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Fast and sensitive high performance liquid chromatography analysis of cosmetic creams for hydroquinone, phenol and six preservatives

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ABSTRACT

A fast and sensitive HPLC method for analysis of cosmetic creams for hydroquinone, phenol and six preservatives has been developed. The influence of sample preparation conditions and the composition of the mobile phase and elution mode were investigated to optimize the separation of the eight studied components. Final conditions were 60% methanol and 40% water (v/v) extraction of the cosmetic creams. A C18 column (100 mm × 2.1 mm) was used as the separation column and the mobile phase consisted of methanol and 0.05 mol/L ammonium formate in water (pH = 3.0) with gradient elution. The results showed that complete separation of the eight studied components was achieved within 10 min, the linear ranges were 1.0–200 μg/mL for phenol, 0.1–150 μg/mL for sorbic acid, 2.0–200 μg/mL for benzoic acid, 0.5–200 μg/mL for hydroquinone, methyl paraben, ethyl paraben and propyl paraben, butyl paraben, and good linear correlation coefficient (≥0.9997) were obtained, the detection limit was in the range of 0.05–1.0 μg/mL, the average recovery was between 86.5% and 116.3%, and the relative standard deviation (RSD) was less than 5.0% (n = 6). The method is easy, fast and sensitive, it can be employed to analyze component residues in cosmetic creams especially in a quality control setting.

1. Introduction

In the last decade public awareness of the potential hazard of chemicals used in everyday life has grown dramatically. The safety of certain components of raw materials used in cosmetic formulations has attracted much attention and compounds such as hydroquinone, phenol and several preservatives have been flagged as unsafe compounds in some countries.

The cosmetic industry produces hundreds of different skin whitening creams and lotions to be sold to the public. Pigmentation of the skin, is due to the amount, quality and distribution of the naturally occurring pigment, melanin, in the skin [1]. Genetic and environmental factors [2] such as the effects of UV sun radiation and scavenging toxic drugs and chemicals [3] can increase melanin production resulting in conditions such as tanning, melasma, chloasma, age spots or uneven skin tone. The most commonly used and effective skin whitening agent is hydroquinone [2]. Prolonged usage of hydroquinone may cause exogenous ochronosis which is in fact further pigmentation of the skin. Other reported dermatological complications include dermatitis, cataracts, pigmented colloid milia, sclera disorders, nail pigmentation and patchy depigmentation. These are the main reasons as to why hydroquinone is no longer authorised for use in cosmetic skin lightening formulations and since the year 2000 it can only be obtained on prescription in the European Union countries [4].

Phenol has to a minor degree a whitening effect. It is used as a disinfectant of medical care, a 3–5% solution can disinfect the skin, but prolonged exposure greatly irritates and harms the skin. Among many preservatives, benzoic acid and sorbic acid are widely used in foodstuffs, drugs, and cosmetic products to prevent their aging and decay, however, they all have some known toxicity [5]. Parabens are esters of p-hydroxybenzoic acid. Because of their broad antimicrobial spectrum with relatively low toxicity, good stability, and non-volatility, they have been extensively used as preservatives for cosmetics in restricted concentration levels. However parabens in creams have also been reported to show androgenic effects and to accumulate through skin exposure in the body [6].

The analytical determination of these additives in cosmetics is important not only for quality assurance purposes but also for consumer interest and protection. Recently methods for the analysis of these components, either hydroquinone, phenol or preservatives have been shown by spectrophotometry [6], GC [7,8], CE [9], CEC [10] and electrochemistry [11]. Among these methods, spectrophotometry and electrochemistry methods are subject to matrix interference, which makes the quantification of the additives difficult. The GC method, which is the traditional technique for the

Abbreviations: BA, benzoic acid; BP, butyl paraben; EP, ethyl paraben; HQ, hydroquinone; MP, methyl paraben; PO, phenol; PP, propyl paraben; SA, sorbic acid.

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analysis, has the drawback of the difficulty of direct determination of some substances – in particular sorbic and benzoic acid due to their poor volatility, polarity, and the thermal instability of the latter. CE and CEC have many advantages, high performance, sensitivity, rapidity and economy. However relative to the former, reproducibility in HPLC is more effective and it is an ideal technique for the residual analysis of these components.

