

Harpur Hill, Buxton  
Derbyshire, SK17 9JN  
T: +44 (0)1298 218000  
F: +44 (0)1298 218590  
W: [www.hsl.gov.uk](http://www.hsl.gov.uk)



**Method Validation for MbOCA analysis on nitrile  
and leather rigger gloves**

**HSL/2006/101**

Project Leader: **John Unwin**

Author(s): **John Unwin**

Science Group: **Health Improvement**

# CONTENTS

<b>1</b>	<b>INTRODUCTION.....</b>	<b>1</b>
<b>2</b>	<b>EXPERIMENTAL .....</b>	<b>2</b>
2.1	Analysis parameters .....	2
2.2	Calibration procedure .....	5
2.3	Glove extraction efficiency.....	7
<b>3</b>	<b>CONCLUSIONS.....</b>	<b>9</b>

## EXECUTIVE SUMMARY

Poor chromatographic resolution of the target analyte was encountered during an on-going survey being carried out as an HSE enforcement exercise investigating exposure to the carcinogen 4,4'-Methylene-bis(2-chloroaniline) (MbOCA), a curing agent used in the manufacture of moulded polyurethane products. The aim of the survey was to investigate airborne and skin exposure as well as surface contamination and the effectiveness of protective gloves. Several of the glove types encountered could not be analysed by the routine high performance liquid chromatographic (HPLC) method (MDHS 75) due to interferences from co-extracted materials. An alternative procedure was required to complete the analysis for the survey for the leather and disposable nitrile polymer based gloves encountered.

The procedure adopted utilised gas chromatography with mass-selective detection (GC-MS) to achieve separation from interferences and an internal standard, 3,3'-dichlorobenzidine (DCB), to ensure good analytical robustness.

### Objectives

- Develop an analytical procedure for the determination of MbOCA on nitrile and leather rigger gloves.
- Determine the extraction efficiency of MbOCA from nitrile and leather rigger gloves.

### Main Findings

- For both leather rigger and nitrile gloves full chromatographic resolution of MbOCA and DCB (internal standard) was observed from the co-extracted components of the glove matrix.
- The analytical method was able to selectively quantify MbOCA in the glove matrix.
- The extraction efficiency of MbOCA from rigger and nitrile gloves was quantitative with a range of than 93-98% for the concentrations studied.
- This method may also be suitable for the analysis of MbOCA on marigold type gloves and many other types of disposable gloves.

### Recommendations

- The procedure described should be adopted for all future analysis of MbOCA where matrix interferences are anticipated or when HPLC analysis is not available.

# 1 INTRODUCTION

As part of the Disease Reduction Programme (Carcinogens) a survey to measure the exposure of workers to 4,4'-Methylene-bis(2-chloroaniline) (MbOCA) during the manufacture of moulded polyurethane products was carried out. MbOCA may cause bladder cancer if it is breathed in, taken into the body with food, drink or cigarettes, or absorbed through the skin and consequently it has a WEL of only 0.005 mg/m<sup>3</sup>. A strict control regime including the use of PPE (gloves and workwear) is required in order to minimise exposure through skin absorption and by inhalation during critical operations such as mixing of MbOCA with polymer resin.

The routine laboratory method of analysis normally carried out for the quantitation of MbOCA on cotton swabs (surface wipes), cotton gloves and acid-coated GFA filters (air monitoring) utilises high performance liquid chromatography (HPLC) with UV-detection (MDHS 75 – Aromatic amines in air and on surfaces). During this survey it was observed that for certain types of gloves such as disposable nitrile and leather rigger gloves that extracts were highly loaded with co-extracted material which interfered with the analysis method by HPLC. An alternative procedure was sought for these difficult samples.

