Analytical Methods and Regulative Viewpoint of Antimicrobial Preservatives in Cosmetics

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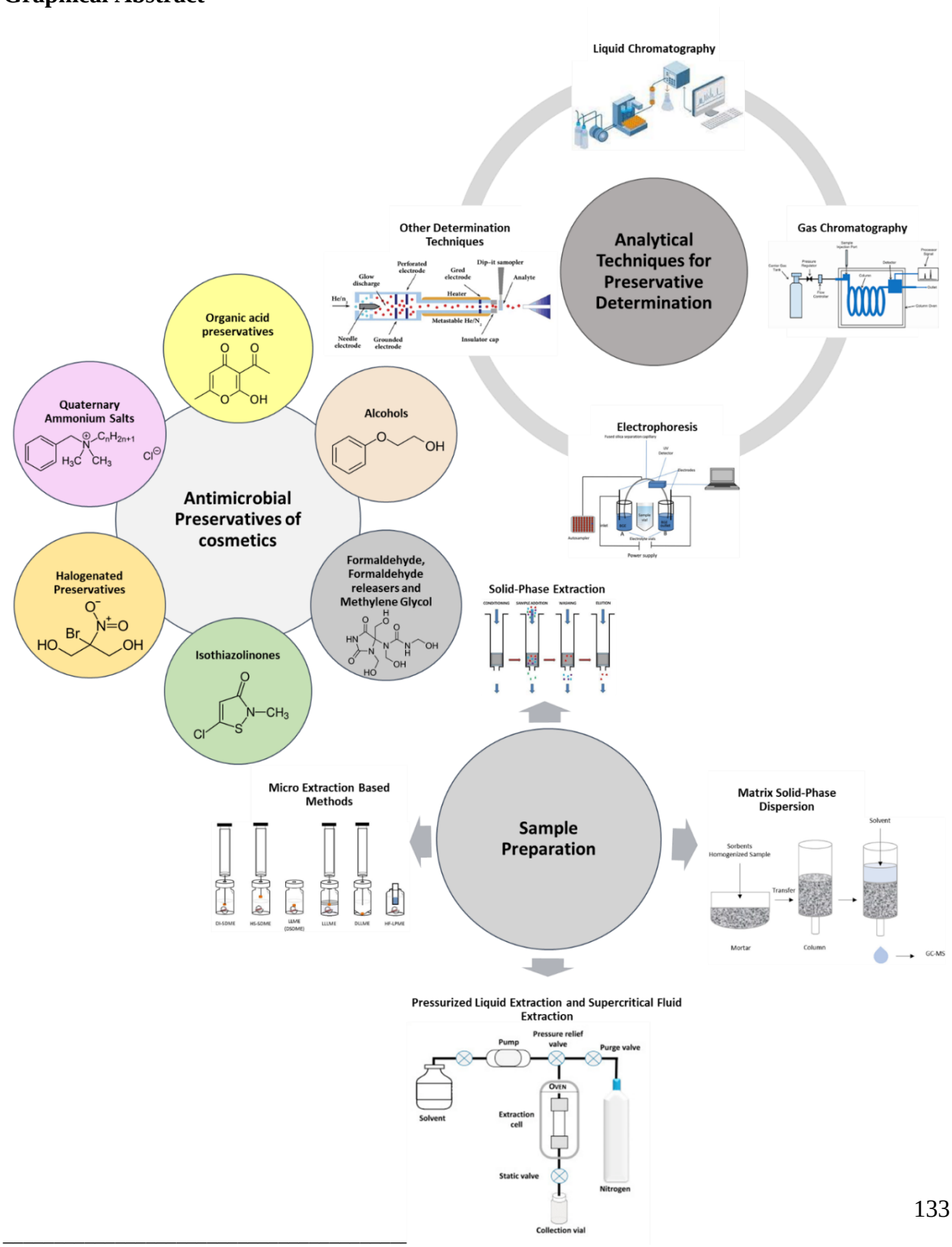
Graphical Abstract



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1.0 Abstract

Cosmetics need to be resistant to microbial contamination to protect consumer health and increase shelf life, much like any other product containing water, organic and inorganic components. The aims of anti-microbiological activity are to protect consumers from potentially harmful bacteria and to preserve products subject to degradation. Chemical, physical, or physicochemical methods are used to ensure this. Organic acid preservatives, alcohols, formaldehyde releasers, halogenated preservatives, isothiazolinones, quaternary ammonium salts and chlorhexidine are among the preservatives included in the legislation. Indeed, high quantities are more successful from a preservation standpoint, nevertheless they are toxic to consumers, whereas low amounts can lead to microbial resistance. Accordingly, the criteria of several international legislation and validation methods for introducing microbiologically safe items to the market have become essential. Although there are many approaches based on gas chromatography (GC) as per literature, the most common methods for the determination of preservatives are based on liquid chromatography (LC). Both of these procedures, as well as capillary electrophoresis (CE) and micellar electro kinetic chromatography (MEKC), have been frequently utilized in the cosmetics industry to determine preservative levels. Analytical approaches have been primarily focused on parabens, whereas the number of available methods to investigate other preservatives is limited. There is a tendency toward the usage of miniature extraction processes where new and improved sample preparation and extraction techniques including matrix solid-phase dispersion, solid-phase extraction, pressurized liquid extraction/supercritical fluid extraction and microextraction-based method have been introduced with high levels of efficiency and extraction capacities. Considering the significance and relevance of preservatives in cosmetics, this study highlights the most recent state-of-the-art information on their safety and regulatory concerns. Given the rising influence on consumer health, sample preparation and analytical methods for preservative detection were also investigated which have been proposed by the international scientific literature.

