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Review

Advances in the analysis of non-allowed pharmacologically active substances in cosmetic products

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ABSTRACT

The state-of-the art analysis of non-allowed pharmacologically active substances in cosmetic products is here presented and a new methodology developed for this type of analysis is proposed. Cosmetic products released on the market should not cause damage to human health when applied under normal conditions of use. With respect to this condition, the definition of "cosmetic product" is reported according to the international and region specific regulatory requirements for the manufacturing and marketing and that of non-allowed substances with particular reference to pharmacologically active substances, with therapeutic indication. The existing methodologies for the analysis of non-allowed products in cosmetic preparations generally include some sort of sample treatment and/or extraction before the analytical step, which always include a separation by liquid chromatography (LC) at ambient temperature followed by detection with ultraviolet spectrophotometric detection or mass spectrometry. A systematic high throughput analysis of non-allowed pharmacologically active substances is finally proposed together with the results of such an analysis performed in the "Drug abuse and doping" Unit of the National Institute of Health in Rome.

Contents

1. Introduction

According to the European Commission Directive 93/35/EEC, Article 1, a cosmetic product is defined as "any substance or preparation intended to be placed in contact with various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition" [1]. On the other hand, the Federal Food, Drug, and Cosmetic Act (FDCA) of the United States of America (USA) defines cosmetics as "articles

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intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance" [2].

These definitions give an indication on the target site of application of a cosmetic product and on its allowed functions [3]. Thus, products such as skin creams, lotions, perfumes, lipsticks, nail polishes, eye and facial make-up preparations, soap products, shampoos, permanent waves, hair colours, toothpastes, deodorants, fall under the category of cosmetic products in the European Union (EU) and USA. More ambiguous product types such as sun tanning preparations, antiperspirants and antidandruff shampoos are also considered cosmetics within Europe, whereas this may differ in other parts of the world [4]. In this concern, The USA Society of Cosmetic Chemists coined in 1961 the term "cosmeceutical" to originally describe "active" and science-based cosmetics. However, with the development of prescription-strength tretinoin for the enhanced appearance of ultraviolet damaged and wrinkled skin the definition was expanded to "a cosmetic that has or is purported to have medicinal properties". The US government has never recognized a separate "cosmeceutical" category [5].

Today's cosmetic market is driven by innovations including the development of new colour pallets, and of unique formulas concentrating on different needs, as well as treatments targeted to specific skin types. Most cosmetic products have a lifespan of less than five years and manufacturers reformulate 25% of their products every year. They need to improve products constantly in order to stay ahead in a highly competitive market where more choice and ever greater efficacy are expected by the consumer.

Consumers of cosmetic and personal care products are protected by strong requirements laid down by regulatory issues such as, e.g. the Cosmetics Directive by EU and USA FDCA cosmetic acts to ensure the safety of cosmetics and by a strong commitment by manufacturers to utilize the best science and latest available research data to assure the safety of a cosmetic product before it is placed on the market [6,7].

Certain cosmetic products require special attention from the regulators due to their scientific complexity or higher potential risk for consumers' health.

Differences in regulatory frameworks can be particularly significant for so-called "borderline products"—a term which refers to products which at first glance might be difficult to classify into a specific category, in a certain country or in several countries (for example products which are presented as chewing gum to keep teeth clean or to reduce bad breath, or products which according to their presentation are intended to stimulate sexual activity or finally the above reported cosmeceuticals) [8].

In actual fact, sometimes it may be unclear whether a product is a true cosmetic product as specified in the Cosmetics Directive or whether it falls into another category under different legislation. In this latter case, each product has to be individually evaluated from time to time.

2. Regulatory issues in cosmetics manufacturing and marketing

2.1. International legislation

The current regulatory framework for cosmetics in the major markets (the EU, United States of America-USA, Japan and Canada) has two definitions of cosmetics:

- a broad definition, with safety ensured through control over ingredients in the form of positive lists, prohibited and restricted lists, specific requirements concerning safety testing and maintenance of data files on safety. This is broadly the model for regulation in the EU;
- a narrow definition, with few restrictions on the ingredients that can be used and the type of safety testing to be undertaken as determined by manufacturers.

Products that do not meet the definition of cosmetics, often on the basis of claims made rather than on composition, are regulated as drugs. This is broadly the model of regulation in the USA (although in the USA, products can be categorised as both cosmetics and drugs therefore subject to both sets of regulations). Regulations in Japan and Canada are somewhat in between these two models. Canada is closer to the USA model but with a longer list of prohibited or restricted ingredients for cosmetics.

