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UV gradient combined with principal component analysis: Highly sensitive and specific high performance liquid chromatography analysis of cosmetic creams

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ABSTRACT

HPLC has been employed to develop a method for the analysis of cosmetic creams, in particular the compounds hydroquinone, phenol and six preservatives have been studied. UV tuning was optimized as a gradient to achieve lower limits of detection compared to those of a previously validated method. In addition the chromatograms were then exported, aligned and visualized in a principal component analysis (PCA) model. The results were the highly efficient separation of the eight studied compounds. All the compounds showed good linear correlation coefficients ($\geq$0.9997), the detection limit was found to be in the range of 15–200 ng/mL, a 10-fold improvement for the preservatives on previous methodology and the average recovery was within limits between 83% and 117% with a relative standard deviation (RSD) less than 3.6% (n = 6). The PCA plot was constructed from the UV optimized cosmetic samples chromatograms from real samples, real samples that were spiked and quality controls. Quality controls contained the eight compounds and showed complete clustering in the PCA and three spiked samples containing six to seven toxic components clustered in the same quadrant. The method is highly sensitive and its potential use as a method that could be employed in the control of cosmetics, particularly those containing banned or suspected toxic additives, has been demonstrated.

1. Introduction

The cosmetic creams industry generates billions worldwide, while at the same time being a largely self-regulated industry. Existing laws on ingredient safety are proposed by the cosmetics industry but organisations like the US Food and Drug Administration (FDA) cannot by law require safety assessments to this very day. Consequently, several products that have been shown to be toxic are not being recalled by any safety product organization [1]. The law is currently being revised and some cosmetic creams, such as skin whitening agents, have been made illegal in the UK, mainly those that contain mercury [2]. In recent years a number of other potentially harmful compounds utilised in skin whitening creams have been identified, including several glucocorticoids, which do not comply with European regulations [3]. Concerns over the toxicity and carcinogenicity of hydroquinone, frequently found in skin whitening agents, have been reported, although these findings are controversial and largely based on *in vivo* and animal studies [4,5]. The use and safety of phenols in topical treatments, again is of concern. In addition, we consider the analysis of a number of preservatives used as antimicrobial agents, including benzoic acid, sorbic acid and parabens. The presence of such additives is regulated by the European Economic Community law and covers the list of allowed preservatives and their maximum allowed concentrations. Concentrations of benzoic acid must be less than 0.5% (w/w) for leave-on products, and less than 0.4% (w/w) or 0.8% (w/w) for parabens depending on whether one or two are present, respectively, but there is little data reported on the biosafety of these additives [6,7]. Especially concerning is the absorption and accumulation of metabolites in the body since in the case of cosmetic cream products these are often applied daily or repetitively to what is in essence our biggest organ. Main health concerns relating to parabens are based on animal studies, these have shown that exposure to certain parabens can cause adverse health effects including effects on development of the male reproductive system [8,9].

There are a limited number of methods which report the sensitive quantification of potentially toxic agents in cosmetic creams, but with increasing efforts devoted to the biosafety of such formulations. With widespread concerns on public safety, the need for sensitive, quantitative analytical techniques to determine the

Abbreviations: HQ, hydroquinone; PO, phenol; BA, benzoicacid; SA, sorbicacid; MP, methylparaben; EP, ethylparaben; PP, propylparaben; BP, butylparaben.

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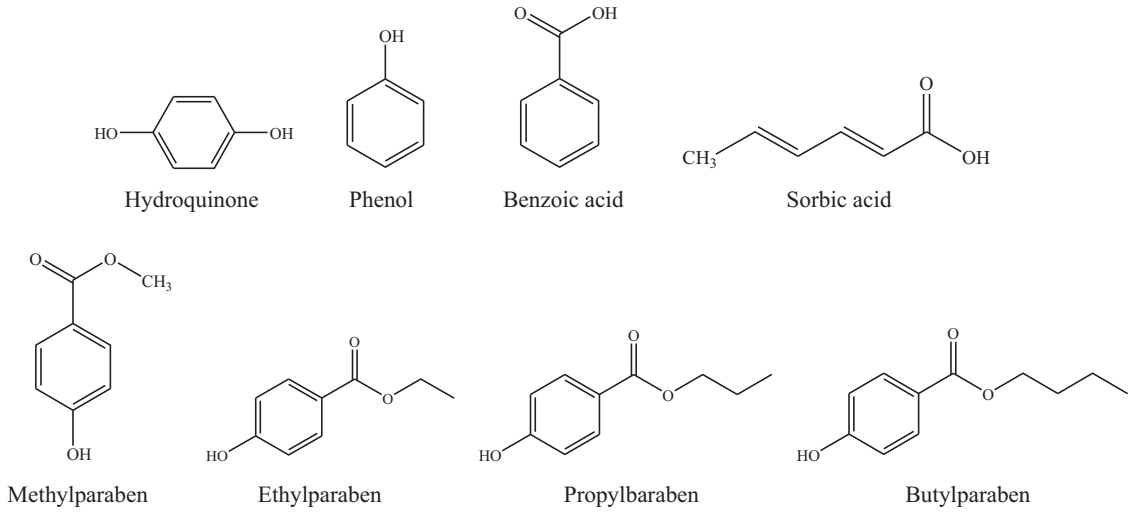

