FALSE POSITIVES II – CHLOROPHENOLS IDENTIFICATION TOWARDS HPLC-DAD-MS ANALYSIS COMPARED TO ISO 17070:2015 TECHNIQUE

Gustavo Adrián Defeo F.S.L.T.C., Miria Borgheresi PhD., Dr. Manila De Cicco, Dr. Bianca Carpignani

Ars Tinctoria SRL – Analytical laboratory

Via del Bosco 125 (IT-56029) Santa Croce sull'Arno (PI) Italy +39 0571 35110

info@arstinctoria.it.

Abstract. The restriction of certain dangerous substances according to REACH (Registration, Evaluation, Authorisation of Chemicals as well as the Restricted Substances Lists (RSL) requirements promoted by various renowned brands obliges tanneries to everyday more numerous analysis with undesired conflictual situations on false positive tests results. This situation is worsened by the voluntary reduction of requested detection limits far below the levels recommended by the accepted ISO methods. On this context, ISO 17070:2015 was extended in its current version from the determination of pentachlorophenol to tetrachlorophenol, trichlorophenol, dichlorophenol and monochlorophenol isomers. Some brands also included under this technique the ortho phenyl phenol (OPP) analysis, requesting a quantification limit below 1 mg/kg for all analytes. The present paper proposes a new HPLC-DAD-MS direct method for the verification of chlorophenols positive cases, and its extension to leather chemicals analysis, as well as the discrimination among false positive cases and real positive ones. The paper also illustrates case studies reporting differences in the quantification of the said analytes and the chlorophenol scission in different analytical conditions

1 Introduction

Chlorophenols is a family of substances traditionally used as pesticides, herbicides and fungicides, but also as intermediates for dyes production. Following the limitation of use in many countries of Pentachlorophenol (PCP), different norms were developed for its determination on different materials.

For Pentachlorophenol determination on Leather, ISO 17070 (IULTCS/IUC 25)[1] norm "*Leather - Chemical tests - Determination of pentachlorophenol content*" was developed as reference method. The normalised test of just PCP remained so until the 2006 version included. This norm intended originally to validate the compliance of Leather to a European limitation of 1000 mg/kg of Pentachlorophenol. In the following different countries and brands requested lower limits for PCP, and thus the method was stressed in its quantification limit (some brands imposed voluntary limitations down to 0,01 mg/kg), but also in its extension to the complete chlorophenols five basic types, including positional isomers (19 analytes in total). From the 2015 version on, ISO 17070 norm was extended in scope to the different chlorophenol types *(ISO 17070:2015 (IULTCS/IUC 25))*[2].

In some voluntary analytical protocols, ISO 17070 method was also extended in scope to the quantification of orthophenylphenol (OPP) and 4-chloro-3-methylphenol (PCMC) known as chlorometacresol (CMK). Both additional analytes have a more accurate dedicated method (ISO 13365:2011 (IULTCS/IUC 29))[3] developed for HPLC, and with a demonstrated higher accuracy than the said adaptation.

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[1] *ISO 17070:2006 (IULTCS/IUC 25) Leather -- Chemical tests -- Determination of pentachlorophenol content*

[2] ***ISO 17070:2015*** *(IULTCS/IUC 25) Leather -- Chemical tests -- Determination of tetrachlorophenol-, trichlorophenol-, dichlorophenol-, monochlorophenol-isomers and pentachlorophenol content*

[3] *ISO 13365:2011 (IULTCS/IUC 29) Leather -- Chemical tests -- Determination of the preservative (TCMTB, PCMC,*

2 Chlorophenols

Chlorophenols include five basic types, depending the number of chlorine substituents, namely mono-chlorophenols (MonoCP), dichlorophenols (DiCP), trichlorophenols (TriCP), tetrachlorophenols (TeCP) and pentachlorophenol (PCP). Including their positional isomers in total sum 19 analytes (Fig. 1).

