

Proceeding Paper

Quality Control in Cosmetics. A Five-Year Screening Survey on the Content of Phthalates in Colognes[†]

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Abstract: Phthalate esters are used in colognes and other cosmetics and related products to maintain fragrance. Additionally, phthalates can dissolve and stabilize certain aroma ingredients and essential oils. The results of a five-year study (2016–2020) conducted in a quality control laboratory on the presence of phthalate esters in various cosmetic products are presented. A total of 1147 samples from four cologne categories: eau de toilette, eau de cologne, fragrance, and perfume were analyzed using a powerful analytical technique such as gas chromatography-mass spectrometry (GC-MS/MS) for the quantification of the nine different phthalate esters (BBP, DEHP, DNOP, DNPP, DBP, DIPP, DMEP, DMP, PIPP) in each category. The results revealed the absence of phthalates at concentrations above the threshold limit ($1 \mu\text{g mL}^{-1}$) in 95% of the samples analyzed. However, different levels of phthalates were detected in 57 samples, mainly DEHP and DBP. Our findings also demonstrate distinct phthalate profiles according to cologne type, with relevant variations among the four cologne categories analyzed.

Keywords: phthalate esters; colognes; determination; GC-MS/MS

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1. Introduction

Cosmetic products consist of substances or combinations of substances designed for contact with the outer layers of the human body (such as epidermis, hair, nails, etc.) as well as teeth and oral mucous membranes. Cosmetics are used for the primary purposes of cleansing, perfuming, altering appearance, protecting, maintaining or correcting body odors. Due to their usage, products like colognes, perfumes, or fragrances, expose humans to their components, potentially causing skin reactions, allergies, or other adverse effects. Some research has suggested that specific phthalates might lead to negative health impacts, such as endocrine disruption and reproductive toxicity [1,2]. Phthalates belong to a category of chemical compounds employed in the production of scented items like colognes and perfumes for various reasons [3,4]. Phthalates aid in maintaining and stabilizing fragrances within products, ensuring that the scent remains consistent and doesn't dissipate rapidly after application. The use of phthalates in the formulation can slow down the evaporation of volatile fragrance components, contributing to a longer-lasting scent on the skin. Moreover, these compounds facilitate the blending of essential oils and other fragrance ingredients, ensuring even distribution in the cologne solution.

Governments and regulatory bodies must ensure and guarantee the safety of cosmetic products, including colognes, fragrances, and perfumes. Therefore, regulatory agencies around the world place restrictions on the use of phthalates in cosmetic and

personal care products. These regulations vary by country, but generally include limitations on phthalate concentrations and labeling requirements. In the case of the European Union, EC regulation 1223/2009 details the prohibited or restricted substances in cosmetics, in addition to indicating the labeling requirements for these products [5]. Among the different categories of chemical products used as additives in perfumes or colognes, European regulations subject phthalates to especially strict monitoring. Six phthalates (dibutyl phthalate (DBP), bis(2-methoxyethyl) phthalate (DMEP), diisopentyl phthalate (DIPP), di-n-pentyl phthalate (DNPP), benzyl butyl phthalate (BBP), and bis(2-ethylhexyl) phthalate (DEHP), have been banned as ingredients in cosmetics and personal care products due to its possible carcinogenic and mutagenic effects on human health.

In this paper, we present the preliminary results of a five-year quality control screening (2016–2020) for phthalate content using GC-MS/MS in a large database of colognes, which includes 1147 samples, encompassing eau de cologne, eau de toilette, fragrances, and perfumes.

2. Material and Methods

2.1. Samples Collection and Sample Preparation

In this study, a total of 1147 cosmetic samples were analyzed in a quality control laboratory specialized in textiles, cosmetics, and related materials during the period of 2016–2020. The samples belong to four different categories: eau de cologne ($n = 41$), eau de toilette ($n = 789$), fragrance ($n = 87$), and perfume ($n = 230$). In all cases, samples were provided directly for the manufacturers or suppliers in their original and properly sealed containers. They were stored in a dark and temperature-controlled environment until analysis. Most of the samples are of Spanish origin, but the sample set also included a significant number from France and Sweden.

