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Characterization of suspected illegal skin whitening cosmetics

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ABSTRACT

An important group of suspected illegal cosmetics consists of skin bleaching products, which are usually applied to the skin of the face, hands and décolleté for local depigmentation of hyper pigmented regions or more importantly, for a generalized reduction of the skin tone. These cosmetic products are suspected to contain illegal active substances that may provoke as well local as systemic toxic effects, being the reason for their banning from the EU market. In that respect, illegal and restricted substances in cosmetics, known to have bleaching properties, are in particular hydroquinone, tretinoin and corticosteroids.

From a legislative point of view, all cosmetic products containing a prohibited whitening agent are illegal and must be taken off the EU market. A newly developed screening method using ultra high performance liquid chromatography-time off flight-mass spectrometry allows routine analysis of suspected products.

163 suspected skin whitening cosmetics, collected by Belgian inspectors at high risk sites such as airports and so-called ethnic cosmetic shops, were analyzed and 59% were classified as illegal. The whitening agents mostly detected were clobetasol propionate and hydroquinone, which represent a serious health risk when repeatedly and abundantly applied to the skin.

1. Introduction

Skin whitening cosmetics are most often applied to lighten uneven skin tone. Sometimes only a limited skin area, such as the neck, the face and the back of the hands is involved, but as well the whole body surface may be exposed.

The usage of these cosmetics is widespread and in particular popular in countries with skin photo types IV, V and VI. Reasons for applying these products are diverse and may be of cultural, political, socio-economic, aesthetic or medical origin [1,2].

Skin bleaching products, positioned on the market either as cosmetics or as a dermatological treatment, contain whitening agents including kojic acid, salicylic acid, nicotinamide, arbutin, hydroquinone, corticosteroids and tretinoin. Because of the well-known side-effects of the latter three compounds, their use in cosmetics is not only forbidden in the EU, but also in several countries outside the European borders [3,4]. Observed local effects consist of ochronosis, which is typical for hydroquinone, irritant dermatitis, leukoderma and post-inflammatory hyper pigmentation while systemic toxicity may lead to kidney and liver diseases [5–8]. Salicylic acid is regulated in the EU and can be used in cosmetics only to a maximum concentration of 2.0% with the exception of rinse-off hair products (3.0%, annex III of Regulation 1223/2009). It is restricted because of its keratolytic effects and its penetration enhancing properties for other ingredients [6,7]. For this reason salicylic acid is mostly used in combination formulations.

Despite their legal ban in cosmetics, hydroquinone, tretinoin and corticosteroids can still be found in skin bleaching cosmetics present on the European market because of their higher efficiency in comparison with legally admitted substances [8]. Also in a number of countries these agents are still considered as legal cosmetic ingredients [1].

Illegal whitening products may also be a combination of a permitted agent and an illegal one. As such salicylic acid is often added to corticoid- or hydroquinone-containing products.

All these products represent a potential risk for human health in particular upon repeated and long-term exposure.

In order to gain an idea about the situation on the Belgian market, samples were collected by inspectors at sea- and

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airports or in so-called ethnic-cosmetic shops. As such 163 samples were analyzed using a newly developed ultra high performance liquid chromatography–time off flight–mass spectrometer (UHPLC–ToF–MS) screening method. All identified whitening agents were quantified using the ultra high performance liquid chromatography–diode array detector (UHPLC–DAD) method previously described by our group [9].

To ensure selectivity and avoid misinterpretation by confounding matrix components with whitening agents, the methodology used was validated in such a way that it was suitable for a variety of cosmetic matrices. This developed method provides the analytical possibility to routinely screen cosmetic or dermatologic samples for the presence of illegal bleaching substances.

2. Materials and methods

2.1. Standards and reagents

The reference standards **kojic acid** (batch 1363411V, purity ≥98%), **arbutin** (batch BCBD1957V, purity ≥98%), **nicotinamide** (batch 0001448241, purity ≥99%), **hydrocortisone 21-acetate** (batch 025K1123, purity ≥98%) were purchased from Sigma Aldrich (St. Louis, USA). **Salicylic acid** (batch 03E37-B02-241946, purity ≥99%), **tretinoin** (batch 09J22-BO5-251745, purity ≥98%), **betamethasone valerate** (batch 10J04-B01-262745, purity ≥97%), **clobetasol propionate** (batch 11G25-B02-264743, purity ≥99%), **dexamethasone** (batch 12C09-B03-269762, purity ≥98%) and **prednisone** (batch 07L19-B10-232010, purity ≥99%) were obtained from Fagron (Waregem, Belgium). **Fluocinolone acetonide** (batch 037K1286, purity ≥98%) was from Fluka (Steinheim, Germany), **betamethasone dipropionate** (batch 10F17-B08-257129, purity ≥97%) from Certa (Braine-L'alleud, Belgium) and **hydroquinone** (batch 10157959, purity ≥99%) came from Alfa Aesar (Karlsruhe, Germany).

