

VALIDATION OF METHOD FOR 4-CHLOROANILINE AND 4,4'-BI-O-TOLUIDINE FROM DYED LEATHER

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Toxicity assessment of leather products designed to obtain leather goods must be in accordance with legislation and environmental standards which require the use of chemicals from semi-finished leather processing. Azo dyes will be checked for two banned aromatic amines (4-chloroaniline and 4,4'-bi-o-toluidine) which can be found as a result of splitting azo dyes used to dye the leather. The method will be validated by gas chromatography (GC-MS) and the following parameters will be tested: accuracy (trueness and reliability), precision, sensitivity, detection limit, limit of quantification, working range, linearity.

Keywords: Validation method, azo dyes, leather, 4-chloroaniline, 4,4'-bi-o-toluidine

INTRODUCTION

The most toxic products, mutagens, carcinogens (Eurachem Guide, 1998) were the first to have alarmed European Union and imposed publication of several directives that prohibit or limit the use of these chemicals (Directive 99/51/EC on pentachlorophenol, Directive 2002/61/EC banning azo dyes, Directive 2009/563/EC on eco-labelling footwear, Directive 2010/75/EU on industrial emissions in tanneries, REACH).

Azo dyes account for about 90% of all dyes (Eurachem Guide, 1998) used for finishing leather and textiles. Azo dyes decompose under reductive conditions to form aromatic amines, derivatives of benzidine. 22 aromatic amines are banned in the EU Regulation 552/2009, and 2 more were added in 2015.

EU Regulation provides test method for each type of substrate and defines the detection limit of 30 mg/kg for each amine that may be found in the leather and therefore this is the limit. Other aromatic amines from leather were investigated (www.cdc.gov; Huber, 2010; Chelaru *et al.*, 2016). The objective of this study is to identify the presence of 4-chloroaniline, 4,4'-bi-o-toluidine used in dyed leather designated for clothing manufacture.

MATERIALS AND METHOD

Materials

Methanol; T-butyl methyl ether; Sodium dithionite, minimum purity 87%; Aqueous solution of sodium dithionite, 200 mg/ml, prepared daily; N-hexane; Amines; Methanolic sodium hydroxide 20% (w/v), 20 g of NaOH dissolved in 100 ml of methanol; Distilled water - grade 3 according to SR EN ISO 3696: 2002 - Water used for analytical laboratories. Specifications and methods of analysis.

Method

Testing method for leather is established by EN ISO 17234-1:2015: Chemical tests for the determination of certain azo colorants in dyed leathers - Part 1 - Determination of certain aromatic amines derived from azo colorants.

RESULTS

The studies were performed on bovine hides experimentally dyed in the laboratory obtained with known concentrations of amine in the study (4-chloroaniline and 4,4'-bi-o-toluidine).

4-chloroaniline

4-chloroaniline (CAS 106-47-8) is part of chlorinated organic compounds. The chemical formula (linear form) is $\text{ClC}_6\text{H}_4\text{NH}_2$ and structural form is displayed in Figure 1.

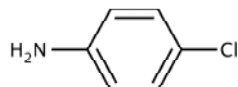


Figure 1. 4-Chloroaniline

4-Chloroaniline is an important compound used in the chemical industry for the production of leather dyestuffs, pesticides and drugs. 4,4' bi-o-toluidine is very toxic, possibly carcinogenic, absorbed through skin (www.cdc.gov). There was a total of 10 specimens of each individual skin sample treated with one of three amines. For each specimen the steps in SR EN ISO 17234-1: 2015 were followed (Table 1).