Keywords: Preservatives, Cosmetics, Analytical Techniques, Derivatization, Extraction

1. Introduction

Most of the day-to-day used cosmetic products consists of a high-water content and organic compositions. Hence, it has the tendency of being easily biodegraded by various types of microorganisms which can lead to high risk with respect to consumer health. Such contaminations can cause skin irritation and infections insisting extra caution when it comes to the exposure of damaged skin and eyes (Lundov, 2009, Matwiejczuk et al., 2020).

To inhibit these microbial growth, addition of preservatives in cosmetics is crucial. Some of such added preservatives can also act as antioxidants and photo protective agents as well. Preservatives are biologically active components so the safety of using these chemicals in day today consumed products should be brought to attention. The preservative chemicals can be irritating, sensitizing or even be toxic to humans. These harmful impact of preservatives has led to various restrictions throughout the years by international regulations worldwide (Union, 2014).

The “permitted” lists of preservatives vary from region to region. However, the most suggestive idea is to use more universally acceptable components. Despite that, public opinion on even the “permitted” preservatives has impacted the present trends of preservative choices of cosmetic formulators (Nowak et al., 2021). Many manufacturers tend to avoid preservatives in the spotlight just to claim their products “preservative free” as it has been observed as a constructive aspect by end users. Thus, utilization of alternative preservatives and other chemical components has been observed in recent years. Moreover, cosmetic ingredients which are added based on other functions than preservation, still with high microbial activity are being used in these “preservative free” cosmetics (Steinberg, 2012, King et al., 2021).

The development of analytical techniques for the determination of preservatives in cosmetic formulations is required to ensure product safety in accordance with regulations and to assess the health risk from potential exposures. Furthermore, personal care products are classified as "emerging organic contaminants," with large levels of these compounds and their metabolites found in the environment. The quantity of recent research on this topic reflects the growing interest in determining the amount of these chemicals. There is a substantial number of preservatives available even though only some of them can be found commonly in applications. When knuckling down to antimicrobial preservatives, they are chemicals used in preserving systems from decomposition processes and fermentation by prohibiting the extension of microorganisms. These types of preservative systems can be categorized further based on the chemical components, molecular structure and functional groups (Geis, 2006).

1.1 Organic acid preservatives

Organic acid preservatives and their salts can be influenced by pH of the medium. Therefore, these can only exhibit their action in acidic form. Usually, these compounds are adjoined to systems in the form of salts in order to improve incorporation. However the antimicrobial activity is not reached until the free acid form is released to the system by lowering of pH (Steinberg, 2012). Some commonly used organic acids in cosmetic systems for preservation are dehydroacetic acid, propionic acid, benzoic acid, sorbic acid and salicylic acid.

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1.2 Alcohols

Hydroxyl group contacting preservatives can have the preserving actions. However, this can be to a lesser extent in comparison with organic acids. Benzyl alcohol, phenoxyethanol, Isopropyl methylphenol are few of the examples for such alcohols in this group.

The alkyl esters of 4-hydroxybenzoic acid, which are commonly referred to as parabens are the most relevant compounds in this category. Currently, the benzyl form and the iso forms are prohibited to use by the European Union (EU) Cosmetic Regulation. They exhibit a high function in antifungal activity and has showed a high activity in baffling gram-negative bacteria. The carbon number of alkyl chain is directly proportional to the antimicrobial activity of the substance (Polati, 2007). However, these compounds are only active in water phase with dependency on pH, so the reduction in water solubility in contrast leads to critical difficulties in formulations. Hence the potassium and sodium salt forms of these compounds are commonly incorporated in formulations.

1.3 Formaldehyde, Formaldehyde Releasers and Methylene Glycol

Formalin is the most commonly referred commercial solution of formaldehyde. It is an anhydrous gas easily reacting to form methylene glycol (Steinberg, 2012). Hence, it is often used in water based cosmetic formulations including shampoo, conditioner, bubble bath, hand wash and shower gels. They convey both antibacterial and antifungal activity in these rinsed-off systems.

Preservative chemicals with n-methyl groups are also categorized under this group as they frequently act as formaldehyde donors and releasers in polar solvent systems. From these imidazolidinyl urea, sodium hydroxymethylglycinate, diazolidinyl urea and benzylhemiformal are commonly found in cosmetic products (Alvarez-Rivera et al., 2018). As per EU regulations, the ‘containing formaldehyde’ warning should be included in labels of formaldehyde or substance carrying products if the concentration of finished product exceed the 0.05% level.

1.4 Isothiazolinones

Isothiazolinones are an effective group of preservatives in water based systems with a wide diversity of applications in both commercial and household basis (Nakashima et al., 2000). They are derived from heterocyclic 2H-isothiazolin-3-one compound accommodating a vital sulphur moiety which is competent in oxidizing thiol containing remnants. Hence, it provides a powerful preservative action abreast a range of fungi and bacteria. The commercially available Kathon CG contains the active ingredients of 2-methyl-3-isothiazolinone (MI) and 5-chloro-2-methyl-3-iso-thiazolinone (CMI) forms in its formulation in 3:1 ratio. This Kathon CG is extensively used in both leave-on and rinsed-off commercially available cosmetics formulations such as shampoos, skin care products and gels. The high effectivity of these substances even at low concentration levels has resulted adequate rise in utilization of them in even industrial products, cleansing agents and other domestic products (Fewings, 1999).

1.5 Halogenated Preservatives

This type of preservative systems allows a strong activity especially towards fungi. 2-bromo-2-nitro-1,3 dioxane (bronopol) and 5-bromo-5-nitro-1,3-dioxane (bronidox) are some of the

examples. The decomposition of these compounds can release nitrosating agents which show reactivity with aliphatic amines such as monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA). These compounds are frequently employed in hair care products and other hygiene based products in order to improve the texture of the product (Polati, 2007).

