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# Determination of 2-Mercaptobenzothiazole (MBT) in Tannery Wastewater by High Performance Liquid Chromatography with Amperometric Detection

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2-(Thiocyanomethylthiol)benzothiazole (TCMBT) is widely used for preservation of partially processed leather ("wet-blue" state) and also for wood preservation. Even though TCMBT itself does not involve major health hazard, its degradation products are considered toxic (Evans et al. 2000). The primary product of TCMBT hydrolysis is 2-mercaptobenzothiazole (MBT), which is both persistent and toxic in the aquatic environment (Reemtsma et al. 1997, Fiehn et al. 1998). Another important source of MBT contamination is the rubber industry. The levels of MBT reported in the industrial wastewater, in the effluents of wastewater treatment plant and in different natural waters ranged from few $\mu g\ l^{-1}$ up to 1.3 mg $l^{-1}$ (Shackelford 1983, Rennie 1988, Fiehn et al. 1994). In addition to incomplete elimination of MBT during the wastewater treatment, this compound was shown to inhibit the degradation of other organic xenobiotics (Reemtsma et al. 1995). Worth noting, MBT was found as the main metabolite of TCMBT in urine of the exposed rats and humans (Manninen et al. 1996).

The determination of MBT has been performed in various industrial samples (Parbey and Taylor 1989, Hinojosa Reyes et. al 2002) and also in the environmental materials (Dietrich et al. 1988, Ferrer and Barceló 1999). In most applications, the clean-up and/or preconcentration protocols were needed, based on different solvent extractions (Fiehn et al. 1994, Ferrer and Barceló 1999, Hinojosa Reyes et al. 2002), solid phase extraction (Martinez et al. 2001), solid phase micro extraction (Bellavia et al. 2000) and volatilization through derivatization (Manninen et al. 1996). The obtained extracts were analyzed by reversed phase high performance liquid chromatography (HPLC) with spectrophotometric detection (Parbey and Taylor 1989, Niessen et al. 1993, Fiehn et al. 1994, Ferrer and Barceló 1999) or by gas chromatography (Dietrich et al. 1988, Manninen et al. 1996). To the best of our knowledge, the combination of liquid chromatography with electrochemical detection (HPLC-ED) has not been explored yet. The benefits of such coupling rely on the high resolution power of HPLC, potential-dependent electro activity of the solutes and outstanding sensitivity of amperometric measurements. HPLC-ED has proved to be successful in the determination of different aliphatic and aromatic compounds in complex chemical systems (LaCourse 1997). In particular, its feasibility for several thio-

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compounds (methionine, cysteine, cystamine and glutathione) in biological samples have been demonstrated (LaCourse and Owens 1995). In this work, the application of HPLC-ED technique is proposed for the determination of MBT in wastewater matrix.

## MATERIALS AND METHODS

A model PM-80 high performance liquid chromatography pump was used with degasification system 2C-26B, EPSILON amperometric detector and flow-through electrochemical cell CC-5 (0.3 μl) (Bioanalytical System Inc.). The working electrodes tested were gold (28 $mm^2$), glassy carbon (28$mm^2$) and glassy carbon (113 $mm^2$). The reference and auxiliary electrodes were Ag/AgCl and stainless steel electrode, respectively. The equipment was controlled by software package Chromgraph 2.34.00 (Bioanalytical System Inc.) The second chromatographic system was a Hewlett-Packard Series 1050 with a multiple wavelength spectrophotometric detector and ChemStation.

The solvents were of HPLC - grade and all others chemicals were of analytical – reagent grade. Deionized water (Labconco, USA) was used throughout. The stock standard solutions of 2-mercaptobenzothiazole and of 2-mercaptobenzoxazole (1000 $\mu g \cdot ml^{-1}$ each) were prepared in acetonitrile. The following Sigma reagents (Sigma, St. Louis, MO, USA) were used: phosphoric acid, acetic acid, monochloroacetic acid and sodium hydroxide. Acetonitrile was from J.T. Baker Chemicals, USA.

