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Broad screening of illicit ingredients in cosmetics using ultra-high-performance liquid chromatography-hybrid quadrupole-Orbitrap mass spectrometry with customized accurate-mass database and mass spectral library

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ABSTRACT

Comprehensive identification and quantitation of 100 multi-class regulated ingredients in cosmetics was achieved using ultra-high-performance liquid chromatography (UHPLC) coupled with hybrid quadrupole-Orbitrap high-resolution mass spectrometry (Q-Orbitrap HRMS). A simple, efficient, and inexpensive sample pretreatment protocol was developed using ultrasound-assisted extraction (UAE), followed by dispersive solid-phase extraction (dSPE). The cosmetic samples were analyzed by UHPLC-Q-Orbitrap HRMS under synchronous full-scan MS and data-dependent MS/MS (full-scan MS^1/dd-MS^2) acquisition mode. The mass resolution was set to 70,000 FWHM (full width at half maximum) for full-scan MS^1 and 17,500 FWHM for dd-MS^2 stage with the experimentally measured mass deviations of less than 2 ppm (parts per million) for quasi-molecular ions and 5 ppm for characteristic fragment ions for each individual analyte. An accurate-mass database and a mass spectral library were built in house for searching the 100 target compounds. Broad screening was conducted by comparing the experimentally measured exact mass of precursor and fragment ions, retention time, isotopic pattern, and ionic ratio with the accurate-mass database and by matching the acquired MS/MS spectra against the mass spectral library. The developed methodology was evaluated and validated in terms of limits of detection (LODs), limits of quantitation (LOQs), linearity, stability, accuracy, and matrix effect. The UHPLC-Q-Orbitrap HRMS approach was applied for the analysis of 100 target illicit ingredients in 123 genuine cosmetic samples, and exhibited great potential for high-throughput, sensitive, and reliable screening of multi-class illicit compounds in cosmetics.

1. Introduction

Although it is often assumed that cosmetics are a recent invention, their history can be traced back to ancient times. Over the years, a wide variety of cosmetic products have been developed and used for personal care, visual modification, and even for boosting self-esteem and well-being. The use of cosmetics is deeply embedded in social and cultural practices, and represent a substantial global industry, with total retail sales value of €247 billion in the main markets of Europe, the U.S., China, Brazil, Japan, India, and South Korea in 2016 [1]. Since cosmetics stay in prolonged contact with the human body (epidermis, hair, nails, lips, mucous membranes, etc.), as a paramount priority, cosmetic products placed on the market must be safe to consumers, and shall not cause any damage to human health under customary or reasonably foreseeable conditions of use. Throughout the world, respective market surveillance authorities, while there may be different legal frameworks, have a common goal of ensuring a high level protection of human health through the compliance of cosmetics with regulatory requirements.

Even though cosmetic products and their ingredients are stringently regulated, unlawful violations occur from time to time, such as intentional application of prohibited substances or excessive use of restricted substances, unauthorized addition of ingredients to inapplicable type of products infringing the conditions laid down

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in regulations, inconsistencies between the ingredients declared on the label and those actually contained in finished products, and lack of warning labels indicating common allergens. In particular, cosmetic products containing illicit ingredients such as antibiotics [2–4], androgens [5], progestogens [6,7], oestrogens [8], glucocorticoids [7,9–14], carcinogenic *N*-nitrosamines [15–17], banned phthalate esters [18–22], forbidden pharmacologically active substances [5,7], illegal whitening agents [13], prohibited synthetic musks [19,22], and fragrance allergens [23,24] can still be found on the market. Although they increase the efficacy of cosmetic products, long-term exposure to these substances can cause skin irritation, allergic reactions, and sensitization. As a result, cosmetics containing substances that pose severe public health risks have been frequently notified or recalled by different authorities, including the rapid alert system for dangerous non-food products (RAPEX) of the EU and the U.S. Food and Drug Administration.

The need to guarantee product safety and to enforce regulatory compliance calls for efficient and reliable methods for identifying illegal ingredients. Analytical techniques that have been reported for chemical analysis of cosmetic samples include thin layer chromatography (TLC) [25], capillary electrophoresis (CE) [26], ion chromatography (IC) with ultraviolet detection [3], gas chromatography (GC) paired with flame ionization detection [27], thermal energy detection [17], or with different types of mass spectrometers [15,19,20,22,24,28,29], high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UHPLC) hyphenated with ultraviolet detection [2,5,7–9,21,25], electrochemical detection [30], and mass spectrometers with various mass analyzers [4–7,9–14,16,18,31,32].