Alternatively, preservatives namely 2-chloro-actamide, chlorobutanol, chlorophenesin, chloroxylenol, p-chloro-m-cresol and dichlo-robenzyl alcohol are some of the chemicals that falls under this category (Alvarez-Rivera et al., 2018). All these compounds have a poor solubility in water despite having an influential microbial activity which makes the incorporation of them to the cosmetic matrix challenging. Furthermore, chlorinated compound such as climbazol, triclocarban and triclosan are especially engaged in execution of microorganisms in cosmetics systems. Climbazol in frequently used in antidandruff shampoos whereas both triclocarban and triclosan are used essentially in systems such as soaps, toothpastes and deodorants.

1.6 Quaternary Ammonium Salts

Quaternary ammonium salts are frequently used in hair care products, especially in hair washing and conditioning due to their characteristic softening and anti-static properties. These compounds contain nitrogen with positive charge which manifest strong antimicrobial action at high pH levels. Some of the substances in this group include alkyl trimethyl ammonium bromides and chlorides, benzalkonium chloride, benzethonium chloride, and others. (Alvarez-Rivera et al., 2018).

1.7 Other Preservatives

Additional to the above microbial categories cosmetic preservative such as dibromopropamidine, hexamidine, dibromohexamidine, chlorohexidine, and cetylpyridinium chloride amidst other preservatives are commonly utilized particularly in mouthwash products.

2. Safety of Cosmetic Preservatives

Despite the indubitable benefits of preservatives in cosmetics, the severe and consistent side effects reported from them has aroused concerns consumers. These effects may emerge just after the use of these products and even years later with the continuous use as well. Roughly around 6% of general public has allergic reactions to preservatives and other allergens. The detrimental effects of them can vary from skin irritation to neurotoxicity (Harvey, 2003, He, 2006, Bilal et al., 2020). Parabens have shown anti-androgenic and oestrogenic properties and also haven been considered as endocrine-disrupting agents (Prusakiewicz, 2007). Some research studies have also shown potential correlation between paraben in cosmetics with allergic reaction and even breast cancers (Savage, 2012, Darbre, 2006).

Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) have shown consequential and extended side effects. Especially, BHA has shown potential modulator and disruptor action on the endocrine system with the magnitude up to damaging lung tissues along

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with causing inadequacy in development of the reproductive system (Jeong, 2005). As for BHT, even if the safety assessments declare that they are unable to categorize preservatives as genotoxic, it has the ability to alter genotoxicity in other agents (Lanigan, 2002). When combined with secondary amine structures including components like DEA, MEA, and TEA, nitrosating preservatives like bronidox and bronopol have demonstrated significant carcinogenic nitrosamine production (Matyska, 2000).

The deregulation inducing ability of triclosan in thyroid function has been demonstrated using animal testing where diminishing thyroxine levels in plasma has been observed (Kreft et al., 2020). Triclosan has shown sexual hormone disrupting ability, anti-androgenic and anti-oestrogenic properties as well (Witorsch, 2010). Triclosan can cause chloroform gas to be produced when it reacts with chlorine in tap water. (Rule, 2005). At this instant it is comprehensible that a special attention should be given in regulating products like toothpastes and mouthwash which are permitted to contain triclosan up to 0.3% and 0.2% (w/w). Furthermore, the excessive toxicity of this compound comes from its capability in decomposing and transforming processes to lead to various by products like dioxins, chlorophenols and polychlorinated hydrocarbons (Alvarez-Rivera et al., 2015).

Formaldehyde releasers containing preservatives can cause nausea, eye irritation, allergies and difficulty in breathing. Asthma attacks and headaches can come to a climax in high concentrations of these preservatives (Hauksson, 2015). 3-Iodo-2-propynyl butylcarbamate (IPBC) show toxicity in aquatic systems rather than carcinogenicity in humans (Warshaw and C.G., 2013). However acute inhalation toxicity has been recognized with this contact allergen. 2-methyl-3-isothiazolinone (MI) and 5-Chloro-2-methyl-4-isothiazolin-3-one (MCI) both has exhibit allergen and skin sensitizing properties (Garcia-Gavin, 2010). The Scientific Committee on Consumer Safety has claimed that with respect to the leave-on cosmetics, there's no significant amount of data to assist the utilization of Kathon CG mixture (SCCS, 2009). Researchers have found that the prolonged exposure of MI can be neurotoxic even at low concentrations. In the end, it is also important to emphasize that the scientific evidences have proven the misuse of these biocidal preservatives can lead to antibiotic resistance of bacteria in all biological systems (SCENIHR, 2009).

3. Regulatory Aspects

The utilization of preservatives in cosmetics as a chemical or as an ingredient in the finished products are exposed to a strict regulatory inspection worldwide. For instance, In the European region the allowed cosmetic preservatives for microbial spoilage are listed under European Unions' Regulation of Cosmetic Products (Scott, 2006).

In the United States, the regulatory issues with regard of cosmetics are regulated parallel with food and pharmaceutical by the same government agency, FDA. There's no preapproval system or a positive list of preservatives in this method (Steinberg, 2012). The FDA directly work with the consumers, Cosmetic Ingredient Review (CIR) system and cosmetic industry. CIR surveys on the chemical compounds and how they are utilized in the product where an expert, open and unbiased assessment and publication of results is being done in a peer-reviewed literature (CIR, 2016). However, the assortment of regulations can lead to circumstances that are unintelligible to the consumer. For example, the contentious parabens like phenyl-, isopropyl-, and benzylparabens

have been outlawed in the EU while being thought to be safe to use in the US.Quaternium-15 which is considered safe below the concentration of 0.2% in US proscribed under amendment No. 288/2015 in the EU (Alvarez-Rivera et al., 2018).