Zhang et al. [12] reported determination of hydroquinone, phenol, antibiotics and preservatives in cosmetics. Their results showed good linearity within the range of 1–200 μg/mL ($r > 0.998$), average recovery was 85.2–114.4%, RSD < 6%, however, fronting peaks for benzoic acid and sorbic acid were observed due to unwanted interactions at the conditions employed for analysis. Zhou et al. [13] analyzed hydroquinone and Nipagin Esters in cosmetics, 5 substances were successfully separated within 11 min.

López García et al. [6] selectively analyzed for hydroquinone in gel and cream, the linearity was in the range of 6.0–30.0 g/mL and presented a correlation coefficient (r) of 0.9999, calculated by least square method. The LOD and LOQ were 0.08 and 0.26 g/mL, respectively.

Conversely to peak fronting exhibited by benzoic and sorbic acids, the analysis of parabens is difficult due to unwanted interactions by RP-HPLC often producing peak tailing [8]. Janjua et al. [14] analyzed urinary concentrations of butyl paraben after cream usage by HPLC–MS–MS, and found increased excretion of butyl paraben following cream usage. Dugo et al. [15] employed superheated water at 100 and 200 °C as mobile phase for RP separation to analyze parabens, the optimized method was finally applied to parabens in a commercial body cream.

Hence, we report on a faster and more sensitive analysis method of a mixture of eight: hydroquinone (HQ), phenol (PO), benzoic acid (BA), sorbic acid (SA), methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) compounds with potential harzardous effects by HPLC. In Section 3, the acronym for the compounds is used. The developed method was applied to the analysis of these components in ten different cream samples purchased in five different countries.

2. Experimental

2.1. Instrumentation

Experiments were performed in an Agilent 1100 HPLC system (Agilent, USA) consisting a quaternary pump, an autosampler, a vacuum degasser, and a column compartment, coupled to a diode array detector. 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 experiments was purified using a Synergy UV Water Purification System (Millipore, MA, USA) and was used to prepare all solutions for the HPLC method. The cosmetic creams were purchased from UK, US, China, Sudan and Thailand.

2.3. Chromatographic conditions

The chromatographic column used was a Zorbax Bonus-RP C18 column, 100 mm × 2.1 mm, i.d. with 3.5 μm particle diameter (Agilent, USA). The mobile phase was a methanol and 0.05 mol/L ammonium formate solution (pH = 3.00), 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. The detection was made at 280 nm. All analyses were performed at room temperature.

2.4. Standard solutions

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. The stock mixture solutions were composed of 0.5 mL HQ, 1 mL PO, 0.2 mL SA, 2.5 mL BA, 0.5 mL MP, 0.5 mL EP, 0.5 mL PP, 0.5 mL BP, respectively and diluted in a 25 mL volumetric flask. Suitable working solutions with concentration in the range of 0.2–200 mg/L were also prepared as standard calibration solutions. The calibration curves were plots of area *vs.* concentration.

2.5. Sample preparation

An amount of 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, v/v) were added, followed by sonication for 30 min. The extract was centrifuged for 10 min at 5000 rpm. The extract was then filtered with a 0.20 μm membrane. The filtrate was then injected into the micro-HPLC instrument.

3. Results and discussion

3.1. Sample preparation and extraction optimization

The extractant and ultrasonication time were optimized for the sample preparation procedure. It has been reported in the literature that sorbic and benzoic acid can be effectively extracted by adding acetic acid in the extractant [16] while other reports used methanol as the extractant of choice [8,12]. Here the extraction efficiency was optimized by varying the extractants and their ratio. The results of the experiments with spiked cream are shown in Fig. 1. Peak height (h) and peak area (A) can directly reflect the extraction efficiency of the studied components. The results showed that with the increase of methanol content, the peak height of components decreased, peak areas except for the four parabens also showed a slight decrease. If methanol and acetonitrile were used as extractants, the peak height was obviously reduced. In addition, acetic acid in the extractant did not influence significantly the extracted amounts of SA and BA. Considering all the above factors, 60% methanol and 40% water was selected as the best extractant.