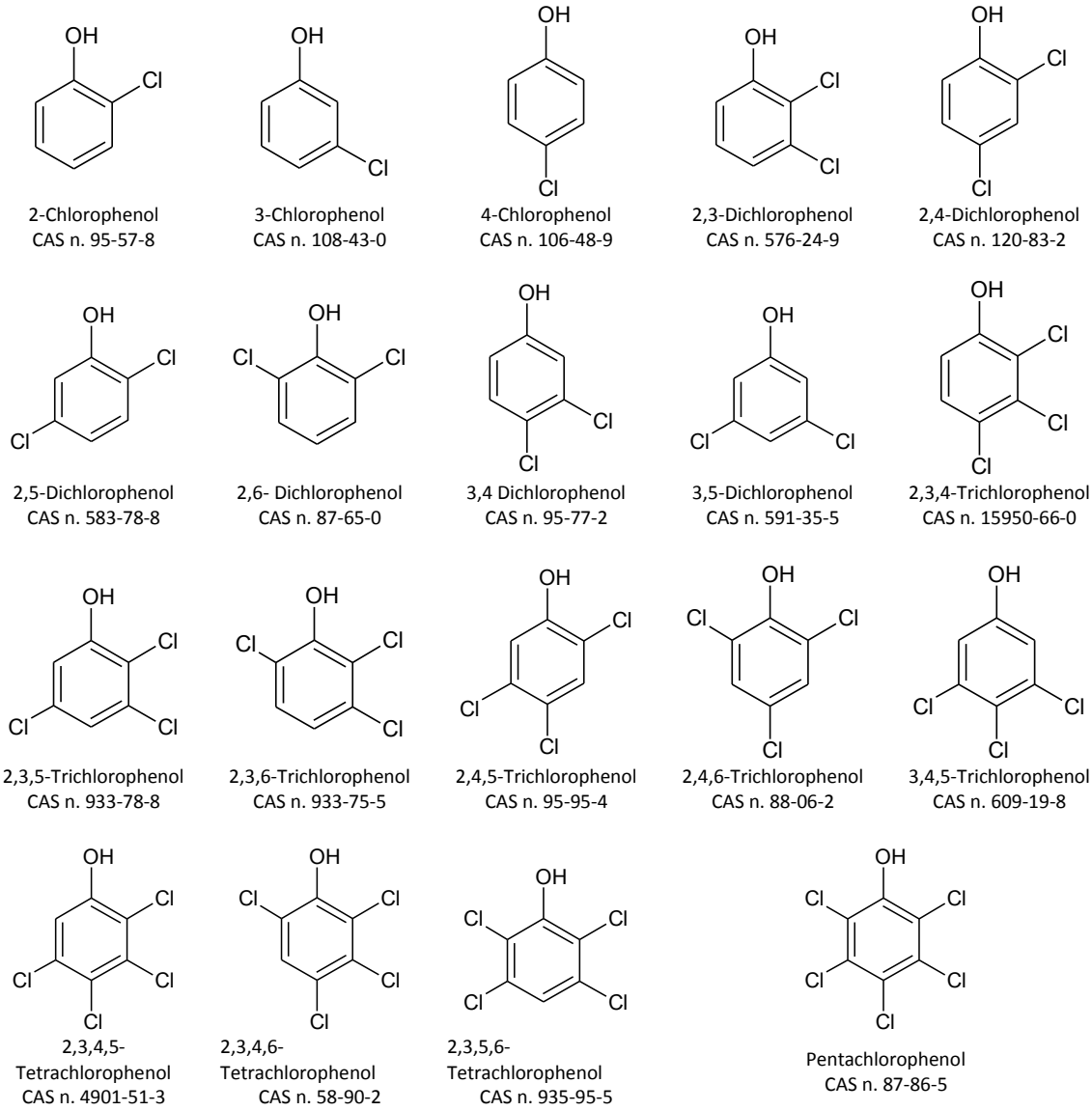

Fig. 1. Chlorophenols basic types and their isomers.

3 ISO 17070:2015 brief description

The grinded sample is dispersed in a 1M solution of H_2SO_4 and steam distilled. Acidic media is needed for the correct solvent separation of this analyte. At least 450 ml of distillate are collected in a K_2CO_3 solution. This solution is brought to a final volume of 500 ml, from where 100 ml are taken to undergo acetylation. This reaction is achieved by adding acetic anhydride

OPP, OIT) content in leather by liquid chromatography

and Triethylamine catalyst in a separating funnel where the carbonate solution is in contact with hexane. For quantification, the hexane phase containing acetylated chlorophenol is then injected in GC-MS, comparing with a similarly prepared internal standard.

Considering that this method includes a relatively long preparation technique uncertainty becomes relatively high below 5 mg/kg leading to frequent false positive results specially if quantification limits are requested below 1 mg/kg. Because of these critical points, we have studied a simpler direct method to confirm the effective presence of chlorophenols in suspected false positive analysis, or in cases where the requested quantification limit enters within the rumor levels of the GC-MS detector.

4 Chlorophenols determination in HPLC-MS direct method

4.1 Preparation

The HPLC-MS direct method for chlorophenols determination, was developed both for Leather and Leather chemicals.

For Leather: a sample was grinded, weighed and extracted in ultrasound bath during 20 minutes with an aliquot of terbutylmethylether, taking the supernatant solvent and repeating the operation other 2 times collecting together all three extractions. The extract is dried with a rotary evaporator and dissolved in 2 ml. methanol. Filtered with a 0,22 µm nylon filter, into e 2 ml vial and analysed in HPLC-DAD-MS.