Nonetheless, most often, this regulatory diversity is rectified in a consistent manner. For example, when it comes to the case of chloroacetamide which is under public discourse in the EU (now authorized up to 0.3%) is already contemplated as unsafe in the US and already banned in Canada (Alvarez-Rivera et al., 2018).

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4. Analytical Techniques for Preservative Determination

The complex nature of cosmetic matrices often necessitates a sample preparation stage in order to effectively carry out multi-component analysis of a broad spectrum of chemicals. For the separation, identification, and quantification of cosmetic preservatives, liquid chromatography (LC), thin-layer chromatography, and electrophoretic methods have always been used. Although LC is still the most often used method for determining preservatives, a growing variety of approaches based on gas chromatography (GC) are being utilized. Both procedures, along with capillary electrophoresis (CE) and micellar electro kinetic chromatography (MEKC), have been widely employed in the cosmetics industry for preservative detection.

4.1 Liquid Chromatography

Usually when analyzing preservative systems reverse-phase liquid chromatography with the use of different types of detectors is occupied. UV detectors are the most abundant detectors among others such as chemiluminescence (CL), inductively coupled plasma (ICP-MS), mass spectrometry (MS), corona-charged aerosol (CCAD) and electrochemical detectors (ED) (Alvarez-Rivera et al., 2018).

Due to the possible presence of a matrix system in the samples the identification as well as the following quantification of the preservatives can be quite challenging. However, the limited availability of some LC-MS systems have shown permitted solving of co-elution problems which has led to clear identification of the preservative system (Ocaña-Gonzalez, 2015).

Preservatives such as isothiazolinone are LC-biddable preservatives which can be precisely analyzed by LC-MS/MS.(Wittenberg, 2015) Thus researchers have presented an extraction method for the preservatives BzI, OI, MI and MCI of several household and cosmetic products. Before the analysis MSPD extraction in positive electrospray ionization mode has been conducted. Also, the recent works that have studied MCI and MI, equipped a samples pre-treatment method based on the steps of dilution where they have achieved a lower detection limit (0.1 μg/g) compared to the MSPD method (0.0066–0.060 μg/g) (Alvarez-Rivera, 2012)

Table 4: Liquid Chromatographic methods for determining preservatives in cosmetics

Analyte	Matrix	Sample preparation remarks	Analytical technique	Recovery	LOD	RSD	References
ethylparaben, methylparaben, isopropylparaben, propylparaben, butylparaben, benzoic acid, 4-hydroxybenzoic acid	Shampoo, Toothpaste, cream	-	HPLC–UV	-	25-250 ng/mL	-	(Memon, 2005)
ethylparaben, methylparaben, propylparaben, benzoic acid, 4-hydroxybenzoic acid	shampoo, Toothpaste, sun block	USAEME with MeOH and buffer solution	HPLC–UV	22.6%-102.1%	0.25-8.30 mg/L	≤9.8%	(Yamini, 2012)
ethylparaben, methylparaben, propylparaben	moisturizing cream, Sunblock, aftershave	solidified floating vesicular co-acervative drop microextraction with tetrabutylammonium and decanoic acid	HPLC–UV	92.2%-108.8%	0.2-0.5 µg/L	3.9%-11.9%	(Moradi, 2012)
ethylparaben, methylparaben, propylparaben	Water based cosmetics	microwave-assisted IL–DLLME	HPLC–UV	68.3%-124.5%	0.6-1.2 mg/L	4.9-5.1%	(Cheng, 2011)
Phenethyl alcohol, phenylpropanol, methylpropanediol, ethylhexylglycerin	Moisturizing creams, bath gels, gels, sunscreen creams	vortexassisted liquid–liquid semi-microextraction	HPLC–UV	84%-118%	0.02-0.06 µg/ mL	3.9%-9.5%	(Miralles, 2016)

caprylyl glycol							
triclosan	Body wash, moisturizing cream, face wash and hand wash	in-tube based ultrasound-assisted salt-induced liquid–liquid microextraction	HPLC–UV	90.4%-98.5%	0.09 ng/mL	0.8%-5.3%	(Chen, 2013)
ethylparaben, methylparaben, isopropylparaben, isobutylparaben, propylparaben, butylparaben	-	-	UPLC–UV	-	-	<1%	(Pedjie, 2010)
ethylparaben, methylparaben, propylparaben, butylparaben	Body creams, sunscreens, antiperspirant creams	stir bar sorptive extraction accompanied by PDMS stir bar	UPLC–UV	17%-99%	30-200 ng/mg	<5%	(Melo, 2010)
ethylparaben, methylparaben, propylparaben, butylparaben	Cream, shower gel	Ultrasound assisted extraction ultra-sonication with methanol	UPLC–UV	91.4%-105.8%	2.25-4.82 ng/mL		(Mincea, 2009)
2-methyl-3-isothiazolinone, 5-chloro-2-methyl-3-isothiazolinone	Shampoo, dental cream, face cleansing gel, baby bath gels, baby liquid soaps,	matrix solid-phase dispersion	HPLC–MS/MS	>80%	0.0066-0.060 μg/g	<7%	(Alvarez-Rivera, 2012)

	hair mask, baby soft shampoo, baby body milks fluid make-up, hand cream, hair gel,						
ethylparaben, methylparaben, propylparaben, butylparaben , butylated hydroxyanisole, butylated hydroxytoluene	Homemade cream samples	supercritical fluid extraction combined with LC-MS	HPLC–MS/MS	-	4.7-142 ng/ g	<18%	(Lee, 2006.)
ethylparaben, methylparaben, isopropylparaben, propylparaben, butylparaben, benzylparaben, triclosan, butylated hydroxytoluene, butylated hydroxyanisole	Hand lotion, foundations, deodorant, lipstick, toothpaste, hand sanitizer	Ultrasound assisted extraction Sample sonication, centrifugation, supernatant filtration	HPLC–MS/MS	-	0.91-4.19 µg/mL	-	(Myers, 2015)