Tannery wastewaters were from León city, Mexico. Two samples were kindly provided by Sistema de Agua Potable y Alcantarillado de León. These were immediately filtered (0.22 μm), kept at 4°C and analyzed within 2 days (triplicate analysis of each sample). After appropriate dilution (1:50, v/v), the samples were introduced to the chromatographic system. Chromatographic column and separation/detection conditions are given in Table 1.

## RESULTS AND DISCUSSION

The method of internal standard (IS) was applied in this work. The selection criterions for IS were: (i) similarity of its physicochemical properties to those of the MBT and (ii) detection of the two compounds under similar conditions. As can be observed in Figure 1, the molecular structures of MBT and 2-mercaptobenzoxazole (MBO) are almost identical, which should assure their similar chromatographic and electrochemical behavior.

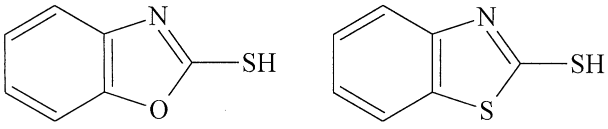
The reversed phase separation of these two compounds was examined using spectrophotometric detection (280 nm for MBO and 324 nm for MBT). The base line resolution was obtained performing isocratic elution with acidified aqueous solution (pH 2 – 4.5): acetonitrile (1:1). No significant differences in elution profiles were observed while using different pH buffers, namely phosphate, acetate buffers and monochloroacetic acid in the concentration range 0.10 – 20

**Table 1.** Instrumental operation conditions for the proposed HPLC-ED procedure.

| **Chromatographic conditions** | |
|---|---|
| Column | Hypersil C18 (250 x 4.6 mm, 5µm) |
| Mobile phase | Phosphate buffer (3.0 mmol $l^{-1}$, pH 2.4):acetonitrile (1:1, v/v) |
| Elution | Isocratic |
| Flow rate | 1.0 ml $min^{-1}$ |
| Injected volume | 20 µl |
| **Amperometric detection** | |
| Working electrode | Glassy carbon, 113 $mm^2$ |
| Reference Electrode | Ag/AgCl |
| Auxiliary electrode | Stainless steel |
| Potential applied | 900 mV |
| Measurement mode | Peak area, nA·s |

mmol $l^{-1}$. However, in the direct application to wastewater samples this procedure lacked both the selectivity and sensitivity. As mentioned in the introduction, to circumvent this problem, different clean-up and preconcentration schemes have been proposed in the literature. In this work, the electrochemical detection was examined as a means to improve analytical performance without necessity of extensive sample pretreatment.

The preliminary experiments carried out by cyclic voltammetry in the solutions simulating the mobile phase composition, the oxidation of MBT and MBO was observed at the potentials higher that 400 mV. The proposed mechanism for the anodic response of thiocompounds is based on catalytic nature of noble metal electrodes. According to this mechanism, the analyte is first adsorbed on the oxide-free surface and then oxidized with formation of respective oxides on the electrode surface (LaCourse and Owens 1995). Not surprising though, a passivation of the noble metal working electrode has often been observed. For cleaning/renovation of the electrode surface, the application of pulsed electrochemical detection has been recommended (LaCourse 1997). In this work, the amperometric detection at constant potential was used. To check for possible passivation effect, the performance of gold and gassy carbon working electrodes was compared (area 28 $mm^2$). The detection conditions were studied at different potentials applied (400 – 1000mV) and varying the composition of mobile phase within the limits assessed in the HPLC–UV experiments. Based on the value of signal-to-noise ratio as the selection criterion, the mobile phase composed of phosphate buffer (3.0 mmol $l^{-1}$, pH 2.4): acetonitrile (1:1, v/v) was chosen and the detection was carried out at 900 mV. The base line separation of MBO and MBT was achieved with the retention times of the two compounds 4.4 min and 5.3 min, respectively. As expected, better detection sensitivity was obtained using the gold working electrode, yet the passivation of the surface occurred (Table 2). Even though lower S/N was observed with glassy carbon electrode, the analytical signals for MBT and MBO was highly reproducible up to 25 consecutive injections and also over several days. These results clearly indicate that the presence of oxide-free noble metal surface is not indispensable for anodic