For dyes and chemicals: In this case we adapted different extraction methods for mono and dichlorophenols respect tri, tetra and pentaclorophenol: For mono and dichlorophenols, a sample is weighed into an Erlenmeyer flask, adding an aliquot of distilled water, dissolving in ultrasound bath at room temperature.

Extraction of Tri-, Tetra- and Pentachlorophenol: a sample is weighed into an Erlenmeyer flask, adding an aliquot of methanol, dissolving in ultrasound bath at room temperature. In both cases, filtered with a 0,22 µm nylon filter, into e 2 ml vial and analysed in HPLC-DAD-MS.

4.2 Chromatographic HPLC-DAD-MS conditions

Chromatographic column: GEMINI C18 - 250 mm x 4,6 mm, 5 µm, 110 Å.
Mobile phase: Sodium bicarbonate 0.01 mmol/L: Methanol
Gradient: time program

Time	Methanol concentration
0 min to 20min	40% to 80% Methanol
20 min to 25 min	80% Methanol
25 min to 30min	40% Methanol

Flow: 800 µL/min
Injection volume: 20µL
Column oven temperature: 40°C

Mass-Spectrometer parameters
Nebulizing gas flow: 1,5 L/min
DL temperature: 250°C
Heat block temperature: 400°C
Drying gas flow: 10 L/min
Measurements were recorded in SIM mode.

In Tab. 1 we have reported the m/z ratios used for the identification of the different chlorophenols basic types.

Table 1. m/z signals for Chlorophenols identification.

	Target m/z	m/z	m/z	m/z
Pentachlorophenol	262.7	264.7	266.7	268.7
Tetrachlorophenols	228.7	230.7	232.7	
Trichlorophenols	194.9	196.9	198.9	
Dichlorophenols	160.9	162.9	164.9	
Monochlorophenols	127.0	128.0		

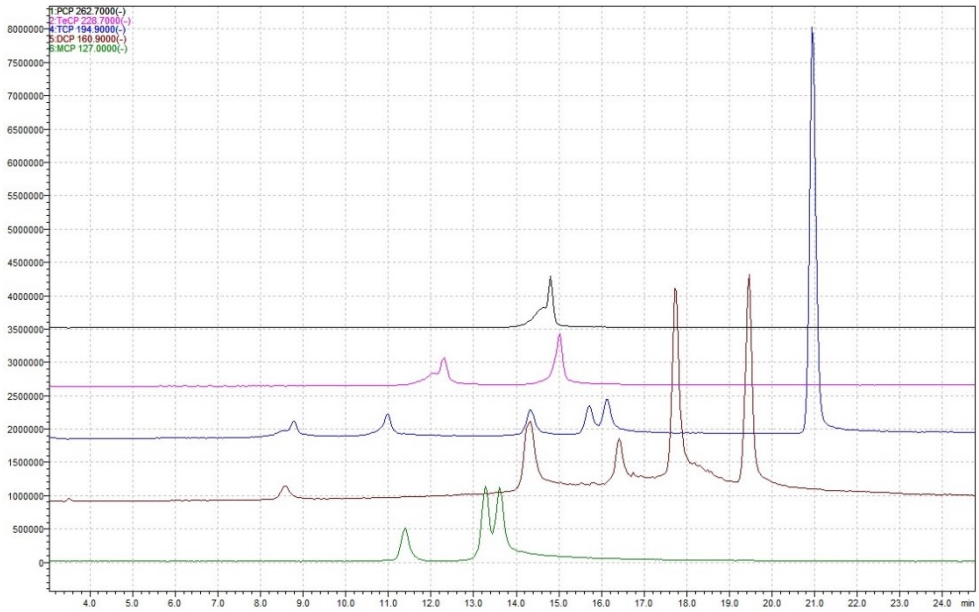

Fig. 2. Chromatogram shifted in the five registered SIM events.

5 Chlorine isotopic masses as identification evidence

The recorded mass signals for each chlorophenol showed the typical behaviour originated in the typical isotopic abundance of the chlorine atom. Each chromatographic peak, showes a signal where the target mass is associated to one or more mass peaks which differs of 2 mass units related to the number of chlorine substituents present on the phenolic ring. This effect, due to the natural abundance of the two stable chlorine isotopes 35Cl and 37Cl respectively in ratio 76%: 24 %. We consider this effect a very clear evidence for chlorophenols identification (Fig. 3).