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ethylparaben, methylparaben, propylparaben, butylparaben	Make-up, shampoo, creams	Ultrasound assisted extraction Sample dilution, sonication and centrifugation. SPE	HPLC–CCAD	82%-104%	0.5-2.1 mg/L	3.3%-7.6%	(Márquez-Sillero, 2010)
ethylparaben, methylparaben, propylparaben, butylparaben	Wash-off cosmetics	Ultrasound assisted extraction Sample dilution , sonication and filtration	HPLC–CL	93.3%-105.9%	1.9-5.3 ng/mL	<4.5%	(Zhang, 2005)
2-bromo-2-nitro-propane-1,3-diol, 5-bromo-5-nitro-1,3-dioxane	Shampoo, body wash, hand soap	Ultrasound assisted extraction Sample dilution, vortex mixing, sonication, centrifugation, filtration	UPLC–ICP–MS	-	3.3 µg Br/L	<2.2%	(Bendahl, 2006)
ethylparaben, methylparaben, propylparaben	Shampoo	SPE C18 cartridges, elution using acetonitrile	HPLC–ECD	93.1%-104.4%	0.01% (w/w)	2.3%-9.8%	(Martins, 2011)

4.2 Gas Chromatography

When focusing on the most abundant alternatives to LC, both GC-MS and GC-MS/MS can be considered as more budget friendly substitute comparative to other systems such as photoionization detector (PID), flame ionization detector (FID) and electron capture detector (ECD) which are equipped less in scope (Alvarez-Rivera et al., 2018).

Derivatization is usually advocated in GC analysis in order to enhance the performance of chromatographic analysis with respect to cosmetic preservatives. Improving the peak separation and peak symmetry is the main attainment (Yang, 2010). Acetylation is one of

the commonly equipped derivatization procedures which is commonly employed in analyzing phenolic preservatives. Acetylation is a more economical alternative in comparison to other methods based on silylation agents including alkylation using butylchloroformate, N,O-bis(trimethylsilyl) trifluoroacetamide and isobutylchloroformate (Abbasghorbani, 2013, Yang, 2010).

Standard GC detectors have been proposed as well despite the fact that it requires conformation with the aid of MS in most cases (Farajzadeh, 2013). It has been reported that GC-FID has been used to detect parabens in cosmetic items. However, due to the lack of unique recognition in contrast to GC-MS, use of this approach has declined since 2006 (Jain, 2013).

Table 5: Gas Chromatographic methods for determining preservatives in cosmetics

Analyte	Matrix	Sample preparation remarks	Analytical technique	Recovery	LOD	RSD	References
ethylparaben, methylparaben, propylparaben	Mouthwash solution, shampoo toothpaste	air-assisted liquid-liquid microextraction Butylchloroformate as derivatization agent/ extraction solvent	GC–FID	59%-116%	0.41-0.62 mg/L	<4.9%	(Farajzadeh, 2013)
ethylparaben, methylparaben, propylparaben, butylparaben	Perfumes	Sample dilution in ethyl acetate	GC–MS	>88%	0.016-0.502 µg/g	-	(Sanchez Prado et al., 2011)
ethylparaben, methylparaben, propylparaben, butylparaben	Hair sprays, deodorants, cream, perfumes, lotion	Ultrasound assisted extraction Sonication -assisted Extraction with MeOH followed by a	GC–MS	85%-108%	10-200 µg/kg	4.2%-8.8%	(Shen, 2007)

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		clean up using solid-phase extraction with LC-C_{18} cartridges					
ethylparaben, methylparaben, isopropylparaben, isobutylparaben, propylparaben, butylparaben, benzylparaben, 2-bromo-2-nitro-propane-1,3-diol, 5-bromo-5-nitro-1,3-dioxane , iodopropynyl butylcarbamate, butylated hydroxyanisole, butylated hydroxytoluene, triclosan	Body milk, moisturizing lotions, creams, sun block, antiperspirant, make-up, liquid and hand soaps, shampoos	Matrix-solid phase dispersion Sorbent, Florisil; solvent, hexane: acetone (1:1); extracts acetylation	GC–MS	>78%	0.15-11 ng/mL	<10%	(Sanchez Prado et al., 2011)
4-hydroxybenzoic acid, ethylparaben, methylparaben, propylparaben	Cream, toothpaste, hair shampoo (wastewater)	Three-phase dynamic hollow fiber-based liquid-phase microextraction	GC–MS	8.4%-31.3%	0.01-0.2 μg/L	3.9%-6.0%	(Esrafili, 2014)
ethylparaben, methylparaben, isopropylparaben, isobutylparaben, propylparaben, butylparaben,	Baby wipes, toilet paper	pressurized liquid extraction (MeOH at 110°C for 5 min)	GC–MS	80%-115%	0.00077-0.051 μg/g	<10%	(Celeiro, 2015)

benzylparabens, butylated hydroxyanisole, butylated hydroxytoluene, 5-bromo-5-nitro-1,3-dioxane, phenoxyethanol, triclosan, iodopropynyl butylcarbamate							
ethylparaben, methylparaben, isopropylparaben, isobutylparaben, propylparaben, benzylparaben phenoxyethanol, , butylated hydroxyanisole, butylated hydroxytoluene, 5-bromo-5-nitro-1,3-dioxane , triclosan,	Shampoos, , baby lotion, toothpaste, shower gel body cream, sunblock, lipstick, deodorants, , regenerative cream, nail varnish remover	Micro-matrix-solid phase dispersion	GC–MS and GC–MS/MS	83%-115%	0.006-0.100 μg^{-1}(GC-MS), 0.00050-0.037 µg/g (GC-MS/MS).	<10%	(Celeiro et al., 2014)
ethylparaben, methylparaben, propylparaben,	Moisturizing cream, baby body milks,	solid-phase microextraction at	GC–MS/MS	>85%	0.000092 %	<13%	(Alvarez-Rivera, 2014)