Japan is closer to the EU model, but has an additional product category of quasi-drugs; their regulation is less rigorous than for drugs but still requires pre-market approval and registration of ingredients. Outside Europe, a number of countries and/or regions have used the EU model in drafting their own cosmetic regulations. These include the Association of Southeast Asian Nations (ASEAN), Mercosur (Argentina, Brazil, Paraguay and Uruguay) and the Comunidad Andina (Andean Pact) regions. Other countries have reproduced certain features of the EU model, including China, Algeria, India, Israel, Morocco and Saudi Arabia [9].

2.2. The EU legislation

Directive 76/768/EEC (Article 2) states that a cosmetic product released on the market should not cause human health damage when used under normal or reasonable conditions. The responsibility is clearly placed upon the industry (i.e. on the manufacturer or his authorised agent or any other person responsible for placing the product on the Community market). The EU Member States have then the duty to take all the necessary precautions to ensure that only cosmetic products which conform to the provisions of Directive 76/768/EEC are placed on the European market [5]. Among other measures, they are responsible for the implementation of a post-marketing surveillance/inspection system to ensure that for every cosmetic product the following information is readily made accessible by the industry [5,6]:

- the qualitative and quantitative composition of the product,
- physico-chemistry, microbiology and purity of the ingredients and the cosmetic product,
- the manufacturing method,
- safety assessment of the finished cosmetic product,
- name and address of the safety assessor,
- existing data on undesirable effects on human health,
- proof of the effects claimed,
- data on animal testing.

The compilation of the above points is commonly referred to as a cosmetic's Technical Information File (TIF) or Product Information File (PIF) [7].

The Directive also imposes label requirements necessary to adequately inform the consumers about the identity of the manufacturer, the safety and stability of the product and its ingredients.

The USA FDCA legislation is similar in the above reported points. A precise review of product safety, good manufacturing practice, the ingredients and product composition and product labelling is done by the Food and Drug Administration (FDA) and by the Cosmetic Ingredient Review (CIR) Expert Panel [5].

2.3. Non-allowed substances in cosmetic products: legislation and examples

The Annexes IV, VI and VII of Directive 76/768/EEC consist of "positive" lists of allowed colourants, preservatives and UV filters, accompanied by their maximum levels and/or conditions of use in finished products. While Annex I lists by category all the cosmetic products, Annex II is a "negative" list containing non-allowed substances, whereas Annex III lists substances which are not allowed to be used in cosmetic products outside the specified restrictions and conditions. The content of the Annexes is regularly updated through amendments and/or adaptations to technical progress of the Cosmetics Directive. According to the European legislator (Council Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products) a cosmetic product must be safe: the assessment of the safety of a finished cosmetic product needs to take in consideration "the general toxicological profile of the ingredients, their chemical structure and their level of exposure" [6]. This means that for every ingredient the necessary physicochemical and toxicological information must be gathered and evaluated. As above reported, in case of USA, it is the CIR Expert Panel which revises and publish the list v ingredients allowed in cosmetic products [5]. Based on the above reported international legislation, in addition to the compounds reported in the list of "non-allowed (or prohibited) substances, there are also pharmacologically active substances, with therapeutic indication which cannot be added to a cosmetic product [10,11].

Some of the non-allowed compounds most commonly found in cosmetic products are anaesthetics, antihistaminics, antimycotics, corticosteroids, steroidal compounds and drugs used for male erectile dysfunction [10–14].

Examples of the non-allowed pharmacologically active substances in cosmetic products are:

- aminophylline, used in the treatment of asthma, is present in many products for cellulite treatment;
- tretinoin and other acne medicines are present in products used for wrinkle reduction;
- botulinum toxin used to treat torsion dystonias and other involuntary movements, is also used for smoothing facial lines and wrinkles. Among dark-skinned African women the cosmetic use of bleaching products is common practice; the use of corticosteroids as depigmenting agents is also very common in this population [12].
- glucocorticosteroids creams are considered pharmaceutical products and not cosmetic products, and thus, can be sold in authorized shops only.

Although the safety and composition of cosmetic products is strictly regulated in several countries, as extensively reported in the previous section, there are still problems related to products sold in illegal market or trough non-controlled internet web sites.

In the next sections, existing analytical methodologies to analyze the presence of non-allowed pharmacologically active substances are described together with a proposal for a systematic toxicological analysis to screen in cosmetics all the possible prohibited substances.