MBO MBT

**Figure 1.** Molecular structures of 2-mercaptobenzothiazole (MBT) and 2-mercaptobenzoxazole (MBO).

oxidation of the two thiols studied. To enhance the detection sensitivity with glassy carbon, the electrode of larger surface area was examined (113 $mm^2$). The obtained parameters are presented in Table 2. Again, excellent reproducibility of

**Table 2.** Comparative characteristics of three working electrodes. Detection was carried out at 900 mV, 45 $\mu g\ l^{-1}$ MBT and 150 $\mu g\ l^{-1}$ MBO.

| Electrode | | Fouling effect | Analytical parameters evaluated | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | MBT | | | MBO | | |
| Material | Surface area | | S/N | CV | DL | S/N | CV | DL |
| Gold | 28 $mm^2$ | YES | 6.2 | - | 5.0 | 12.2 | - | 5.6 |
| Glassy carbon | 28 $mm^2$ | NO | 3.9 | 0.45 | 8.0 | 8.8 | 0.31 | 7.7 |
| | 113 $mm^2$ | NO | 23 | 0.51 | 1.0 | 54 | 0.34 | 1.4 |

**Key:** S/N – signal-to-noise ratio; CV, % – coefficient of variance for 25 consecutive injections; DL, $\mu g\ l^{-1}$– detection limit, based on three standard deviations of the noise level

**Table 3.** Analytical parameters evaluated for MBT by the proposed HPLC-ED procedure and the analytical results obtained in the analysis of two wastewater samples. (instrumental conditions given in Table 1)

| Parameter | With internal standard | | Without internal standard | |
|---|---|---|---|---|
| Calibration equation | $9.92 \cdot 10^{-3}\, c_{MBT} - 17.3 \cdot 10^{-3}$ | | $36.6\, c_{MBT} - 28.7$ | |
| $R^2$ | 0.9997 | | 0.9989 | |
| Standard error for slope | $9.27 \cdot 10^{-5}$ | | 0.656 | |
| Standard error for intercept | $4.43 \cdot 10^{-3}$ | | 9.8 | |
| QL, $\mu g\ l^{-1}$ | 3.2 | | 4.1 | |
| Analytical results, mg $l^{-1}$ MBT (RSD, %) | | | | |
| | Ext. Cal.[1] | St. Add.[2] | Ext. Cal.[1] | St. Add.[2] |
| Sample 1 | 0.42 (3.8) | 0.45 (5.9) | 0.40 (7.5) | 0.47 (13) |
| Sample 2 | 0.84 (2.9) | 0.81 (4.5) | 0.83 (6.4) | 0.88 (10) |

**Key:** $c_{MBT}$ – analyte concentration, $\mu g\ l^{-1}$; QL – quantification limit; RSD – relative standard deviation; [1,2] – results obtained in the analysis of this same sample, using two different calibration techniques: Ext. Cal. – calibration curve and St. Add. – standard addition method.