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methyl benzoate, butyl benzoate, ethyl benzoate, phenyl benzoate, phenoxyethanol, , 5-bromo-5-nitro-1,3-dioxane, benzylparaben, triclosan, , butylated hydroxyanisole, butylated hydroxytoluene, isopropylparaben, isobutylparaben	antiperspirants, sun cream, eye make-up remover, make-up, shower gel, toothpaste, shampoo, child bath wash, hair conditioner, Aftershave, cleansing milk	40°C with NaCl (20%, w/v), Divinylbenzene/Carboxen/Polydimethylsiloxane fibre coating			(w/w) (0.00091 % (w/w) for Bronidox)		
ethylparaben, methylparaben, propylparaben, butylparaben , isopropylparaben	Hand cream and mouthwash solution	solid-phase extraction Solvent-assisted dispersive micro-solid-phase extraction	GC–PID	87%-103%	50-300 ng/L	<8%	(Abbasghorbani , 2013)

4.3 Electrophoresis

Electrophoresis methods has been widely equipped in both hydrophobic compounds and charged compounds of cosmetic products. CE has been suggested for the determination of parabens as well as ionic preservative systems such as cetylpyridinium and benzethonium. Monolithic capillary columns and fused silica has been employed in these analysis (Huang, 2013).

In separation of the pair of ionic and neutral preservatives MEKC can be equipped as a substitute where the carrier buffer has been incorporated with a surfactant such as sodium dodecyl sulphate in a micellar medium. This method is often subsumed in analyzing parabens, phenoxyethanol, benzyl alcohol, benzoic acid (BA), imidazolidinyl urea, TCS, salicylic acid (SA) dehydroacetic acid (DHA) and MI (Cheng, 2011).

Table 6: Electrophoresis methods for determining preservatives in cosmetics

Analyte	Matrix	Sample preparation remarks	Analytical technique	Recovery	LOD	RSD	References
Triclosan	Toothpaste, facial cleanser, lotion	Ultrasound assisted extraction Sample dilution and ultra-sonication	NACE–UV	94.2%-97.7%	0.075 µg/mL	-	(Ma, 2014)
Formaldehyde, glyoxal	Skin care products, toothpaste, baby lotion	Ultrasound assisted extraction Sample dilution and ultra-sonication; derivatization reaction (2-thiobarbituric acid) and centrifugation	Mini–CE–AD	94%-105%	1.64-2.80 ng/mL	-	(Li, 2014)
ethylparaben, methylparaben, propylparaben, , benzylparaben, isobutylparaben, isopropylparaben	Lotions	Sample dilution ; successive dilution in water	MEKC	81.0%-113.6%	4.32-7.78 nM	<2.96%	(Wu, 2014)
ethylparaben, methylparaben, propylparaben, butylparaben,	Shampoos, perfumes, gels,	Ultrasound assisted extraction Sample dilution,	MEKC	89%-115%	1.10-11.04 µg/mL	2.4%-16.7%	(Lopez-Gazpio, 2015)

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sorbic acid, salicylic acid, benzoic acid	creams, soaps, dog shampoo, air freshener	ultra-sonication, internal standard addition filtration and dilution when necessary					
2-methyl-3-isothiazolinone, triclosan, sorbic acid, butylated hydroxyanisole, butylated hydroxytoluene , ethylparaben, methylparaben, propylparaben, butylparaben, salicylic acid, benzoic acid	Shampoos, soaps, hair gels, perfumes, creams, toothpastes, dog shampoo, air freshener	Ultrasound assisted extraction Sample dilution, agitation, ultra-sonication, IS addition filtration and dilution when necessary	MEKC–UV	90%-115%	0.91-2.80 μg/ mL	<9%	(Lopez-Gazpio, 2015)

4.4 Other determination Techniques

There are several other determination methods including flow injection analysis (FIA), microwave-induced plasma desorption ionization (MIPDI)–MS, electrochemical detection and direct analysis in real time–MS (DART–MS) which has been applied in the cosmetic preservative determination frequently (Alvarez-Rivera et al., 2018). There have been several unconventional electrochemical detection methods proposed for the detection of parabens in the literature. Researchers has implemented a paraben sensor with the aid of a molecularly imprinted polymers on film where total paraben content has been determined.

Even with the limitations of resolution capacity of FIA in comparison to HPLC it has been utilized in several research work in order to enhance the sample throughput. Monolithic columns are often equipped in order to achieve an efficient separation at low pressure conditions (Wang et al., 2010).