2.2. Reagents and Apparatus

All solvents used were of chromatography grade and were supplied by Merck (Darmstadt, Germany). Ultra-pure water was produced using a Milli-Q system (Millipore, Bedford, MA, USA). Standards for the different phthalate esters analyzed were obtained from LGC Group (Middlesex, UK), Sigma-Aldrich (Madrid, Spain), and Scharlab (Barcelona, Spain). Deuterated di-n-octyl phthalate (DNOP-d4) and deuterated bis-(2-ethylhexyl) phthalate (DEHP-d4), both used as internal standards, were provided by Laboratorios CIFGA S.A. (Lugo, Spain). Anthracene-d10, also used as an internal standard, was purchased from Sigma-Aldrich (Vallensbaek Strand, Denmark). Phthalate determinations were carried out using an Agilent 7890B gas chromatograph coupled to a 7000 Series Triple Quad Mass Spectrometer detector (GC-MS/MS) from Agilent Technologies (Agilent, Santa Clara, CA, USA).

2.3. Analytical procedure

Phthalates in cosmetics, specifically in perfume, were determined following the British Standard EN 16521:2014 [6]. Samples were prepared for analysis using the following procedure: 1.0 mL of the liquid sample was diluted with ethyl acetate to a total volume of 10 mL in a volumetric flask. The resulting solution was then filtered through a 0.45 μm syringe filter. Subsequently, 980 μL of this sample solution were mixed with 20 μL of a 50 mg L^{-1} internal standard working solution and injected into the GC-MS/MS system. The analytes present in the sample were evaporated in the inlet of the gas chromatograph at high temperatures and introduced into the chromatographic column. Separation of the analytes occurred on a fused silica capillary column HP-5MS (30 m \times 0.25 mm ID, 0.25 μm) using a temperature gradient program. The analytes were then separated and introduced into the electron impact ion source of the mass spectrometry. The ions generated were introduced into the mass spectrometer analyzer, where they were selected based on the m/z ratio and subsequently quantified using a detector. Signal acquisition by the GC-

MS/MS equipment was carried out in MRM mode. The working conditions used in the analysis are detailed in Table 1.

Table 1. GC-MS/MS experimental conditions.

Injection volume	1.0 μL		
Carrier gas	1 mL min^{-1} , Helium (constant flow)		
Injection mode	Split 10:1 – Split flow: 10 mL min^{-1}		
Inlet temperature	290 $^{\circ}\text{C}$		
Temperature ramp	Initial: 50 $^{\circ}\text{C}$ (hold 1 min)		
	Ramp 1: 50 $^{\circ}\text{C}$ to 190 $^{\circ}\text{C}$ (20 $^{\circ}\text{C min}^{-1}$)		
	Ramp 2: 190 $^{\circ}\text{C}$ to 220 $^{\circ}\text{C}$ (8 $^{\circ}\text{C min}^{-1}$)		
Total time	Ramp 3: 220 $^{\circ}\text{C}$ to 310 $^{\circ}\text{C}$ (30 $^{\circ}\text{C min}^{-1}$, hold on 2 min)		
	16.75 min		
MSD temperature	280 $^{\circ}\text{C}$		
MSD mode	Electronic impact (–70 eV)		
Acquisition mode	MRM		
MS Quad temperature	150 $^{\circ}\text{C}$ (max: 200 $^{\circ}\text{C}$)		
MS Source temperature	230 $^{\circ}\text{C}$ (max: 250 $^{\circ}\text{C}$)		
Analyte	Retention time (min)	Quantification transition (m/z)	Qualification transition (m/z)
BBP	14.22	206–149	149–65, 149–93
DEHP	14.88	279–149	167–149, 279–93
DNOP	15.51	279–149	279–93
DNPP	13.25	237.2–149	149–65, 149–93, 149–121
DBP	11.98	223–149	205–149
DIPP	12.75	237.2–149	149–65, 149–93, 149–121
DMEP	12.30	176–149	207–149
DMP	8.36	163–77	163–133
PIPP	13.00	237.2–149	149–65, 149–93, 149–121
Anthracene-d10	10.68	188–160	188–186
DEHP-d4	14.87	171–153	153–69
DNOP-d4	15.45	283–153	283–125, 283–97

The analytes were quantified by comparing them to an external calibration curve while correcting for the signal of the internal standard. The identification of the analytes was carried out by comparing the samples against the external calibration standards based on the relative signal obtained for the selected characteristic ions using the MRM detection mode. Following this procedure, nine phthalate esters were determined: benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), di(n-octyl) phthalate (DNOP), di-n-pentyl phthalate (DNPP), dibutyl phthalate (DBP), diisopentyl phthalate (DIPP), bis(2-methoxyethyl) phthalate (DMEP), dimethyl phthalate (DMP), and isopentyl pentyl phthalate (PIPP).