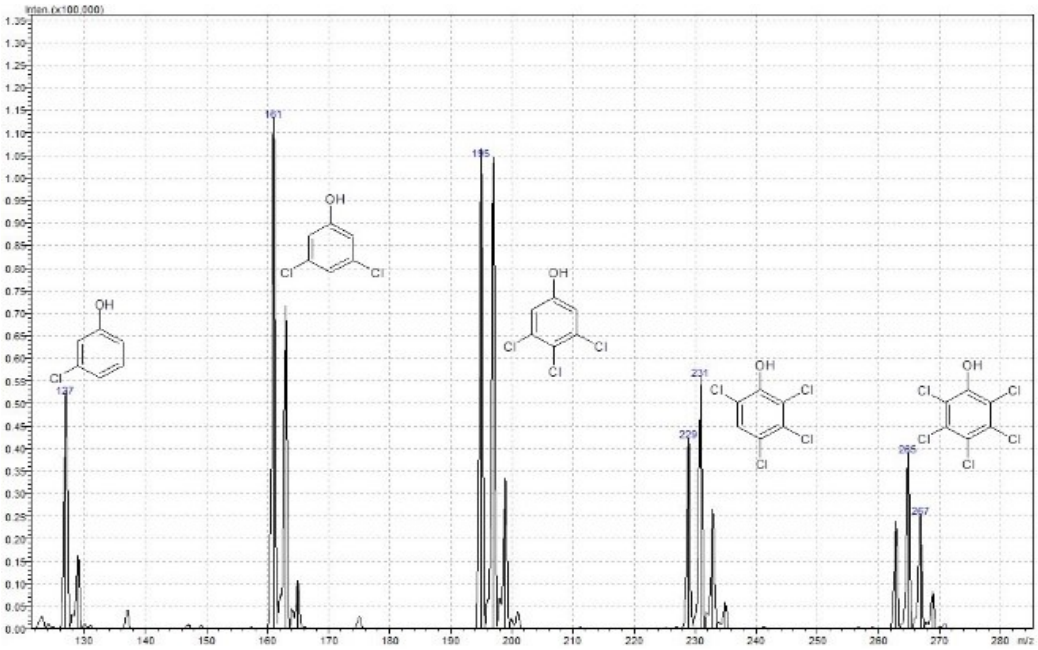

Fig. 3. m/z peaks to identifying the different Chlorophenols.

6 Comparison between ISO 17070:2015 and direct HPLC-DAD-MS methods.

Results obtained by means of ISO 17070:2015 and HPLC-DADMS direct method, were aligned within a reliable quantification limit of ca. 1 mg /kg of tetrachlorophenol and considering a workable uncertainty. Many retests done on samples reported positive with ISO 17070:2015 below 1 mg/kg and demonstrated in many cases the absence of the typical isotopic masses pattern, thus showing the false positive result. In some cases, the method allowed to determine the presence of pentachlorophenol and tetrachlorophenols within the 0,1 mg/kg // 1 mg/kg range, where the said effect was very evident. The HPLC-DAD-MS direct method helped for a quick quantification of chlorophenols traces on dyestuffs, and dyes intermediates.

7 A suspect case of 4-chlorophenol development from a halogenated dyestuff

Most tests carried out for the validation of this test method gave reasonable results on Leather samples and chemicals, considering a reasonable uncertainty level.
In the positive cases found it was demonstrated the use of the detected chlorophenol as precursor of related dyes intermediates. A peculiar case was observed with a monoazo dye, which gave positive results in the order of 100 mg/kg with the indirect method, but negative results with the direct method.
In this case we suspect break of the C-N bond of the monoazo dye during distillation or acetylation.
An analogous case of C-azo coupling reversibility was published in 1998 by V.S. Mokrushkin and M.A.Bezmaternikh[4]. One possible interpretation of 4-chlorophenol generation is described in Fig. 4. Further tests are being performed to demonstrate this eventual radical degradation.

4 *V.S.Mokrushin, and M.A.Bezmaternikh: 'The first example of reversible C-azo coupling in a series of aromatic and heteroaromatic compounds', Mendeleev Commun., 1998, 8(5), 197–198*

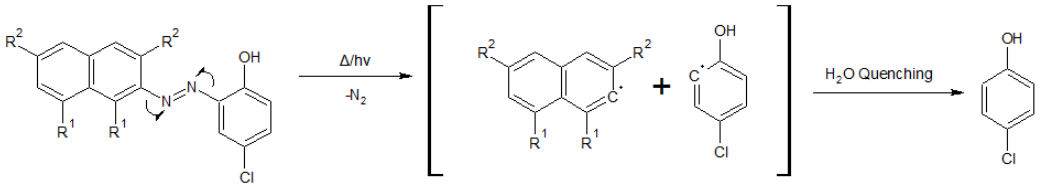

Fig. 4. Proposed radical degradation mechanism.

Conclusions

The proposed direct HPLC-DAD-MS method shown quick and reliable results, demonstrating eventual false positive results on the lower detection limits. Further tests are being performed to detect the possible generation of chlorophenols during distillation and acetylation when applying ISO 17070:2015 on halogenated substances.

Acknowledgement

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