3. Analytical methodologies for the determination of non-allowed pharmacologically active substances in cosmetic products

In recent years, a few number of methods for the determination of non-allowed pharmacologically active substances in various types of samples have been described in the scientific literature.

The reported methodologies generally consist on some sort of sample treatment and/or extraction before the analytical step, which always include a separation by liquid chromatography (LC) at ambient temperature followed by detection with ultraviolet spectrophotometric detection or mass spectrometry (MS).

3.1. Sample treatment and/or extraction procedures

In recent literature, aliquots of cosmetic samples (1 ml for lotions and 1 g for creams), are accurately weighed into a 100 ml volumetric flask, taken to volume with methanol or a solution of 8:2 (v/v) acetonitrile/water containing 1% trifluoroacetic acid (TFA) pH 2.5 and sonicated for 10 min. After centrifugation, the supernatant is filtered through 0.45 μm nylon filter (Superchrom S.r.l., Milano, Italy), as in the case of antimycotics [13], cosmetic preparations for topical use containing sildenafil, vardenafil or tadanafil [10], cosmetic creams and lotions to prevent hair loss and hormone-dependent skin diseases, like acne and hirsutism [11]. Alternatively, the supernatant can be extracted using a solid phase extraction (SPE) cartridge conditioned with 3.0 ml acetonitrile followed by3.0 ml of a 1:9 (v/v) solution acetonitrile/water containing 1% TFA, as in the case of antihistaminic and local anaesthetics particularly for the after-sun cosmetics formulations [14]. The cartridge is washed with 5.0 ml water containing 1% TFA and the analytes of interest are eluted with 4.0 ml of an 8:2 (v/v) acetonitrile/water solution containing 1% TFA. Subsequently the eluate is diluted to 5.0 ml with the same solvent for further analysis.

For glucocorticosteroids, depending on the active ingredient in the cosmetic samples, there are several choices for extraction procedures. Creams containing clobetasol propionate, fluocinonide and fluocinolone acetonide are dissolved in methanol, while for creams containing betamethasone-dipropionate, a methanolic solution with 0.1% acetic acid is used and samples are heated at 60 °C on a water bath to achieve complete extraction of the active ingredient. Products containing methyl-prednisolone acetate are simply diluted in methanol taking care to mix well the suspension before dilution. Dexamethasone based products are dissolved in absolute ethanol, heated at 54 °C on a water bath under constant magnetic stirring. Prior to the analysis, all the solutions are pre-filtered on 1 μm lass filters (Acrodisc, Pall, German Laboratory, USA) and then on 0.45 μm nylon filters (Superchrom S.r.l., Milano, Italy) [12].

3.2. LC separation of analytes

The analytical methods reported in the literature for the analysis of non-allowed substances in cosmetic products are essentially based on LC determinations. Chromatographic separation is achieved using reverse phase columns; the mobile phase used in separations is always a mixture of water and acetonitrile with different percentages of TFA. Most often the chromatographic separation is achieved by gradient elution. Antimycotics can be separated on a stainless-steel column: Discovery RP-amide C16 (150 mm × 4.6 mm × 5 μm) using a linear gradient up to 46% acetonitrile within 70 min, followed by an increase in acetonitrile concentration to 50% in 80 min which was found to be optimal [13]. At the end of the elution, the initial mobile phase is passed for 10 min through the column to allow for the re-equilibration of the chromatographic conditions. For sildenafil, vardenafil and tadalafil in cosmetic preparations for topical use, the chromatographic separation can be achieved using a Waters Sunfire (150 mm × 4.6 mm × 5 μm) column; the mobile phase used is a mixture of (A) water (0.02% TFA) and (B) acetonitrile (0.02% TFA) programmed as follows: 90% A for 1 min, decreased to 75%

A in 5 min and then decreased to10% in 25 min with 10 min to re-establish the initial conditions [10]. In the chromatographic separation for cosmetic products used for hair loss prevention the column was Zorbax SB-CN (250 mm × 4.6 mm × 5 μm); in this case the mobile phase used in the separation consisted of (A) water (0.1% TFA) and (B) acetonitrile programmed as follows: 90% A in 1 min, decreased to 10% in 40 min, then increased again to 90% in 10 min [11]. For the determination of anaesthetics and antihistamines in cosmetic products two eluting conditions were adopted:

(A) Initial mixture: acetonitrile/water containing 1% TFA (10:90, v/v); then a linear gradient elution up to 60% acetonitrile in 30 min. The final mixture was maintained for 10 min before re-equilibrating the column with the initial mobile phase.
(B) Initial mixture: acetonitrile/water containing 10 mM sodium perchlorate pH 3.0, 60:40 (v:v), this proportion was kept for 10 min followed by a linear gradient elution up to 70% acetonitrile in 20 min. The final mixture was maintained for 10 min before re-equilibrating the column with the initial mobile phase [14].