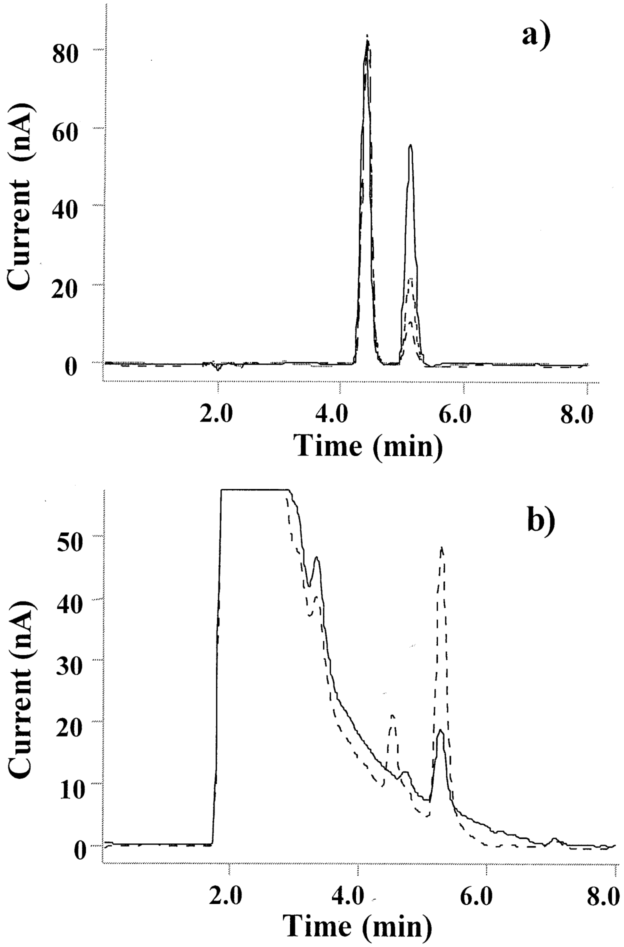


**Figure 2.** Typical HPLC-ED chromatograms of mixed standard solutions (a) and wastewater sample (b). The instrumental conditions given in Table 1.

(a) (⋯) 15 µg l$^{-1}$ MBT and 75 µg l$^{-1}$ MBO, (- - -) 30 µg l$^{-1}$ MBT and 75 µg l$^{-1}$ MBO, (——) 75 µg l$^{-1}$ MBT and 75 µg l$^{-1}$ MBO.

(b) (⋯⋯) 1:50 diluted sample and (——) diluted sample with standard addition: 50 µg l$^{-1}$ MBT and 10 µg l$^{-1}$ MBO.

the MBT and MBO signals was observed, confirming that the oxides formed during anodic reaction did not cause surface inactivation.

Once the experimental conditions were selected (Table 1), the analytical parameters of the proposed HPLC-ED procedure were evaluated using two types of analytical signal. In the first case, the area of MBT chromatographic peak was taken, and in the application of internal standard technique, the ratio between MBT and MBO chromatographic peaks was used. For calibration, the mixed standard solutions were used that contained 0; 7.5; 15; 30 and 75 $\mu g\ l^{-1}$ of MBT and 75 $\mu g\ l^{-1}$ of MBO. Typical chromatograms of these solutions are presented in Fig. 2a. In Table 3 the linear regression functions, regression coefficients, standard errors for slope and intercept as well as quantification limits obtained for the two signal types are presented. As can be observed in this Table, the application of MBO as an internal standard enabled a slight improvement of all these parameters.

The determination of MBT was carried out in two tannery wastewater samples. Owing to the high sensitivity of electrochemical detection, the dilution (1:50, v/v) of samples was needed. This procedure together with enhanced selectivity of amperometric as compared to spectrophotometric detection enabled to observe well resolved elution of the two target compounds, at these same retention times as the standards. Typical chromatograms of the sample and the spiked sample are presented in Fig. 2b. The recoveries at two levels of MBT (20 and 40 $\mu g\ l^{-1}$) were 93 ± 6 % and 96 ± 4 %, respectively. The quantification was carried out by ex ternal calibration and by the three point standard addition (15; 30 and 50 $\mu g\ l^{-1}$ MBT, 10 $\mu g\ l^{-1}$ MBO in the diluted sample). As can be observed in Table 3, a good agreement was obtained between the results obtained by two quantification techniques and also using two different types of analytical signal (1 - peak area of MBT and 2 - ratio MBT/MBO). For statistical evaluation, the analysis of variance was carried out, showing no statistically significant differences (ANOVA, $p < 0.05$). These results indicate that the use of internal standard did not affect the accuracy of determination, yet it assured the enhanced precision (compare relative standard deviations in Table 3).

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