For quantification with a DAD detector, HPLC-grade acetonitrile was used from Biosolve (Valkenswaard, the Netherlands). Water was obtained using a milliQ-Gradient A10 system (Millipore, Billerica, USA). For screening with a TOF detector, ULC/MS-grade acetonitrile and water were purchased from Biosolve (Valkenswaard, the Netherlands). Boric acid and ammonia solution 25% (v/v) was from Merck (Darmstadt, Germany). Leucine enkephalin and phosphoric acid used for the calibration of the MS were purchased from Sigma Aldrich (St. Louis, USA).

2.2. Sample set of suspected illegal whitening cosmetics

The sample set consisted of 163 samples which were taken by inspectors from the Belgian federal public service "Animal, Plant and Food Directorate-General" (DG4) and the Belgium Federal Agency for Medicinal and Health Products (FAMHP). Collection of samples took place between 2009 and 2012.

As summarized in Table 1, 48% of the samples were creams, 18% lotions and 13% soap bars. Other formulations such as serums, oils and gels were also present.

Due to the confidential nature of inspection data it is not possible to give precise information about each sample individually. In general, half of the samples were taken at high risk sites such as customs at cargo and passenger airports while the other half were taken in retail, wholesale business or so-called "ethnic cosmetic" shops in Belgium. These cosmetic products were taken during specific control operations and made part of larger quantities. A cargo sample usually was part of several palettes or containers. Samples taken in retail stores were representative of several units of the same product that were available in the shop.

2.3. Sample preparation

Sample preparation was developed and validated previously in our laboratory [9]. In short, 1.0 g of formulation was accurately weighed and diluted with 25 ml acetonitrile. For quantification 5 ml of a 0.1 mg/ml prednisone in acetonitrile was added as internal standard.

This solution was stirred for 10 min, ultrasonificated (Branson 8510) at 50 °C for 30 min and stored at −20 °C for 1 h. The preparation was then filtered through a 0.2 μm polytetrafluoroethylene syringe filter (25 mm). Before injection, the solution was five times diluted with a water/acetonitrile mixture (90/10, v/v).

For the extraction of soap, 1.0 g was dissolved in 10 ml of water and immediately neutralized with hydrochloric acid, to stabilize hydroquinone. The solution was brought to a total volume of 25 ml with acetonitrile. The extraction procedure was then continued as described above.

2.4. Validation samples

For the validation of the screening method 32 blank cosmetic matrices were used. These products were kindly provided by pharmacists. They were selected in such way that the ratio of the different matrices present in the validation test set was representative for the different matrices found in the suspected illegal samples. An overview of the number of cosmetic formulations present in the validation test set is shown in Table 1.

The validation samples were analyzed before and after spiking. The selected concentration levels for the spiked samples were kept lower than the usage concentrations, which was for the legal agents the cosmetic usage concentration and for the ingredients banned in cosmetics the medical usage concentration. Both concentration levels are shown in Table 2 [10–12]. Spiking was done by adding 100 μl of the spike solutions to 1 g of cosmetic formulation in a light-protected recipient to avoid photo degradation of retinoid like substances.

Concentrations of the spiked solutions are given in Table 2. The spiked solutions were diluted in 90/10 (v/v) acetonitrile/water. They were homogeneously distributed using a stirrer before further dilution with acetonitrile to a total volume of 25 ml was carried out. The extraction was then carried out, as described in Section 2.3.

2.5. Instrumental conditions

a. Screening method

Method development and validation were performed on an Acquity UPLC™ system (Waters, Milford, USA), consisting of a

Table 1
Cosmetic formulations present among the suspected samples and the validation samples.

Cosmetic formulation	Number of samples	% of total sample set (n = 163)	Number of validation samples	% of total validation set (n = 32)
Cream	78	48	15	47
Lotion	29	18	6	19
Soap	22	13	5	16
Serum	14	9	2	6
Oil	12	7	2	6
Gel	8	5	2	6