3.3. Detection of analytes

As reported in the literature, the instrument most often used is a photo-diode array detector (DAD).

A diode array consists of a number of photosensitive diodes placed side by side and insulated from one another in the form of a multi-layer sandwich. Each diode may be only a few thousands of an inch thick and the output from each diode can be scanned, stored and subsequently processed by a computer in a number of different ways. Generally, the use of DAD facilitated the evaluation of peak purity factors, very useful in the analysis of real samples to confirm the absence of interfering co-eluting compounds and the selection of appropriate wavelength to obtain the best sensitivity for all the investigated compounds.

In order to produce a pharmaceutical effect, the concentration of non-allowed ingredient in a cosmetic product should be comparable to the minimum of these pharmacologically active substances usually administered in pharmaceutical preparations. UV–DAD detection is usually adequate to detect these ingredients in the investigated products at these concentrations. To detect them at lower concentrations and to have a high degree of specificity and additional information about the structure of the analytes, the use of electrospray ionisation (ESI) before MS is also suitable [10,11]. The chromatographic conditions used for LC–ESI-MS (e.g. column, injection volume, column temperature and mobile phase) were the same as the ones used for LC–DAD. All chromatographic solvents were degassed with helium before use.

The purity and identity of compounds under investigation (MS characterizations) were determined as follows. The substances, dissolved in mobile phase, were infused through an integrated syringe pump into the interface ESI (+), ESI (−), atmospheric pressure chemical ionization (APCI) (+) and APCI (−) in single quadrupole mode at the rate of 1 ml/min. Based on these experiments, the following optimized conditions were used: capillary voltage at 3.0 kV, cone voltage at 15 V, source temperature and desolvation temperature at 350 °C. The cone and desolvation gas flows were set at 50 and 400 l/h, respectively.

4. A proposal for systematic toxicological analysis of non-allowed substances in cosmetic products

Recently, many cosmetic products of doubtful origin and composition, have been sold via Internet where scams are widespread and difficult to control. To address this concern, the Italian anti-adulteration and safety bureau (Carabinieri per la tutela della salute-NAS) seized several illegal cosmetic preparations sold via Internet web sites or through illegal venues (e.g. private doctors, fitness centers and odd stores). Although in the majority of cases the ingredients in the products were not listed, a strong suspicion that the pharmacologically active substances previously mentioned could be used illegally in these formulations, prompted the bureau to request a specific analysis of the seized products [10,11].

To solve this problem, a systematic high throughput analysis of non-allowed pharmacologically active substances has been proposed to be generally applied to cosmetic preparations.

Systematic toxicological analysis (STA) of non-allowed pharmacologically active substances in cosmetic products is an important routine task in analytical toxicology, due to the increase of the traditional and web market of these latter products worldwide.

Since the compounds that have to be analyzed are often unknown, the first step before quantification is identifying the compounds of interest. High-throughput procedures in analytical toxicology mean that thousands of relevant toxicants can simultaneously be screened using one single procedure. The analytical strategy often includes a screening test and confirmatory test before quantification. In analytical chemistry, when unknown compounds of supposedly low molecular weight (such as the compounds reported above as the most common non-allowed pharmacologically active substances detected in cosmetic products) have to be screened and reliably identified, particularly in small amounts and/or in complex matrices, gas chromatographic (GC) systems coupled with MS detection are the most commonly used techniques. While GC–MS is the most frequently used technique in analytical toxicology for such high-throughput screening, single-stage or tandem LC–MS with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) are passed the development stage and are becoming increasingly important in routine toxicological analysis, especially for quantification of the identified analytes [15].

The “Drug abuse and doping” Unit of the National Institute of Health in Rome has developed and validated a two-step methodology for analysis of non-allowed pharmacologically active substances in pharmaceutical and cosmetic products (drugs, drugs of abuse, cosmetic products and doping agents) seized by the Italian anti-adulteration and safety bureau (NAS).

4.1. Sample preparation, extraction and derivatization in case of GC screening

With few exceptions, chromatographic techniques require some isolation procedures to separate the compounds to be analyzed from the matrices.

