 ml in 100 ml water) which was again diluted and subsequently passed onto the preconditioned C_8 cartridges. Absolute concentrations and relative recoveries are given in table 1. The mean recovery is 88 ± 4%.

Table 1. Observed recoveries of the solid phase extraction of MBT

	Standard recovery (mg l^{-1})	Relative recovery (%)
1	3.64	91.1
2	3.48	87
3	3.52	88.2
4	3.74	93.7
5	3.37	84.3
6	3.55	88.8
7	3.34	83.6
8	3.80	95.2
9	3.34	83.6
10	3.35	83.8

We have also compared dichloromethane extractions, SPE C_{18} and SPE C_8 column extractions. The best results were obtained with SPE C_8.

An analysis of the repeatability was carried out with 4 equal parts of one single soother. MBT migration of each soother part was done in agreement with the recommended procedure, extraction of MBT on cartridges was done as described in the «experimental» section.

The observed concentrations amounted to 20, 21, 20 and 16 mg MBT per kg of rubber respectively, meaning an average concentration of 19 ± 2 mg MBT kg^{-1}.

Discussion

The dichloromethane extraction procedure is tedious. Moreover, for reasons of sustainable environmental quality and safety the use of chlorated solvents cannot

be recommended. In contrast herewith, the SPE extraction is a rapid procedure, it uses but small solvent volumes and, last but not least, this method allows for a significant concentration of the substrate under examination.

The proposed method was successfully applied for analyses of soothers and teats, presently available on the Belgian marked. We analysed 19 soothers and teats from different supply companies. The measured concentrations vary between 0.05 and 45.13 mg MBT kg^{-1} (table 2). Here must be added that the acceptable limit of 0.05 mg MBT kg^{-1}, proposed by the Belgian legislation, is very often largely exceeded.

Table 2. Frequency distribution of MBT concentrations in commercially available soothers and teats

Results of MBT	Number of sample
< 0.050 mg/kg	2
between 0.050 mg/kg and 1 mg/kg	9
between 1 mg/kg and 10 mg/kg	5
> 10 mg/kg	3

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Summary

2-mercaptobenzothiazole (MBT) is a widely used vulcanization agent in the rubber industry. MBT can cause allergic reactions after skincontact. From a toxicological point of view, no decision was taken by the working group concerning the classification and labelling of dangerous substances (Directive 67/548/CE).

Blosczyk (6) determines MBT by HPLC after extraction of the simulating liquid with dichloromethane. A more elegant and more environment friendly method is proposed whereby MBT in the simulating liquid is passed onto a SPE C_8 column and eluted with acetonitrile.

Zusammenfassung

2-Mercaptobenzothiazol (MBT) ist ein Vulkanisierungsmittel und wird häufig in der Gummiindustrie verwendet. In Kontakt mit der Haut kann MBT Allergien hervorrufen. Was die Giftigkeit des MBT betrifft, ist bis jetzt von den Toxikologen der Arbeitsgruppe, die sich mit Klassifizieren und Etikettieren gefährlicher Substanzen befasst (Richtlinie 67/548/CE), kein Beschluss gefasst worden.

Blosczyk (6) bestimmt MBT durch HPLC nach Extraktion der Simulansflüssigkeit mit Dichloromethan. Eine elegantere und umweltfreundlichere Methode wurde entwickelt, wobei MBT aus der Simulansflüssigkeit an einer SPE-C_8-Säule absorbiert und mit Acetonitril extrahiert wird.

Résumé

Le 2-mercaptobenzothiazole est un agent de vulcanisation largement utilisé dans l'industrie du caoutchouc. Le MBT peut causer des allergies au contact de la peau. Du point de vue de la toxicité du MBT aucune décision n'a encore été prise par les toxicologues participant au groupe de travail concernant la classification et l'étiquetage des substances dangereuses (Directive 67/548/CE).

Blosczyk (6) détermine le mercaptobenzothiazole par HPLC après extraction du liquide simulateur au dichlorométhane. Une méthode plus élégante et moins polluante est mise au point en passant le liquide simulateur sur SPE C_8 et en éluant ensuite avec l'acétonitrile.

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