Table 7: Other techniques for determining preservatives in cosmetics

Analyte	Matrix	Sample preparation remarks	Analytical technique	Recovery	LOD	RSD	References
ethylparaben, methylparaben, propylparaben, butylparaben	Cleansing tissues, hair foam, cleaning gel	Hair foam samples - LLE with diethyl ether; cleaning towel samples - sonication with ACN	FIA–UV	89.0%-103.3%	11.2-33.7 μM	0.65%-3.55%	(Garcia Jimenez, 2010)
4-hydroxybenzoic acid, ethylparaben, methylparaben, propylparaben, butylparaben	Sun cream, suntan oil, sun milk, make-up, face cream, lipstick	-	DART–MS; confirmation by GC–MS	-	-	-	(Haunschmidt, 2011)
2-phenylphenol, hexetidine, benzyl-4-chlorophenol, 2-methyl-3-isothiazolinone, climbazol	Facial cream, moisturizer, sunscreen	-	MIPDI–MS	-	-	-	(Zhao, 2015)

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5. Sample Preparation for cosmetic preservatives

Sample preparation for cosmetic preservatives usually consists of dilution of the sample in a suitable solvent followed by agitation or vortex mixing in order to homogenize the sample. Ultrasound techniques are commonly equipped in these methods in the sample extraction process. Methanol is the most frequently employed solvent amongst other solvents such as ethanol, diethyl ether, water, acetonitrile and mixtures of the solvents as well (Alvarez-Rivera et al., 2018).

In some cases, these obtained extracts are not homogenous and pure enough for the analysis. The presence of insoluble matrix compounds and other interferences can cause contamination which can lead to errors and lack of reproducibility in the results. So, execution of systematic sample preparation techniques beyond the basic methods like filtration and centrifugation is essential to reach the desired sensitivity and selectivity in data.

5.1 Solid-Phase Extraction

Solid Phase Extraction (SPE) is often used in studies to remove the interferences of contaminated solutions after dilution and sonication of the sample. However, a pre-treatment step is essential to steer clearing of clogged cartridges.

SPE methods are highly dependent on the sorbent used. Researchers have used C_{18} cartridges in order to separate sorbic acid, benzoic acid and salicylic acid from mouthwash products and shampoo samples. Methanol has been used to elute the analytes where they have been separated using ion-exclusion chromatography (Martins, 2011). The paraben contents of cosmetic samples have been also analyzed by research groups using C_{18} columns. In both of these works, sample dilution and sonication has been done as a pre-treatment step before SPE. Moreover, an automated method of sample pre-treatment followed by the determination of three different parabens in an oil-based cream, water-based lotions, lotion and gel has been conducted by a research group. SPE coupled with FIA has been incorporated where MEKC separation has been performed using C8-bonded silica column. Recoveries in the range of 92.2-102% has been obtained where the buffer consumption has been minimized and the buffer contamination has been avoided as well (Han, 2008).

There have been new discoveries on materials with high efficiency, selectivity and loading capacity for cosmetic preservative extractions especially for parabens. A group of researchers have experimented using multi walled carbon nanotubes for SPE which has been equipped for purification prior to analyzing the sample using HPLC coupled with C-CAD. Here an adequate amount of recovery has been recorded (82%–104%) with the added advantages of cartridge reusability and low amount of stationary phase requirement which has led to rationalization of cost analysis as well (Márquez-Sillero et al., 2010). Graphene has also shown an outstanding adsorption capacity with recoveries in the range of 63-100% where it has been used as a sorbent in SPE followed by CE for parabens in cosmetic samples (Ye, 2013).

5.2 Matrix Solid-Phase Dispersion

Matrix Solid-Phase Dispersion (MSPD) has been used as one of the suitable extraction methods for cosmetic preservatives as well. Here, the viscous, solid to semi-solid sample is blended which is accompanied by a solid support to dissolve as well as to disperse the sample components onto the bound organic phase of the exterior of the particle. This lead to total disruption and dispersion of the sample

(Kristenson, 2006, Liu et al., 2020). The main advantage of this method is the capability of carrying out extraction and clean-up both together with simplicity in procedure.

Matrix Solid-Phase Dispersion has been utilized in analyzing multi-class preservatives in a wide variation of leave-on as well as rinse-off cosmetics (Sanchez-Prado et al., 2011). Both the bromine and antioxidant preservative content have been determined in this study. The dispersive agent has been Florisil where the column has been eluted using hexane/acetone (1:1). Finally, the acetylated extract has been analyzed using GC-MS. Another MSPD method using Florisil has been employed in determining isothiazolinones (CMI and MI) where methanol has been used as the eluting solvent. The extract has been analyzed using HPLC–MS/MS where the general recovery has been higher than 80% with a lower extraction yield of 60% for MI (Alvarez-Rivera, 2012).

To reduce the usage of solvents and generation of residues an approach based on micro-MSPD and in-vial micro- MSPD has been applied (Celeiro, 2015). About 0.1 g of sample and a little quantity of florisil as the dispersant sorbent has been used in Pasteur glass pipettes in the character of micro-MSPD columns. Only 1 mL of ethyl acetate has been used to elute the sample with the solvent reduction by five times compared to regular MSPD. Mean recoveries of 90% has been obtained by GC–MS as well as GC–MS/MS analysis with RSD values ranging below 10%. This method has taken less than 5 mins for extraction get around to a more economical and rapid extraction procedure with minimum loss of volatile compounds(Celeiro et al., 2014).

5.3 Pressurized Liquid Extraction and Supercritical Fluid Extraction

Pressurized Liquid Extraction (PLE) is a systematic extraction technique that allows for enhanced analytical automation while also reducing the numberof organic solvents. Loading different solvents into extraction cell can also allow control towards the extraction selectivity.

Researchers have used one step sample preparation method using PLE to analyze several classes of preservatives in leave-on cosmetic products. Acetylation has been conducted by adding acetic anhydride along with pyridine directly into the PLE cell (Sanchez-Prado et al., 2011). Under optimal conditions, florisil has been utilized as the dispersing sorbent, after which it was extracted with ethyl acetate at 120°C for 15 minutes. A PLE based multi-component method has been advanced for concurrent analysis of divergent cosmetic additives including preservatives by a research group with adequate performance with 90% mean recovery has been recorded when extracted with MeOH at 110°C temperature for 5 minutes (Celeiro, 2015).