Isolation can be performed by liquid-liquid extraction (LLE) or solid phase extraction (SPE) after suspending the samples in 2 ml 0.1 M phosphate buffer at three different pH: acid (pH = 2.5) basic (pH = 10–12) and neutral (pH = 7) pH.

The LLE procedure is not time consuming, although it is difficult to automate, requires high-purity solvents, and can result in the formation of emulsions with incomplete phase separation, leading to impure extracts. However liquid-liquid extraction has been and is still a very common sample workup procedure in STA especially in analytical toxicology [16].

The samples at different pH are placed in an ultrasonic bath for 15 min, then the solutions are extracted with three different aliquots of 2 ml chloroform/isopropanol (9:1, v/v). After centrifugation the organic layer is divided into three aliquots of 2 ml and is evaporated to dryness at 40 °C under a nitrogen stream.

For GC–MS screening analysis, two aliquots are used: the dried residue of the first is derivatized in capped test tubes with 100 μl of *N,O*-bis-trimethylsylyl-trifluoroacetamide (BSTFA) + 1% (trimethylsilyl (TMS) at 70 °C for 30 min.

The extracts obtained by LLE or SPE are usually derivatized prior to GC–MS analysis to increase the volatility and thermal stability of the compounds. Derivatization is mandatory for polar and thermolabile compounds to make them suitable to chromatographic analysis. The reduction in polarity can also improve the gas chromatographic properties of the compounds by minimizing the undesirable and non-specific column adsorption and by allowing for better peak shape and reduction in appearance of ghost peaks. The most common derivatization reactions in STA are acylation, silylation an alkylation [17]. In any case, a second dry aliquot is dissolved in 100 μl ethyl acetate to be analyzed without derivatization by GC–MS.

In case of LC–MS analysis, the third evaporated aliquot is simply redissolved in mobile phase.

In common practice, all the samples and reagents used for treatment and extraction are tripled in order to create different extraction aliquots: two for GC–MS screening analysis and the other for LC–MS analysis of thermolabile compounds.

4.2. Separation and identification of analytes

For GC/MS analytes separation is achieved on a fused silica capillary column (HP-5MS, 30 m × 25 mm i.d., film thickness 0.25 m) (Agilent Technologies, Palo Alto, CA, USA). The oven temperature is programmed at 100 °C for 2 min and increased to 290 °C at 10 °C/min. Split injection mode (15:1) is used. Helium (purity 99%), with a flow rate of 1 ml/min is used as carrier gas. The injection port, ion source, quadrupole, and interface temperatures are: 260, 230, 150 and 280 °C, respectively.

The electron-impact (EI) mass spectra of the analyte and I.S. are recorded in total ion monitoring mode (scan range 40–550 *m*/*z*) to determine retention times and characteristic mass fragments.

The full-scan data files acquired by GC–MS system are screened for the presence of peak and mass spectra of substance by use of Data Analysis Agilent Chem Station software. A first manual screen of the total ion current (TIC) by an experienced toxicologist is followed by identification of unknown or illegal compounds. Even in the absence of reference substances, this can be achieved by computer-assisted comparison of the peak underlying mass spectra with those in the mass spectra library. The GC–MS screening analysis is performed to exclude the presence of:

- common drugs of abuse like opiates, cocaine, amphetamines, cannabinoids, ketamine;
- hormones and steroids;
- anaesthetics;
- glucocorticosteroids.

For systematic analytical toxicology we used several instruments like GC–MS in association to HPLC–DAD or tandem LC–MS with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), especially for quantification of unstable, low-dose, high molecular weight and/or polar compounds.

LC/MS separation is achieved using Zorbax SB-CN (250 mm × 4.6 mm × 5 μm) (CPS analitica, Milan, Italy). The mobile phase used in the separation, at a flow rate of 1.2 ml/min, consists of (A) water (0.1% TFA) and (B) acetonitrile programmed as follows: 90% A for 1 min, decreased to 10% in 40 min, then increased again to 90% A in 10 min. The injection volume is 20 μl.

The mass spectrometer is operated in positive ESI mode with selected ion monitoring (SIM) acquisition. The following ESI parameters are applied: drying gas (nitrogen) heated at 350 °C at a flow rate of 10.0 l/min; nebulizer gas (nitrogen) at a pressure of 40 psi; capillary voltage at 4000 V. MS characterization (purity and identity) of compounds under investigation is achieved using flow injection analysis (FIA). The substances, dissolved in mobile phase, are infused through an integrated syringe pump into the ESI probe at rate of 1 ml/min. In FIA experiments, full scan acquisitions were made over the (*m*/*z* 50–550) range using both negative and positive ionisation. On the basis of these experiments, the best acquisition parameters are selected.

