Analysis of Mercaptobenzothiazole (MBT) Compounds from Sulfur Cured Rubber by a Liquid Chromatography – Tandem Mass Spectrometry (LC-MS-MS) Method

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INTRODUCTION

Mercaptobenzothiazole (MBT) and other benzothiazoles are common vulcanization accelerators for rubber materials that are used in pharmaceutical container/systems, such as the gaskets in the pressurized Metered-Dose Inhaler (pMDI). MBT is of particular concern since it is considered a potential carcinogen and has been shown to migrate into drug formulations.
Due to the toxicological concern and leachability of MBT and other benzothiazoles, analytical methods have been developed to study these types of compounds in the fields of food additives and contaminants (1), contact dermatitis caused by the rubbers (2), as well as pharmaceutical packaging systems (3). MBT can be analyzed by gas chromatography (4) but many other benzothiazoles are thermally-labile and readily decomposed in the GC inlet. HPLC methods are commonly used to study. MBT and other benzothiazoles (2,5) and showed good sensitivity, accuracy and repeatability.
MBT, nitrosamines and polynuclear aromatic (PNAs) are considered extractable/leachables of special toxicological concern by US FDA (6,7) that require special attention. In practice, analytical methods with greater sensitivity and selectivity need to be developed in the controlled extraction study and leachable study to quantify these compounds in different drug development phases.
In this study, a method using liquid chromatography with tandem mass spectrometer (LC-MS-MS) was developed to analyze MBT in the sulfur cured rubber. The method is capable of detecting ng level of MBT in the rubber extracts. This study demonstrates the feasibility of using detector with high selectivity, such as LC-MS-MS method, for extractable/leachable with special toxicological concern that requires greater sensitivity and specificity.

EXPERIMENTAL

Method Conditions

HPLC Parameters

Mobile Phase A: Acetonitrile: Water: Formic Acid 20:80:0.05
Mobile Phase B: Acetonitrile: Water: Formic Acid 90:10:0.05
Flow Rate: 0.2 mL/minute
Column: Waters Symmetry C18, 3.5 µ, 2 x 50 mm
Column Temperature: 40°C
Autosampler Temperature: Ambient
Injector Volume:1 µL
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PE Sciex API 2000/API365 Triple Quadruple Mass Spectrometer

Ionization Mode:	Positive ElectroSpray
Detection Mode:	MRM MBT @ m/z 168/135 MBTS @m/z 333/167

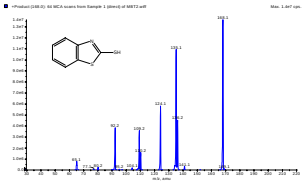

Figure 1: MS-MS Spectrum of MBT

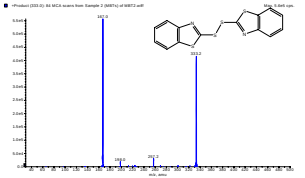

Figure 2: MS-MS Spectrum of MBTS

Selectivity/Specificity

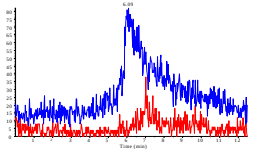

Figure 3. MRM Chromatogram of Extraction Blank: MBT (blue trace) and MBTS (red trace)

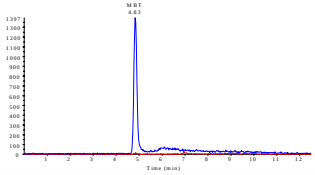

Figure 4. MRM Chromatogram of MBT (250 ng/mL): MBT (upper trace) and MBTS (lower trace) – no MBTS detected in the MBT standard

RESULTS

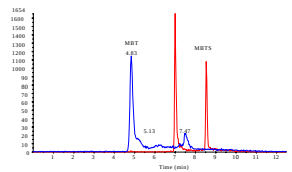

Figure 5. MRM Chromatogram of MBTS (500 ng/mL): MBT (upper trace) and MBTS (lower trace) – MBT was detected in the MBTS standard

Extraction Method

Hansson et al (2) has studied the extraction of MBT/MBTS using different solvents. They found out that Methyl tert-Butyl Ether (MTBE) is a good solvent for MBT/MBTS due to its:powerful extraction medium

•Low toxicity

•Inertness to MBT/MBTS

•High volatility

In this study, the rubber was cut into 3 x 3 mm particles. One gram of the rubber was extracted with 10 mL MTBE for 30 minutes by sonication. After extraction, the extract was diluted to different volume using Methanol: Water 50: 50 diluent to give varying MBT concentrations and filtered using glass fiber syringe filters for LC-MS study.

