Phthalates in tattoo and Permanent Make Up inks: quantification and validation by GC/MS.

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Introduction

Over the last few years, the huge diffusion of the practice of tattooing and permanent make-up (PMU) led EU Member States to focus the attention on possible risks for human health that may arise from the injection of inks into the dermis. Then, a possible exposure to potentially hazardous substances may occur. Among these, phthalates are substances that may be contained in inks that are classified as toxic for reproduction under the regulation (EC) N.1272/2008 (CLP) [1] and listed in Annex II (List of Substances Prohibited in Cosmetic Products) of the regulation (EC) N.1223/2009 on Cosmetic products [2]. The EU introduced a number of measures in order to make an attempt to face the increasing concerns about these risks for the population. In 2008, the Council of Europe issued the Resolution ResAp (2008)1 [3], that defined requirements and criteria for the evaluation of the safety of tattoos and PMU and drew a list of substances that should not be present in tattoo/PMU inks. A forthcoming restriction according to Annex XVII of the regulation (EC) N. 1907/2006 (REACH) on substances in tattoo and PMU ink will definitely regulate the sector [4]. The Laboratory for Chemical Safety (Istituto Superiore di Sanità), as National Reference Laboratory for the implementation of REACH and CLP regulations, carried out the development and in-house validation of a GC-MS method for the quantification of nine phthalates in this matrix. Method validation was performed according to requirements of ISO/IEC 17025 [5] and Eurachem Guide "The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics 2nd" [6].

Materials and Methods

Phthalates selection

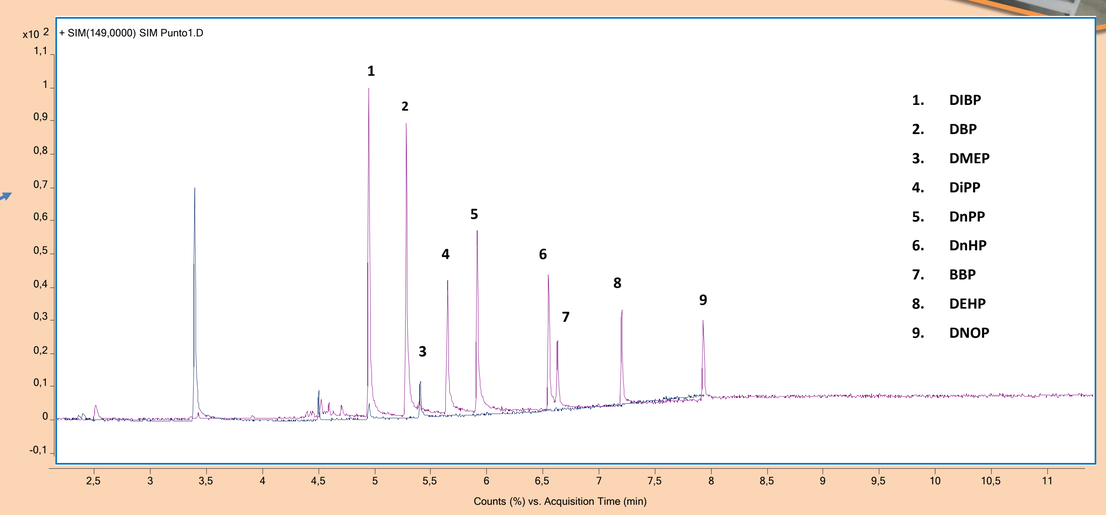
CAS	Phthalates
84-69-5	Diisobutyl phthalate (DIBP)
84-74-2	Dibutyl phthalate (DBP)
117-82-8	Bis(2-methoxyethyl) phthalate (DMEP)
605-50-5	Diisopentyl phthalate (DIPP)
131-18-0	Di-n-pentyl phthalate (DnPP)
84-75-3	Dihexyl phthalate (DnHP)
85-68-7	Benzylbutyl phthalate (BBP)
117-81-7	Bis(2-ethylhexyl) phthalate (DEHP)
117-84-0	Di-n-octyl phthalate (DNOP)

GC/MS method

GC Conditions	
Column	Zebron Semivolatiles 30 m x 0.25 mm d_c x 0.25 µm d_f
Flow Mode	1 mL/min, constant flow (He gas)
Inlet Mode	20:1 Split
Injection Amount	1 µl
Inlet Temperature	290° C
Solvent Delay	4.5 minutes
Initial Oven Temp, Hold Time	150° C, 1 min
Ramp 1	30° C/min, 280° C
Ramp 2	15° C/min, 310° C
Final Hold Time	3 minutes or longer