MbOCA has been analysed by gas chromatography as the heptafluorobutyric acid anhydride derivative (OSHA method 71) using electron capture detection utilising a derivatisation step to increase sensitivity and selectivity. A more direct procedure requiring no additional sample pre-treatment other than extraction would be preferable for extensive surveys of workplaces involving large numbers of samples. A direct procedure involving the use of 3,3'-dichlorobenzidine (DCB) as the analytical internal standard was investigated. Replicates of nitrile and leather gloves were spiked with MbOCA, extracted with solvent and an aliquot removed and analysed by GC/MS after addition of the internal standard. Quantitation was carried out using matrix matched standards in order to overcome any potential instrument inlet or chromatographic discrimination during the analysis and to ensure a linear detector response.

## 2 EXPERIMENTAL

### 2.1 ANALYSIS PARAMETERS

Figures 1a and 1b show the chemical structures of MbOCA and the internal standard DCB respectively. These differ by only one  $-\text{CH}_2-$  group and have identical functionality which makes DCB effective as an analytical internal standard.

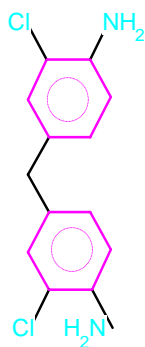


Figure 1a. Structure of 4,4'-Methylene-bis(2-chloroaniline) (MbOCA).

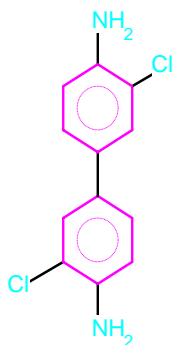


Figure 1b. Structure of 3,3'-Dichlorobenzidine (DCB) internal standard.

Figure 2a and 2b show the mass spectra of MbOCA and DCB respectively. Both compounds show strong molecular ion peaks  $(\text{M}-\text{H})^+$ . The limited amount of fragmentation of these molecules is beneficial for compound identification and analytical sensitivity.

Almost full co-elution of the two analytes occurs using the specified column, however complete spectral resolution is achieved enabling accurate quantitation. A chromatogram of a matrix matched MbOCA standard with internal standard is shown in Figure 3. The full GC-MS instrumental conditions for the analysis are given in Table 1.

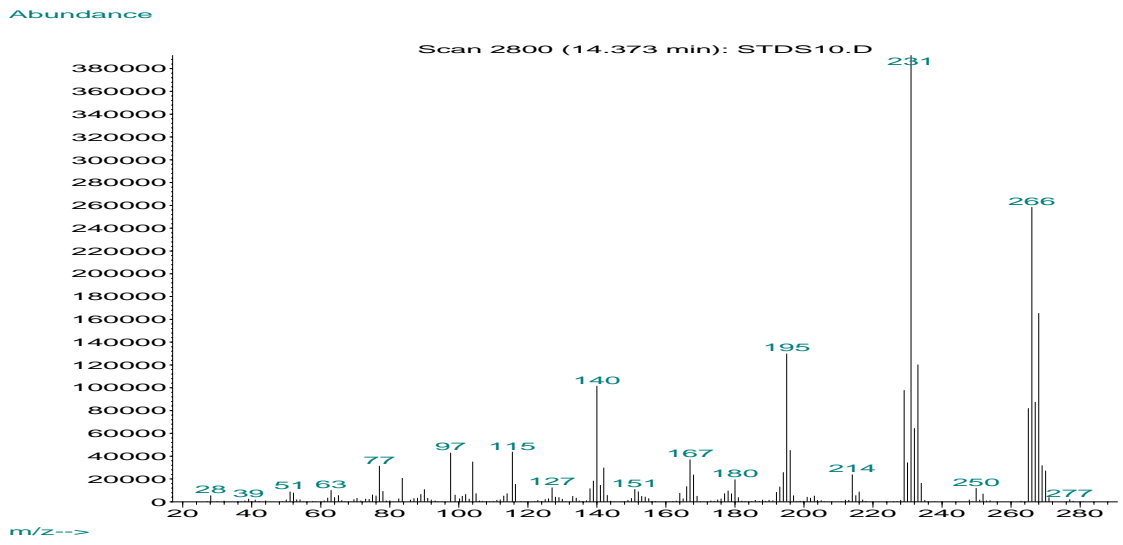


Figure 2a. Mass spectrum of MbOCA ((M-H)<sup>+</sup>= 266).