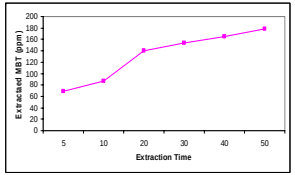

Figure 6. The Extraction Study of of MBT by MTBE from Sulfur-Cured Rubber

Accuracy - Filter Study

A Filter study was conducted to verify that the syringe filter used in the sample preparation did not reduce the recovery of MBT and MBTS. Three 500 ng/mL standards were analyzed before and after the filtration and the area responses of MBT and MBTS were compared. The percent differences between the filter and non-filtered samples are less than 2.5% indicating that filtration does not affect the method accuracy.

Compounds	Mean area responses	
	MBT	MBTS
Before Filtration	4082	2130
After Filtration	3990	2151
% Difference	2.3	1.0

Accuracy- MBT Recovery

Approximately 360 ng/mL of MBT was spiked into the extract. The sample was prepared using the sample preparation procedure and analyzed. Three replicates of spiking samples were prepared and analyzed. The mean recovery of MBT was 87.3%

Table 2: Recovery Results of MBT

	Extract (ng/mL)	Replicate 1 (ng/mL)	Replicate 2 (ng/mL)	Replicate 3 (ng/mL)
Expt'l	219.5	567.8	552.6	553.2
%RSD	3.5 (n=7)	2.3	2.4	3.0
%Rec	NA	89.9	86.0	86.1

LOQ/LOD

The DL of MBT was calculated using S/N ratio = 3.
DL = 6 ng/mL in the solution or 12 pg on column.

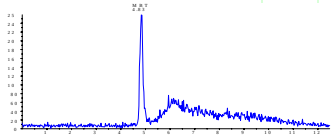

Figure 9. MRM Chromatogram of MBT Standard (50 ng/mL)

Accuracy - Spiking Study of MBTS

MBTS (410 ng/mL) was spiked into the extract and analyzed using the LC-MS-MS method. No MBTS was recovered but ~204 ng/mL of MBT was observed. It is concluded that MBTS was not stable under the experimental conditions and underwent homolytic cleavage of the S-S bond to form MBT. One molecule of MBTS would form one molecule of MBT.

At present, a study is being conducted to investigate the byproduct of MBTS under the current experimental conditions.

Table 3. Spiking Study of MBTS
MBT Concentration (ng/mL)

	Extract (ng/mL)	Replicate 1 (ng/mL)	Replicate 2 (ng/mL)	Replicate 3 (ng/mL)
Calculated (ng/mL)	219.5	428.8	422.4	421.6
MBT Recovered (ng/mL)	NA	209.2	202.9	202.1
Average MBT Recovered	204.7			
MBTS Spiked	410.7 (equivalent to 206.7 ng/mL MBT)			
%Recovery	99.0%			

Repeatability

Four replicates of the sulfur cured rubber samples were extracted and analyzed by the LC-MS-MS method. The mean concentration of MBT was 56.4 ppm and the %RSD (n=4) was 7.1%

Table 1. Calculated MBT Concentration (PPM) in Four Replicates of Extract

Calculated Concentration (PPM)				Mean	%RSD (n=4)
Extract 1	Extract 2	Extract 3	Extract 4		
61.2	52.7	53.4	58.0	56.4	7.1

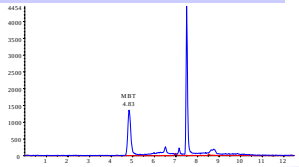

Figure 7. MRM Chromatogram of a Representative Rubber Extract: MBT (blue trace) and MBTS (Red trace). No MBTS was observed in the extracts.

Linearity

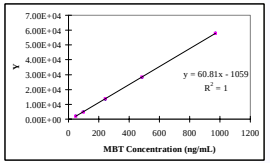

Figure 8. Linearity Plot of MBT (50 – 1000 ng/mL)

CONCLUSION

1. A LC-MS-MS method was developed to analyze MBT in the MTBE extracts of sulfur cured rubber.
2. The method is specific for MBT and it is linear over 50 – 1000 ng/mL.
3. The method is reproducible for extractable MBT determination.
4. The percent recovery of MBT is 87.7% at 400 ng/mL level.
5. The DL of MBT is 6 ng/mL or 12 pg on column.
6. MBTS is not stable under the current experiment condition. It forms MBT in a 1 to 1 ratio. This method quantifies the total MBT in the extract.
7. Further study to identify the by product of MBTS and to develop a MBTS compatible method is in progress.

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