Supercritical fluid extraction (SFE) has also been suggested where several parabens in cream and skin milk samples has been analyzed. Low detection limits (4.7–142 ng/g) has been recorded in this analysis when operated with a CO2 supercritical fluid at 65°C and 14,000 kPa (Lee, 2006).

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5.4. Micro Extraction-Based Methods

5.4.1. Solid-Phase Micro extraction

Solid-Phase Micro extraction (SPME) is a miniaturized sample preparation technique which has been introduced by Pawliszyn's group (Bruheim et al., 2003). Coupling SPME with GC permit complete automation of extraction. Dilution is often required in the cosmetic samples when using direct sampling in order to minimize fiber damage. Performance of various commercial fiber coating such as polyacrylate (PA) has been evaluated by researchers when extracting phenolic preservatives like parabens in cosmetic products. Sample pretreatment has to be conducted in this case by diluting the sample with methanol which has also improved the matrix dispersion in the following dilution in water. From all the fibers namely PA, PDMS, CAR/PDMS, PDMS/DVB and CW/DVB, PA has shown the highest extraction efficiency (Tsai, 2008).

A hyphenated method using SFE *in situ* derivatization and online headspace SPME to GC-MS coupled has been incorporated by researches in order to determine paraben preservatives in cosmetics. The preservatives were extracted from the cosmetic matrices using supercritical carbon dioxide, and the supercritical fluid extraction was carried out for 10 minutes of static extraction and 15 minutes of dynamic extraction. The extractant was then derivatized in situ using the silylation reagent. The product was then adsorbed in the headspace on a polyacrylate SPME fiber before being analyzed using GC-MS. (Yang, 2010).

5.4.2 Dispersive Liquid–Liquid Micro extraction

In comparison to traditional liquid extraction techniques, Dispersive Liquid-Liquid Micro extraction (DLLME) can be incorporated when a rapid mass is being transferred between the extraction solvent and the sample system which leads to a high extraction efficiency and a higher pre-concentration as well (Rezaee, 2006). Researchers have devised a DLLME approach that incorporated acetone and octanol as an extraction and dispersive solvent mixture, enabling the pre-concentration of ethyl-, methyl-, and propylparaben from a mouth rinsing solution. A capillary tube has been used to separate the centrifuged solvent from the water surface for GC-FID analysis. (Farajzadeh, 2013).

Making use of the trichloromethane and isopropyl alcohol mixture as the solvent which is denser than water a DLLME method has been developed to determine four parabens, SA, SOA and BA in cosmetic products. The lower layer has been collected, dried by evaporation and analyzed using high performance CE (Xue, 2013). Researchers have devised a microwave assisted modification together with an ionic liquid extraction for the analysis of parabens in water-soluble cosmetic samples. This has shown accelerated dispersion in the extraction solvent which has led to an increased speed in mass transfer (Cheng, 2011).

6. Future Perspectives

This literature review reveals that over the past few decades, there have been a remarkable research advancement in analysis of antimicrobial preservatives in cosmetics and also a growing awareness on the regulations in general public. However, some areas of regulation and analysis is yet to be addressed in the future. The scalability, practical applicability and cost effectiveness on such utmost important area can be refined with further advancement in those areas. These findings contribute to a better knowledge of

cosmetic preservatives, nevertheless further research is needed to study the possibility of lowering the concentration of preservatives in cosmetics and thereby lowering their impact on consumer health.

Identification of preservatives in cosmetics can be quite challenging due to the presence of matrix components in the analyte that might potentially interfere the analysis. Some of these challenges, involving co-elution problems can be resolved by the use of selective detectors which leads to an unambiguous identification of the preservative. However, employment of LC-MS methods are still slender for preservative determination in cosmetic products.Derivatization is highly advocated especially, in GC analysis to refine the chromatographic performance. Acetylation stands as a viable option above all due to its low cost compared to other derivatization techniques based upon silylation agents.

Focusing on the sensitivity enhancement in analysis of cosmetic preservatives, online sample stacking methods have shown progressive outcomes where a large volume sample stacking MEKC methods with lower detection limits have been announced in comparison to regular MEKC techniques. Even with the limited resolution capacity of FIA in contrast to HPLC, it has been occupied in several research studies to improve the sample throughput where monolithic columns are commonly employed due to its efficient separation especially under low pressure conditions.

7. Concluding Remarks

The major purpose of a cosmetic preservative is its antimicrobial activity. The intrinsic toxicity of these compounds, on the other hand, is a concern for the cosmetics business. As a result, the quest for non-toxic and effective preservatives must continue. As the incidence of contact allergies has increased, preservatives have been re-evaluated, resulting in a reduction in the maximum concentration permitted or, in certain circumstances, a complete prohibition on their use. However, this is a lengthy procedure that will take several years to complete. In this case, it is important to develop rapid analytical methods and to validate them for the regular and simultaneous determination of preservatives. Because the majority of synthetic preservatives are ionized substances, more attention should be paid to chromatographic separation performance. Liquid chromatography is the separation technique of choice for the analysis, but gas chromatography adds significant sensitivity and selectivity to many of the preservatives as well. In terms of sample preparation, micro-extraction techniques are becoming more popular because they use less solvents and reagents. The simplicity of the new micellar electro kinetic chromatography methods should be emphasized, since samples can potentially be evaluated without any pretreatment after adequate dilution. When present regulations are paired with research into the concentration of preservatives in cosmetics, it is possible that some cosmetics are excessively preserved. Hence, manufacturers have to investigate a product's capacity to withstand microbial contamination in order to ensure that it is adequately preserved.

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