Table 1. Information about tested component

Component	Concentration	No. of analyses
4-chloroaniline	200 $\mu\text{g/ml}$	10

Table 2. GC-MS and concentration integrated values

Integrated mass units	Det. ($\mu\text{g/ml}$)	Integrated mass units	Det. ($\mu\text{g/ml}$)
23159019	205,64	19992053	203,13
22909319	205,44	19847365	203,01
23659174	206,04	20579603	203,59
19778586	202,96	20077222	203,19
21984956	204,71	22441986	205,07

Table 3. Values for accuracy formula

Accuracy	Domain	Value
Accuracy % = $\frac{X_{average}}{\mu} \times 100$	90-110%	102.14
Bias % = $\frac{X_{average} - \mu}{\mu} \times 100$	-	2.14 %

Table 4. Values for fidelity formula

Fidelity	Domain	Value
CV (RSD) % = $\frac{s}{X_{average}} \times 100$	2-20%	0,58%

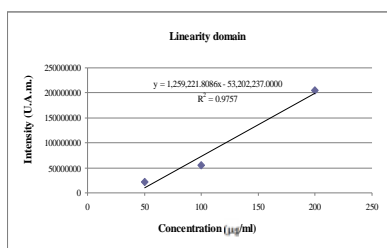
Table 5. Values for repeatability formula

Repeatability	Domain	Value
$r = 2.8 \times s_r$	-	3.42 µg/ml

Linearity Domain

Table 6. GC-MS and concentration integrated values for linearity domain

Integrated mass units	(det., µg/ml)
21236364	50
55503678	100
204380880	200



The form of linear regression equation function:

$$y = 1259221.88x - 53505237$$

b= slope calibration

$$b = 1259221,88 \text{ areas units} \times \mu\text{g}^{-1}$$

$$R^2 = 0.9757$$

Figure 2. 4-chloroaniline linearity domain

Table 7. Values for detection, quantification limit and work domain formula

	Formula	Value
Detection limit	LoD = 3s	3.67 µg /ml
Quantification limit	LoQ = 10 s	10.22 µg/ml
Work domain		
Lower limit	LI = 50 µg/ml	
Upper limit	LS = 200 µg/ml	

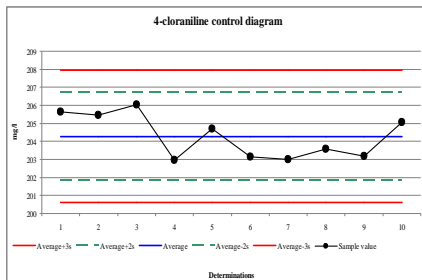


Figure 3. 4-chloroaniline control diagram

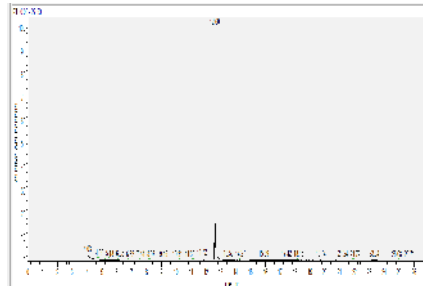


Figure 4. 4-chloroaniline GC-MS chromatogram

4,4'-bi-o-toluidine

4,4'-bi-o-toluidine (CAS 119-93-7) is part of organic compound. The chemical formula (linear form) is $(C_6H_4(CH_3)NH_2)_2$ and structural form is displayed in Figure 5.

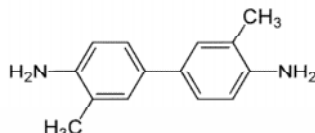


Figure 5. 4,4'-bi-o-toluidine

4,4'-bi-o-toluidine is an intermediate for the production of soluble azo dyes and insoluble pigments used particularly in the textile, leather and paper industries. It is toxic and possibly carcinogenic. It is listed as an IARC Group 2B carcinogen, meaning it is "possibly carcinogenic to humans". Animal studies have shown that animals exposed to 4,4'-bi-o-toluidine developed tumors in the liver, kidney, and mammary glands (www.cdc.gov).