The analytical measurements underwent a stringent quality control process throughout the measurement period to ensure the reliability of the obtained results.

3. Results and Discussion

The samples from the four categories (eau de cologne, eau de toilette, fragrance, and perfume) were analyzed for the content of the 9 phthalates as outlined in Section 2.3. For the 1147 samples measured, phthalate esters were only detected in concentrations higher than the LOD (1 $\mu\text{g mL}^{-1}$) in 57 samples, which represents 4.97% of the total. In 39 of these samples, only one phthalate was detected, while in the remaining 18, the simultaneous presence of two different phthalates was observed. None of the samples showed the

presence of more than two phthalates. A summary of the positive analyzed samples (with phthalate content exceeding $1 \mu\text{g mL}^{-1}$) can be found in Table 2. Depending on the type of samples analyzed, the number of positive results in each category was as follows: no sample showed any phthalate content in eau de toilette, 22 samples indicated the presence of phthalates in eau de cologne, 9 samples in fragrance, and 26 in perfume, with the latter equally likely to have one or two phthalates detected. The percentage of positive samples in each category varies, increasing from the eau de cologne category, where no positive samples were detected, to the perfume category, where 11.34% of the samples exhibited some PAE.

Table 2. Summary of the analyzed samples.

	Eau Cologne	Eau Toilette	Fragrance	Perfume
Number of samples analyzed	41	789	87	230
Number of positive samples	0	22	9	26
% positive in the category	0	2.79	10.34	11.30

The frequency of occurrence of different PAEs is summarized in Table 3. DEHP is the PAE that appears in the highest number of positive samples, with a total of 43 appearances (25 of them in samples containing a single PAE and 18 in samples containing two PAEs). DBP is the second most frequently detected phthalate, present in 22 out of the 57 positive samples (in 5 samples as the sole PAE and in 17 other samples containing two PAEs).

Table 3. Distribution of the number and type of PAEs in the positive samples.

	1 PAE (39 Samples)				2 PAEs (18 Samples)			
	Eau de Cologne	Eau de Toilette	Fragrance	Perfume	Eau de Cologne	Eau de Toilette	Fragrance	Perfume
BBP	-	-	-	-	-	-	-	-
DEHP	-	13	3	9	-	1	6	11
DNOP	-	-	-	-	-	-	-	-
DNPP	-	-	-	-	-	-	-	-
DBP	-	3	-	2	-	1	6	10
DIPP	-	-	-	-	-	-	-	-
DMEP	-	2	-	1	-	-	-	1
DMP	-	3	-	3	-	-	-	-
PIPP	-	-	-	-	-	-	-	-

DMP and DMEP are the other two PAEs detected in the analyzed samples, although their occurrence is much lower compared to DEHP and DBP. Specifically, DMP was detected in 6 positive samples, while DMEP was only found in 4 samples. It's noteworthy that in the 18 positive samples in which two different phthalates were detected, the combination in all cases was DEHP-DBP, except for one instance of DEHP-DMEP. The remaining PAEs considered (BBP, DNOP, DNPP, DIPP, and PIPP) were not detected in any of the samples analyzed.

Furthermore, when considering the percentage of positive samples within each category, a direct correlation is observed with the concentration of aromatic essence in each product. The percentage of positive samples relative to the total number of samples analyzed in each category is as follows: 0% for the eau de cologne category, 2.79% for the eau de toilette category, 10.34% for the fragrance category, and 11.30% in the case of the perfume category. This phenomenon can be explained by considering that the addition of PAEs allows for the fixation of aromas produced by the essences contained in the product. The essence content increases from eau de cologne (2–5%), eau de toilette (5–15%),

fragrance (15–20%), up to the highest value in perfume (20–30%). Additionally, this justifies the observation that the majority of samples containing two PAEs are fragrances (33.33%) and especially perfumes (61.11%).