Phthalates extraction and Sample preparation

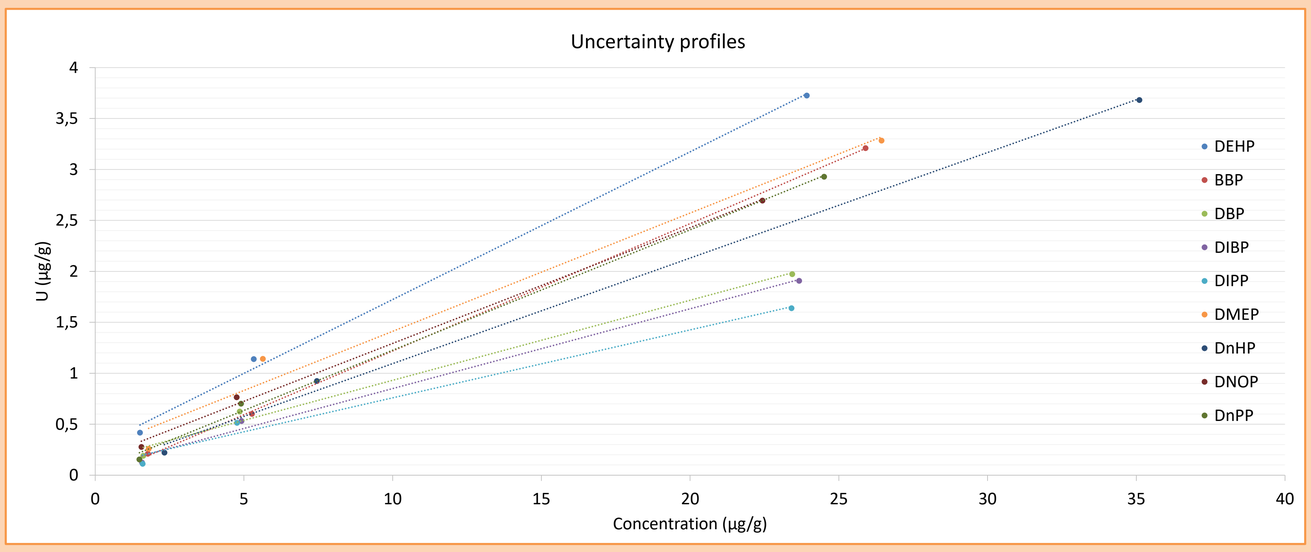
- ✓ Dissolution in tetrahydrofuran
- ✓ Precipitation of any dissolved polymer with a second solvent
- ✓ Analysis by Gas Chromatography-Mass spectrometry (GC-MS)



Results

For all substances of interest, performance characteristics such as Limit of Detection (LoD, 0.044 µg/g - 0.115 µg/g), Limit of Quantification (LoQ, 0.148 µg/g - 0.384µg/g), Working Range (1.48 µg/g – 35.10 µg/g), Intermediate Precision (CV % 3.29% - 13.60%), Recovery (89.1 % - 93.0%) were assessed.

Uncertainty profiles for quantitative analysis of 9 phthalates



Measurement uncertainty was evaluated using the best available estimate of overall precision and bias (recovery) according to the Eurachem guide "Quantifying uncertainty in analytical measurement (QUAM: 2012)" [7].

$$u_c = \sqrt{(u_{rec}{}^2 + u_R{}^2)}$$

$$U = u_c * k$$

K = 2 (95%)

Conclusions

In spite of the challenging matrix to be analyzed, the present in-house validated method is found to be accurate and sensitive, furthermore, it allows a fast processing of samples and it is cost-effective which makes it particularly suitable for use in the official controls on tattoo/PMU inks.

This method will be a reliable tool in view of the REACH restriction. On 15 March 2019 the Opinion on an Annex XV dossier proposing restrictions on substances used in tattoo inks and permanent make-up was adopted by the Committee for Risk Assessment (RAC) and the Committee for Socio-economic Analysis (SEAC). The document proposed an option to establish limits of concentration.

References:

1. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures. OJ L 353, 31.12.2008.
2. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009.
3. Council of Europe. Resolution ResAP (2008)1 on requirements and criteria for the safety of tattoos and permanent make-up
4. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006.
5. European Standard. General requirements for the competence of testing and calibration laboratories ISO/IEC 17025:2017.
6. Eurachem Guide: The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics Second edition (2014).
7. EURACHEM/CITAC "Guide Quantifying Uncertainty in Analytical Measurement QUAM:2012 3rd Edition".

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