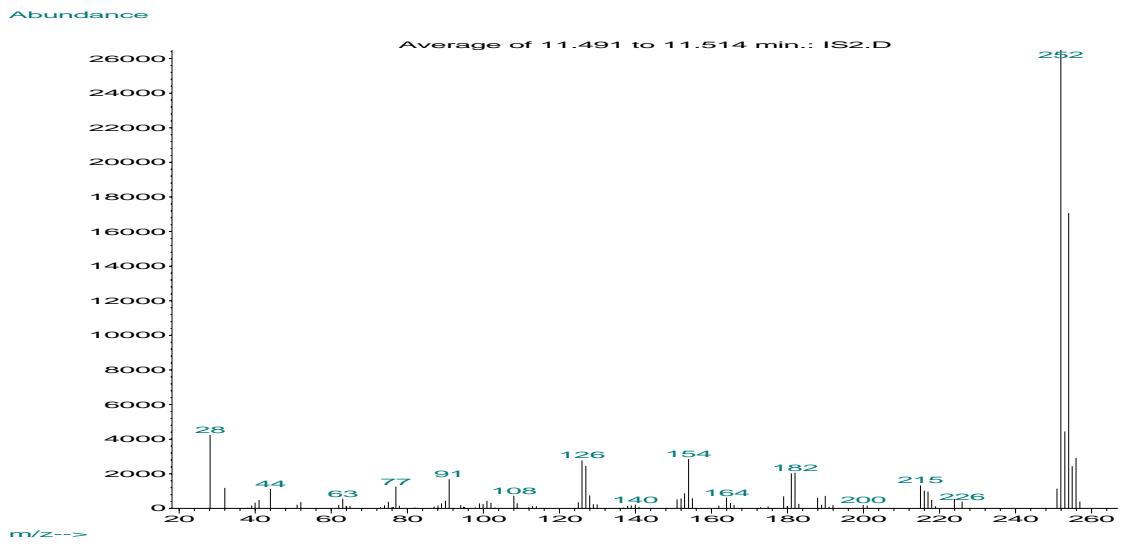


Figure 2b. Mass spectrum of DCB ((M-H)<sup>+</sup>=252).

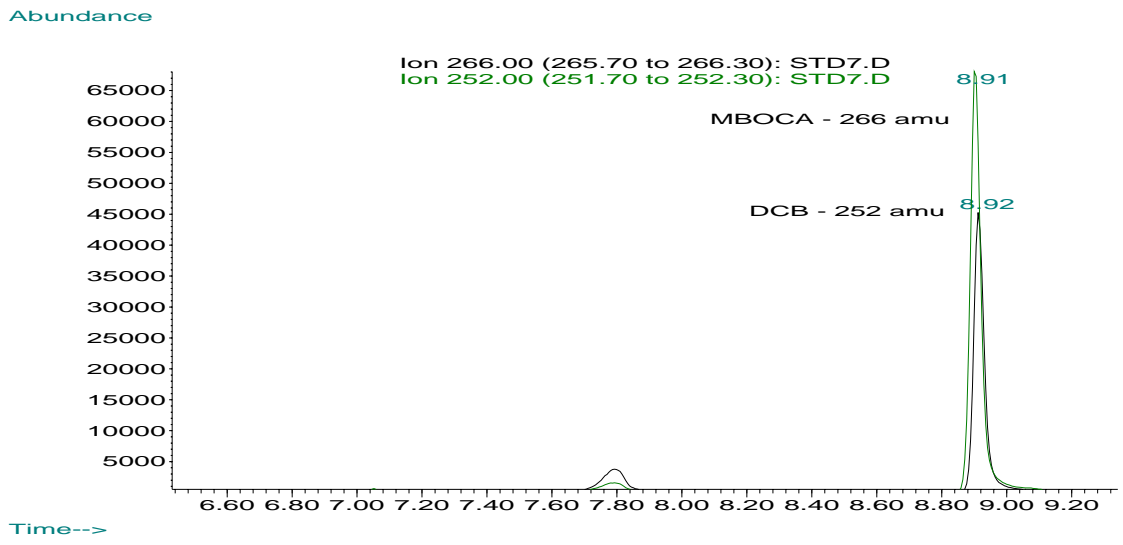


Figure 3. A chromatogram of a nitrile glove matrix matched standard.