The concentration levels for each PAE in the 57 positive samples from the four categories are summarized in Table 4. As shown, DEHP is not only the PAE with the highest frequency of appearance but also the one found in the highest concentration, an order of magnitude higher than any other detected PAE. When considering different types of products, it's also observed (see Figure 1) that DEHP concentrations are higher in categories with a higher percentage of aromatic essence. Consequently, higher concentrations were measured in perfumes, followed by fragrances and eau de cologne. A similar concentration pattern was observed for DBP. The other two detected phthalates (DMEP and DMP) were not commented upon due to the small number of samples in which these PAEs appeared. The results obtained presented comparable levels to those reported by Koniecki et al. [7] in fragrance samples and by Al-Saleh and Elkhatib [8] in branded perfumes. However, in the latter case, the frequency of appearance was reported as BBP > DMP > DEP > DEHP > DBP (using a different threshold limit of 0.1 $\mu\text{g mL}^{-1}$ for BBP, DMP, and DBP).

Table 4. Distribution of the number and type of PAEs in the positive samples.

CATEGORY		DEHP	DBP	DMEP	DMP
Eau de toilette (<i>n</i> = 789)	Positive Samples	14	4	2	3
	Mean	64.0	4.1	5.0	2.0
	Standard Deviation	151	3.3	1.4	0.0
	Minimum	1.0	1.2	4.0	2.0
	Maximum	500	7.0	6.0	2.0
Fragrance (<i>n</i> = 87)	Positive Samples	9	6	0	0
	Mean	17	18	-	-
	Standard Deviation	5.7	4.4	-	-
	Minimum	5.0	9.0	-	-
	Maximum	20	20	-	-
Perfum (<i>n</i> = 230)	Positive Samples	20	12	2	3
	Mean	186	14	6.0	1.7
	Standard Deviation	412	7.6	1.4	0.6
	Minimum	1.0	1.0	5.0	1.0
	Maximum	1785	21	7.0	2.0

All concentrations in $\mu\text{g mL}^{-1}$.

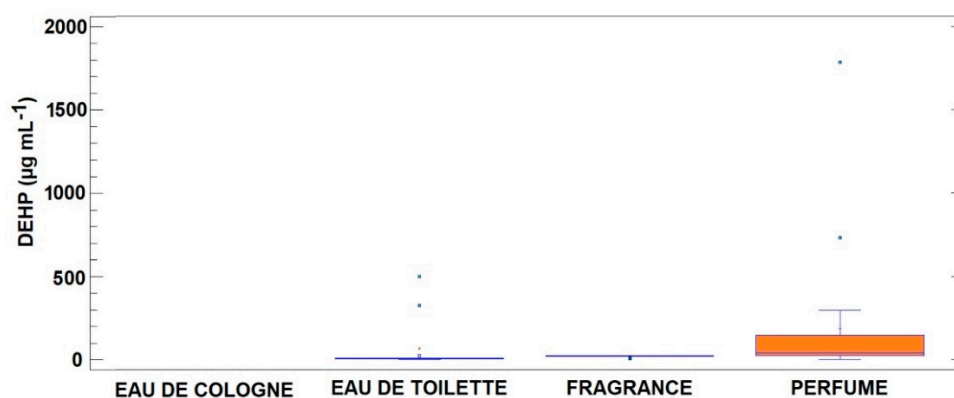


Figure 1. Box and whisker plot for the concentrations of DEHP according to the different categories considered in this study. All concentrations are in $\mu\text{g L}^{-1}$.

In addition, ANOVA studies were conducted to assess the potential impact of the sample's origin and the year of analysis on phthalate content. The results indicated that there are no significant differences (at a 95% confidence level) in the concentration of phthalates with respect to the country of origin and the year of analysis.

4. Conclusions

The results of a quality control system applied for five years to verify the phthalate content of a large set of samples from different types of colonies yielded the following conclusions. GC-MS/MS has been demonstrated as an appropriate analytical technique for the measurement of phthalates in cosmetic samples, and particularly in different types of colonies. Moreover, the percentage of non-compliant samples, with total phthalate content greater than $1 \mu\text{g mL}^{-1}$, was 4.97% of the total samples analyzed. These batches must be reprocessed before being marketed, with the consequent economic cost. In most of the samples, a single phthalate was detected, which was DEHP in most cases. The samples in which two phthalates appear correspond to the categories with the highest concentration of aromatic essence: fragrance and perfumes, which in turn also present the highest concentrations.

Finally, it is clear that the control of phthalates in cosmetic products is relevant to guarantee consumer safety, ensuring compliance with legal regulations, ensuring product quality and contributing to increasing transparency and informed use of cosmetics.

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