5. Discussion

The first observation regarding the analysis of non-allowed pharmacologically active substances is that international literature on this topic is scarce and that few methods have been presented in the last year regarding the investigation of prohibited substances in cosmetic products. It can be speculated that this issue is relatively new and the emergency of the problem is facing now with the speed of web marketing of non-controlled products.

In this concern, systematic toxicological analysis has been used in our laboratories for the analysis of some cosmetic preparations sold via the Internet or in "Smart Shops" and seized by Italian anti-adulteration and safety bureau (NAS). The most recent case has been that of four different bubble baths and four shampoos seized for suspicion of containing cannabis ingredients. The STA analysis revealed the presence of the psychoactive ingredient of cannabis, delta 9-tetrahydrocannabinol (concentration range in both the four different bubble baths and shampoos: 1.8–3.1 μg/ml) which is an illegal substance included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

As reported above, the use of STA helped us to identify non-allowed pharmacologically active substances in cosmetic products for preventing hair loss and other hormone-dependent skin diseases and drugs in cosmetic creams for topical use sold on Internet web sites or through illegal channels (e.g. private doctors and fitness centers) as promising remedies for male erectile dysfunction, premature ejaculation and female orgasmic dysfunctions [10,11].

In case of products used for hair loss prevention [10], the initial screening analysis allowed us to detect in the hair lotion the presence of minoxidil (concentration range: 68.1–82.3 mg/ml lotion) progesterone (concentration range: 29.3–34.3 mg/ml lotion or 37.4–49.0 mg/g cream), canrenone not included in the label (concentration range: 11.1–24.0 mg/ml lotion), and spironolactone (concentration range: 6.3–25.8 mg/ml lotion). The results of our study were two fold and unexpected: first we detected a variety of forbidden substances (hormones, diuretics and minoxidil) in cosmetic products, as well as anaesthetics and antihistamines illegally added to cosmetics used after-sun exposure [14]. The second important unexpected result was that the percentages of the non-allowed pharmacologically active substances in the examined products were extremely high, one order of magnitude higher than those usually employed in cosmetic samples, and comparable to the percentage of pharmacologically active substances in pharmaceutical preparations.

Also, in the case of cosmetic preparations for topical use purchased on the Internet market [10], systematic toxicological analysis has identified the presence of many substances, some of which were not listed. In all the examined products, more than one forbidden compound was present; lidocaine was the local anaesthetic most frequently found in the cosmetic creams (three out of five preparation with a range concentration: 19.3–22.9 mg/g cream), in one occasion together with prilocaine (concentration range: 24.9–25.0 mg/g cream). Procaine was found in one out of the five products (concentration: 21.3 mg/g cream), while benzocaine was never found. Four out of the five creams contained sildenafil (concentration range: 8.5–10.5 mg/g cream), in one case together with testosterone (concentration: 10.7 mg/g cream). In only one case the PDE-5 inhibitor was vardenafil (concentration: 10.1 mg/g

cream) while tadalafil was never present in these illegal preparations. The result of the study is remarkable and surprising; it demonstrates the presence of a variety of forbidden substances in cosmetics sold for topical use.

The presence of two other type of compounds (testosterone and local anaesthetics) in cosmetic creams is expressly forbidden (Annex II) by the Article I from CEE Cosmetic Directive 76/768/EEC, aiming at regulating the manufacturing of cosmetic products [14]. In addition, even though not mentioned in the above reported Annex II, PDE-5 inhibitors should not be added to a cosmetic product, since they are also pharmacologically active compounds requiring medical prescription even when present in topical preparations.

The use of a combination of two detection methods was paramount to the success of these studies:

- diode array detection which can be used by all control laboratories not equipped with an HPLC–MS instrument for the routine control of substances forbidden in cosmetic products, such as the ones detected in our study;
- ESI-MS detection, which identifies with a high degree of accuracy unknown substances which can be illegally added in cosmetics based on their structure and molecular weight.

6. Conclusion

Hyphenated mass spectrometric techniques are and will be indispensable tools in clinical and forensic toxicology and doping control. GC/MS in the EI mode plays a major role particularly in comprehensive screening procedures because a very large collection of reference spectra is available and the cost of the instrument is not excessive. LC/MS with different mass analyzer types will become more and more standard technique for automated target screening procedures and particularly for high-throughput quantification. In fact LC/MS has shown to be an ideal supplement, especially for detection of more polar, unstable or low-dose drugs.

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