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Development and validation of a fast chromatographic method for screening and quantification of legal and illegal skin whitening agents

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ABSTRACT

During the last years, the EU market is flooded by illegal cosmetics via the Internet and a so-called "black market". Among these, skin-bleaching products represent an important group. They contain, according to the current European cosmetic legislation (Directive 76/768/EEC), a number of illegal active substances including hydroquinone, tretinoin and corticosteroids. These may provoke as well local as systemic toxic effects, being the reason for their banning from the EU market. To control this market there is a need for a fast screening method capable of detecting illegal ingredients in the wide variety of existing bleaching cosmetic formulations.

In this paper the development and validation of an ultra high pressure liquid chromatographic (UHPLC) method is described. The proposed method makes use of a Waters Acquity BEH shield RP18 column with a gradient using 25 mM ammonium borate buffer (pH 10) and acetonitrile.

This method is not only able to detect the major illegal (hydroquinone, tretinoin and six dermatologic active corticosteroids) and legal whitening agents, the latter having restrictions with respect to concentration and application (kojic acid, arbutin, nicotinamide and salicylic acid), but can also quantify these in a run time of 12 min.

The method was successfully validated using the "total error" approach in accordance with the validation requirements of ISO-17025.

During the validation a variety of cosmetic matrices including creams, lotions and soaps were taken into consideration.

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1. Introduction

Whitening products have been used for decades to lighten skin color, especially in people with photo type IV, V or VI and are increasingly popular [1,2]. The reasons for skin-bleaching are very diverse and complex and cover as well medical, esthetic, cultural, socio-economic as political issues [1,3]. Indeed, aside from their application in cosmetics, whitening agents are also used in pharmaceuticals to treat skin diseases such as melasma and inflammatory hyperpigmentation [4,5]. Whitening agents, including corticosteroids, hydroquinone and tretinoin may, however, provoke unwanted local effects (ochronosis, irritant dermatitis, leukoderma, post-inflammatory hyperpigmentation, ...) and systemic toxicity (kidney and liver problems). Therefore in Europe, these substances have been placed on annex II of the Cosmetic Directive 76/768/EEC and are thus banned from the EU market [6]. But despite this ban, products containing these ingredients can still be bought through illegal circuits. Knowing that formulations usually include combinations of the above mentioned substances together with legal ingredients and penetration enhancers, their potential risk for human health cannot be excluded, in particular after repeated and long-term exposure.

A range of new agents has been developed including kojic acid, arbutin and nicotinamide. For some of these substances the safety was investigated at the commission level by the Scientific Committee on Consumer Safety (SCCS) and health concerns were expressed, e.g. for arbutin which is considered to be able to release hydroquinone [7,8].

As stated by Chisvert et al. [2], the development of performing methods allowing efficient analysis of this type of cosmetics is highly needed in order to control the market. Methods that detect and quantify hydroquinone alone are not sufficient [9,10].

The same is true for screening methods that only detect corticosteroids or a selected group of bleaching agents consisting mainly of kojic acid, arbutin and hydroquinone [11,12].

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Until now, to the best of our knowledge, no method has been published nor validated to both identify and quantify the most important illegal and legal skin whitening agents while taking into account the variety in composition of the different cosmetic formulations that may contain these bleaching agents e.g. creams, lotions and soaps. Such a generic method may save time and resources, and provide regulatory authorities with more flexibility to survey the (illegal) market and better inform the consumer about potential health risks.

The present paper describes such a method. It is a UHPLC application that enables qualitative and quantitative analysis, of eight illegal (hydroquinone, tretinoin and six dermatologic active corticosteroids) and four legal (kojic acid, arbutin, nicotinamide and salicylic acid) skin whitening agents present in different types of cosmetic preparations and this in a single run of 12 min.

2. Materials and methods

2.1. Standards and reagents

The reference standards kojic acid (batch 1363411 V, 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%), 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%) from Alfa Aesar (Karlsruhe, Germany).