Mass spectrometry (MS) is a highly sensitive and selective technology, and has been used for both qualitative and quantitative analysis of cosmetics. In particular, large numbers of MS-based analytical methods reported in literature are based on tandem mass spectrometry (MS/MS), with a majority being tandem-in-space triple quadrupole (QqQ) instruments. QqQ-MS/MS has been extensively accepted as a main tool in compound confirmation, structural elucidation, and quantitative analysis owing to its superior sensitivity and wide linearity range. The function of selected reaction monitoring (SRM) enables accurate quantification of target analytes at trace levels. Data acquisition of two SRM transitions, together with relative ion intensities and chromatographic retention time, gives sufficient information for reliable identity confirmation [33]. There are current trends in analytical sciences toward the development of high-throughput screening protocols capable of simultaneous detection of multi-class analytes in one single run. Although QqQ-MS/MS has been conventionally recognized as the workhorse of chemical analysis, it presents some limitations in terms of analysis time, scan speed, and sample throughput; and only the presence of limited known compounds of interest can be monitored with commercially-available reference standards [34]. Furthermore, the analytical results from QqQ-MS/MS are not retrospective because of its inherent way of ion selection and thus cannot be used for the analysis of non-target compounds [33].

These drawbacks can be overcome by the advent of high-resolution MS (HRMS), such as Orbitrap mass spectrometry, which have gained wide acceptance due to sensitive full-spectrum acquisition with high mass resolution, increased mass accuracy, sufficient dynamic range, and fast polarity switching [35,36]. Based on the ability to provide accurate mass measurements in MS and MS/MS stages, hundreds of compounds can be screened based on theoretical exact mass in a customized database, which greatly enhances the reliability of confirming the molecular composition. Moreover, the presence of certain compounds initially not considered can be further investigated from previously acquired raw datasets without the need for additional analysis. The development and validation of multi-component methodologies with suitable analytical features, covering wider ranges of different families of ingredients, is a field under development for analysis of cosmetics [37].

The present study aimed to develop a comprehensive analytical approach for the simultaneous screening of 100 illicit ingredients in cosmetics including 39 antibiotics, 40 glucocorticoids, 9 androgens, 8 progestogens, and 4 antifungal drugs, which are potential adulterated substances for immediate cosmetic efficacy, but of great concern for regulatory control [38]. Ultrasound-assisted extraction (UAE) and dispersive solid-phase extraction (dSPE) were employed in sample preparation procedures, followed by UHPLC coupled with hybrid quadrupole-Orbitrap (Q-Orbitrap) HRMS. Target screening based on the use of an in-house accurate-mass database and a user-created mass spectral library allowed rapid and automatic identification, confirmation, and quantitation of the 100 compounds.

2. Materials and methods

2.1. Chemicals and reagents

Authentic reference standards of 100 multi-class compounds of different chemical classifications (quinolones, nitrofurans, chloramphenicols, nitronidazoles, glucocorticoids, androgens, progestogens, and imidazoles) were purchased from Sigma-Aldrich (St Louis, MO, USA), Dr. Ehrenstofer (Augsburg, Germany), AccuStandard (New Haven, CT, USA), and National Institute for Food and Drug Control (Beijing, China) for method development and validation (purities all above 91%). Various dSPE sorbents, including C_{18}, primary secondary amine (PSA), graphitized carbon black (GCB), and hydroxylated multi-walled carbon nanotubes (MWCNTs-OH) were purchased from Macherey-Nagel (Düren, Germany) and Miltenyi Biotec (Köln, Germany). LC–MS grade methanol and acetonitrile were supplied by Fisher Scientific (Pittsburg, PA, USA). Ultrapure water was obtained using a Millipore Direct-Q Water Purification System (Bedford, MA, USA), with a specific resistivity of 18.2 MΩ cm. HPLC grade formic acid and ammonium acetate were purchased from Dikma (Lake Forest, CA, USA). Analytical grade anhydrous magnesium sulfate and sodium chloride were obtained from Xilong Scientific (Guangzhou, China).

Individual stock standard solutions at concentrations ranging from 500 to 1000 $\mu g\,mL^{-1}$ were prepared using appropriate solvents and stored in the dark at 4 °C. The solvents used for each analyte, as well as their molecular formula, molecular weight, CAS number, and octanol/water partition coefficients (log*Kow*), are listed in Table S1. A 10 $\mu g\,mL^{-1}$ multi-compound mixture stock solution for each compound was made daily as required by combining aliquots of each individual stock standard solutions. A series of working standard solutions were subsequently obtained for construction of matrix-matched calibration curves by progressive dilution with blank sample extracts on the day before use.

2.2. Sample collection and preparation

A wide variety of cosmetic samples of different brands and categories were purchased online or locally. All the samples were kept intact in their original packaging prior to laboratory analysis. In this study, (UAE) was employed for a rapid, simple, efficient, and inexpensive sample extraction. Aliquots of 0.2 g homogenized samples were weighed into a 10-mL centrifuge tube (Beckman Coulter, Brea, CA, USA). 2 mL of saturated sodium chloride solution was added, then the sample was shaken for 30 s using an IKA MS 2 minishaker (Staufen, Germany). 5 mL of acetonitrile containing 0.1% (v/v) formic acid and 0.5 g anhydrous magnesium sulfate were

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