Table 1. GC-MS Instrumental condition used in the analysis of MbOCA on disposable nitrile and rigger gloves.

<b>GC</b>	Agilent 6890
Column:	HP5-MS. 30m x 250 $\mu$ m, film thickness 0.25 $\mu$ m
Inlet:	250 °C, Splitless, constant flow (initial pressure 10 PSI)
Oven temperatures (°C):	
Initial:	60
Rate:	40
Final:	250
Hold time (min):	5
Run time (min):	9.75
Retention time (min):	
MbOCA:	8.91
DCB:	8.92
<b>MS</b>	Agilent 5973
Electron Multiplier:	+ 200 V
Monitored ions (amu) :	
MBOCA:	266*, 268 (qualifier).
DCB:	252*.
	* ions used in quantitation.
Limit of Detection: ( $\mu$ g/glove):	
Disposable purple nitrile :	1.0
Leather rigger:	4.0

## 2.2 CALIBRATION PROCEDURE

### 2.2.1 Matrix Matched Calibration Standards

Matrix matched calibration standards are prepared by the addition of known amounts of MbOCA to appropriate volumes of a glove extract which has not been exposed to MbOCA. The blank glove extract is prepared in the same way as gloves which have been exposed to MbOCA. The calibration range used in the determination of extraction efficiency for nitrile and rigger gloves is given in Table 2 and the resulting linear calibration plot for nitrile gloves is shown in Figure 4.

Table 2. Typical calibration range for MbOCA analysis on nitrile and rigger gloves

Standard No.	Volume of stock solution (b or c) ( $\mu$ l)	Volume of matrix match solution ( $\mu$ l)	[MbOCA] (ng/ml)
1	100 <sup>c</sup>	900	66.7
2	200 <sup>c</sup>	800	133.3
3	300 <sup>c</sup>	700	200
4	25 <sup>b</sup>	975	666.7
5	50 <sup>b</sup>	950	1333
6	100 <sup>b</sup>	900	2666
7	200 <sup>b</sup>	800	5332