HPLC-grade acetonitrile and methanol were purchased from Biosolve (Valkenswaard, the Netherlands). Boric acid and ammonia solution 25% (v/v) were from Merck (Darmstadt, Germany). Water was obtained using a milliQ-Gradient A10 system (Millipore, Billerica, USA). The cream base used to prepare the validation samples and the ingredients used to formulate lotions and soaps were purchased from Fagron (Waregem, Belgium).

2.2. Sample preparation

2.2.1. Calibration standards

The compounds were divided into two groups. Group A, containing the more hydrophilic compounds (kojic acid, hydroquinone, nicotinamide, salicylic acid and arbutin) and group B the more hydrophobic ones (corticosteroids and tretinoin). The concentrations used for each calibration standard are shown in Table 1. Solutions were prepared in 100% acetonitrile except for the arbutin solution which contained 0.05% water. Calibration was done by adding 5 ml of the standard solutions to 1 g of placebo cream in a light-protected recipient to avoid photo degradation of tretinoin [13,14]. 5 ml of an internal standard solution, containing 0.1 mg/ml prednisone dissolved in acetonitrile, was added and the solution was further diluted with acetonitrile to a total volume of 25 ml. To stay within the dynamic range of the detector, samples containing compounds from group A were diluted. The final concentrations obtained for each level are shown in Table 1. Further sample treatment is described under Section 2.3.

Table 1
Concentrations of the validation samples and the corresponding calibration standards for three concentration levels. *Solutions containing arbutin were made in acetonitrile containing 0.05% water.

Compounds	Concentrations	
	Validation samples (g/100 g)	Final calibration standards (μg/ml)
Kojic acid	0.5	6
	1	13
	2	26
Arbutin	4	53
	7	93
	10	133
Hydroquinone	1	20
	5	100
	7	140
Nicotinamide	1	13
	2	26
	3	40
Salicylic acid	1	13
	3	40
	5	66
Corticosteroids and tretinoin	0.03	12
	0.05	20
	0.1	40

2.2.2. Validation samples

The spiked validation samples were prepared according to the art of pharmacy profession by mixing the solid whitening ingredients with the anionic hydrophilic cream base (oil in water (o/w) emulsion).

The selected concentration levels for the validation samples correspond to the usual concentrations present in cosmetics. To reduce the workload, creams with multiple components were made and the concentrations of the individual components in the different creams are listed in Table 1 [15,16]. Whenever tretinoin was involved, all work was carried out avoiding light exposure [13,14].

As skin whitening cosmetics are available in different cosmetic formulations (e.g. creams, lotions, soaps, ...), with creams being the most important ones. A recovery study was carried out using as well creams, lotions and soaps. These cosmetic formulations were prepared at an intermediate concentration level as shown in Table 1. They were analyzed in triplicate. The ingredients and composition of the three cosmetic formulations are given in Table 2.

2.3. Sample treatment

One gram of cream or lotion was accurately weighed in a brown conical flask. 5 ml of the internal standard (IS) solution was added and the total volume was brought to 25 ml with acetonitrile. This solution was stirred for 10 min and ultrasonificated (Branson 8510) at 50 °C for 30 min. After ultrasonification, the solution was stored at −20 °C for one hour to precipitate the greasy ingredients of the cream/lotion. The preparation was then filtered through a 0.2 μm Polytetrafluoroethylene (PTFE) 25 mm syringe filter. The concentrations obtained at this point were the same for the validation sample and the corresponding calibration standard.

For compounds of group B the filtered solution could directly be used. For group A, further dilution with a water:acetonitrile mixture (90:10, v/v) was necessary to stay within the linear dynamic range of the detector.

For soaps, 1 g of formulation was dissolved in 10 ml water and immediately neutralized with hydrochloric acid, since hydroquinone is not stable in alkaline solution. IS was then added and the

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