Table 8. Information about tested component

Component	Concentration	No. of analyses
4,4'-bi-o-toluidine	200 µg/ml	10

Table 9. GC-MS and concentration integrated values

Integrated mass units	(det., µg/ml)	Integrated mass units	(det., µg/ml)
1572098500	200,84	1392618607	178,51
1497543548	191,56	1377947260	176,56
1569498199	200,51	1285159747	165,15
1492700267	190,96	1302565826	167,31
1466693430	187,72	1258529985	161,83

Table 10. Values for accuracy formula

Accuracy	Domain	Value
Accuracy % = $\frac{X_{mediu}}{\mu} \times 100$	90-110%	91.05
Bias % = $\frac{X_{mediu} - \mu}{\mu} \times 100$	-	-8,95 %

Table 11. Values for fidelity formula

Fidelity	Domain	Value
CV (RSD) % = $\frac{s}{X_{mediu}} \times 100$	2-20%	7.87%

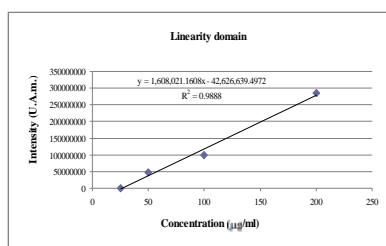
Table 12. Values for repeatability formula

Repeatability	Domain	Value
$r = 2.8 \times s_r$	-	40.15 $\mu\text{g/ml}$

Linearity Domain

Table 13. GC-MS and concentration integrated values for linearity domain

Integrated mass units	det., ($\mu\text{g/ml}$)
12882.326	25
47825938	50
98829960	100
285832597	200



The form of linear regression equation function:

$$y = 1608021.1608x - 42626639.4972$$

b= slope calibration

$$b = 1608021.1608 \text{ areas units} \times \mu\text{g}^{-1}$$

$$R^2 = 0.9888$$

Figure 6. 4,4'-bi-o-toluidine linearity domain

Table 14. Values for detection, quantification limit and work domain formula

	Formula	Value
Detection limit	$\text{LoD} = 3s$	4.45 $\mu\text{g/ml}$
Quantification limit	$\text{LoQ} = 10s$	14.83 $\mu\text{g/ml}$
Work domain		
Lower limit	$\text{LI} = 50 \mu\text{g/ml}$	
Upper limit	$\text{LS} = 200 \mu\text{g/ml}$	

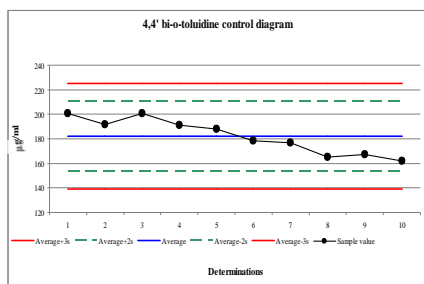


Figure 6. 4,4'-bi-o-toluidine control diagram

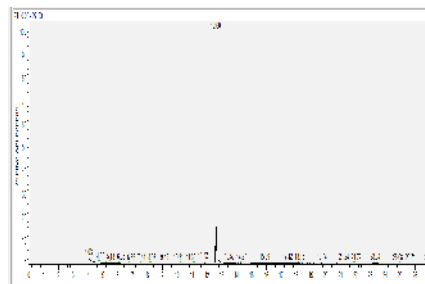


Figure 7. 4,4'-bi-o-toluidine GC-MS chromatogram

CONCLUSION

This paper presents a study for the implementation of gas chromatographic instrumental method for determination of aromatic amines derived from azo colorants used for dyeing leather and its validation. Official testing method is EN ISO 17234-1:2015: Chemical analysis for the determination of certain azo colorants in dyed leathers - Part 1 - Determination of certain aromatic amines derived from azo colorations.

From the calculation method performance parameters we can see the following results:

- Linearity range of the method is between 25-200 µg/ml range in which the correlation coefficient was in the range 0.9757 to 0.9888. For a better linearity, correlation coefficient must be between 0.9800 and 1.0000.
- Detection limit of the amines ranges from 3.67 µg/ml and 4.45 µg/ml;
- The limit of quantification of the amines ranges from 10.22 µg/ml and 14.83 µg/ml;
- The accuracy is 91.05% and 102.14% and is approximation of the actual value and the value found in the sample. For the gas chromatograph method for the performance must be within 85-110%;

Validated analytical method for the determination of aromatic amines in leather meets all the conditions required to be used and applied.

Acknowledgment

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