Fig. 1. Chemical structures of the compounds considered in this study.

level of dangerous agents in creams is required, particularly if regulations will soon be employed to restrict the use of such agents. Here we consider eight compounds some commonly found in cosmetic creams, which we believe to be of importance in health and safety, namely hydroquinone (HQ), phenol (PO), benzoic acid (BA), sorbic acid (SA), methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) (see Fig. 1). Previously we have reported the validation of a HPLC-DAD method to separate these eight compounds of interest [10]. Since some of these components, such as hydroquinone, are not well suited to mass spectrometric detection due to poor ionisation, this study aims to increase sensitivity of the previously reported HPLC-DAD method by tuning the UV in a gradient for these particular components. This optimized, highly sensitive method allows for the simultaneous detection and quantification of the eight components and was applied to the analysis of 10 different cream samples for different cosmetic purposes. A collection of quality control samples, spiked and real samples were subsequently analyzed and visualized using a PCA model to understand the variability and test the model.

2. Experimental

2.1. Instrumentation

Chromatography was performed using on an Agilent 1100 HPLC system (Agilent, USA) consisting of a quaternary pump, an autosampler, a vacuum degasser, and a column compartment, coupled to a diode array detector. An SFE 590/1 ultrasonicator (Ultrawave Limited, Cardiff, UK) and 5415C centrifuge (Eppendorf, Germany) were used in the experiments.

2.2. Chemicals

All chemicals employed with the exception of ammonium formate were obtained from Sigma–Aldrich (St. Louis, MO, USA). Hydroquinone (HQ), phenol (PO), sorbic acid (SA), benzoic acid (BA), methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP) were 99% purity. Methanol and acetonitrile were HPLC grade. Ammonium formate (98.2% purity) was obtained from Prolabo (VWR, UK). The water used in these experiments was purified using a Synergy UV Water Purification System (Millipore, UK) and was used to prepare all solutions for the HPLC method. The 10 cosmetic creams were purchased from UK, US, China, Spain, Sudan and Thailand and ranged from creams and lotions for skin whitening, hydration and anti-aging.

2.3. Chromatographic conditions

The chromatographic column used was a Zorbax Bonus-RP column, 100 mm × 2.1 mm I.D. with 3.5 μm particle diameter (Agilent, USA). The mobile phase consisted of a mixture of methanol and 0.05 mol/L ammonium formate solution (pH = 3.0), and the gradient elution details were as follows: 0 min, 45% methanol; 2 min, 45% methanol; 5 min, 70% methanol and maintained to a max. 20 min. The flow rate was set at 200 μL/min and the injection volume was 5 μL. All analyses were performed at room temperature. The detection wavelength conditions are shown in Table 1.

2.4. Standard solutions for quantification

Standards were prepared in 60% methanol and 40% water (v/v). Stock solutions of each standard at a concentration of 1000 mg/L were prepared. A mixture solution of the components comprised of 1.0 mL HQ, 2 mL PO, 0.2 mL SA, 1.0 mL BA, 0.5 mL MP, 0.5 mL EP, 0.5 mL PP, 0.5 mL BP, respectively, and fixed volume in a 25 mL volumetric flask in 60/40 methanol/water (v/v). Suitable working solutions with concentration in the range of 0.01–200 mg/L were also prepared as standard calibration solutions. The calibration curves were plots of area *versus* concentration and errors were calculated in OriginLab (Northampton, USA). The LODs were established at a signal-to-noise ratio (S/N) of 3.

2.5. Sample extraction

0.2 g of the cosmetic cream was accurately weighed in a glass tube. After this 5 mL of extraction solvent (methanol:water = 60:40,

Table 1
The program table for a gradient in detection wavelength.

Time	Wavelength channel	Detection wavelength	Reference wavelength
0.0	B	290	360
2.5	B	272	360
3.4	B	256	360
4.0	B	232	360
4.5	B	256	360