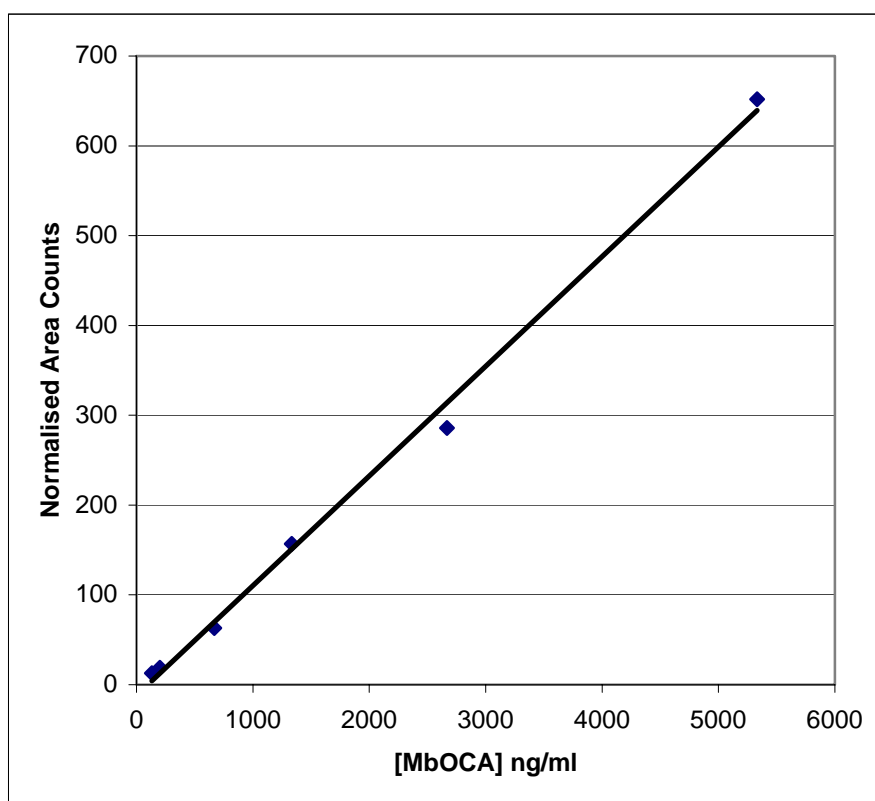


Figure 4. Matrix matched calibration plot for nitrile gloves ( $r^2 = 0.999$ ).

## 2.2.2 Preparation of Stock and calibration solutions

### 2.2.2.1 MbOCA

4,4'-Methylene-bis(2-chloroaniline), 26.66 mg, was dissolved in acetonitrile and made up to 10 ml (solution A). Two further dilutions, 0.1 ml solution A in 10 ml acetonitrile (solution B) and 0.1 ml standard 4 (see Table 2) in 1 ml acetonitrile (solution C) was used in the preparation of the calibration range.

### 2.2.2.2 DCB

3,3' Dichlorobenzidine, 3.54 mg, was dissolved in acetonitrile and then made up to 100 ml. This solution, 50 µl, was added to 1 ml of each sample and standard prior to analysis which resulted in a DCB concentration of approximately 1.8 µg/ml.

## 2.3 GLOVE EXTRACTION EFFICIENCY

Three replicates of each type of glove were spiked at 2 levels with MbOCA using stock solution A. The gloves were then extracted in PTFE bottles using acetonitrile (500 ml and 100 ml for nitrile and leather gloves respectively) for 1-hr on a roller-extractor. The spiking levels are shown in table 3.

The extracts, 1 ml, were spiked with internal standard, 50 µl, then analysed using their respective matrix matched standards. Matrix matched performance samples were analysed after every 3 sample analyses.

Table 3. Spiking levels of MbOCA on leather and nitrile gloves

Leather		Nitrile	
Volume solution A (ml)	Mass of MbOCA (mg)	Volume solution A (ml)	Mass of MbOCA (mg)
1.0	2.666 mg	0.2	0.5332 mg
0.1	0.2666 mg	0.02	0.0533 mg

### 2.3.1 Results and discussion

The extracts from the nitrile gloves were highly coloured (pink) originating from the dye used in the fabric part of the glove and also contained high levels of phthalates and other materials which had caused the interferences observed in the liquid chromatographic analysis. The leather gloves yielded extracts with a pale purple coloration which contained several discrete unidentified components.

Analysis of the extracts without the use of matrix matching of calibration standards and the internal standard resulted in poor precision of the analysis and non-linear calibration lines.

The results of the determination of extraction efficiency are given in Table 4. The extraction efficiency for nitrile gloves ranged from 92 – 98% and 93 – 99% for leather nitrile gloves.

Table 4. Recovery of MbOCA from leather and nitrile gloves

Recovery ( %)			
Leather		Nitrile	
Level 1	Level 2	Level 1	Level 2
98	95	98	93
99	93	95	92
97	95	95	95
<b>MEAN 98</b>	<b>94</b>	<b>96</b>	<b>93</b>

### 3 CONCLUSIONS

- For both leather rigger and nitrile gloves full chromatographic resolution of MbOCA and DCB (internal standard) was observed from the co-extracted components of the glove matrix.
- The analytical method was able to selectively quantify MbOCA in the glove matrix.
- The extraction efficiency of MbOCA from rigger and nitrile gloves was quantitative with a range of than 93-98% for the concentrations studied.
- This method may also be suitable for the analysis of MbOCA on marigold type gloves but a further evaluation is required.