3.2. Optimization of the ultrasonication time

The ultrasonication time for the extraction was optimized in order to get the best extraction efficiency. We continued to investigate the extraction efficiency using peak area. The results showed that as time lengthened, peak area of the studied components increased, but it did not significantly change from 20 min to 40 min. Considering both extraction efficiency and time, 30 min was selected as the parameter for the experiment.

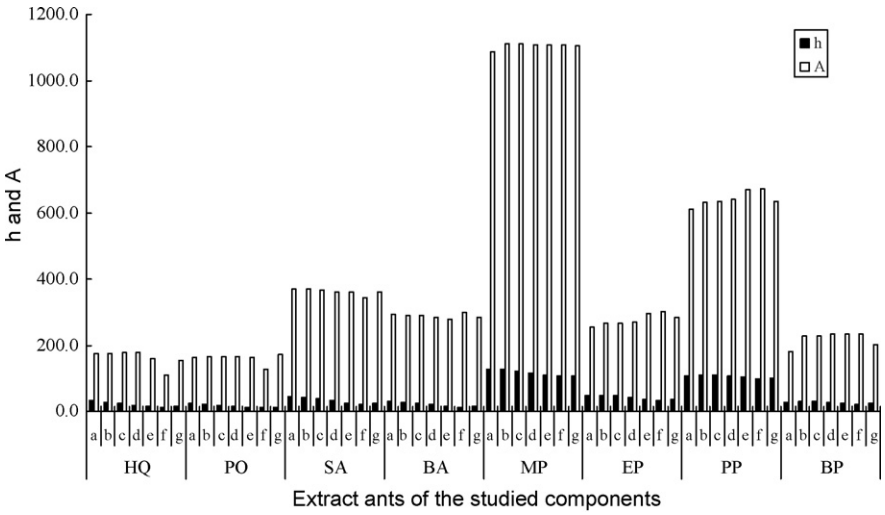

Fig. 1. Effect of different extraction solvents on the extraction of 8 components in creams. h is peak height, A is peak area. a. methanol:water = 50:50 (v/v), b. methanol:water = 60:40 (v/v), c. methanol:water = 70:30 (v/v), d. methanol:water = 80:20 (v/v), e. methanol, f. acetonitrileg.methanol:water:acetic acid = 60:39.5:0.5 (v/v). The background was not deducted in this experiment.

3.3. Optimum detection wavelength

To obtain good sensitivity, the absorption spectrum obtained under optimized condition was investigated and showed that the maximum and characteristic absorption wavelength of the studied components was 223 and 290 nm for HQ, 217 and 270 nm for PO, 228 nm and 274 nm for BA, 256 nm for SA and the four parabens. Considering the absorptions of the eight components, 280 nm was selected.

3.4. Optimum HPLC mobile phase

In order to obtain the best separation of the eight studied components, several mobile phases were investigated, including methanol and water (50:50, v/v), methanol and 0.01 mol/L phosphate solution (55:45, v/v), methanol and 0.05 mol/L ammonium acetate solution (45:55, v/v), above using isocratic elution, methanol and 0.05 mol/L ammonium formate solution (see Section 2.3).

When the mobile phase consisting of methanol and 0.01 mol/L phosphate solution or methanol and water were used, the eight studied components could be separated, however serious peak tailing for SA and BA was observed. When methanol and 0.05 mol/L ammonium acetate solution was employed a baseline separation was obtained but the analysis time was considered too long.

Finally in the case of a methanol and 0.05 mol/L ammonium formate solution (pH = 3.00) the eight components were successfully separated and the problem of peak tailing was solved. As the analysis time of near 23 min was considered too long for an isocratic method, a gradient elution was adopted (see Section 2.3 for details). The influence of the buffer's pH on the retention time of the eight components was investigated. The ammonium formate was employed as the buffer. As shown in Fig. 2, buffer pH does not affect the retention of HQ, PO and four parabens, however, it influences the retention of SA and BA, because it can change the dissociation degree of the acid, and affects the distribution of acid in the stationary and mobile phases, affecting the retention and resolution of these acids.

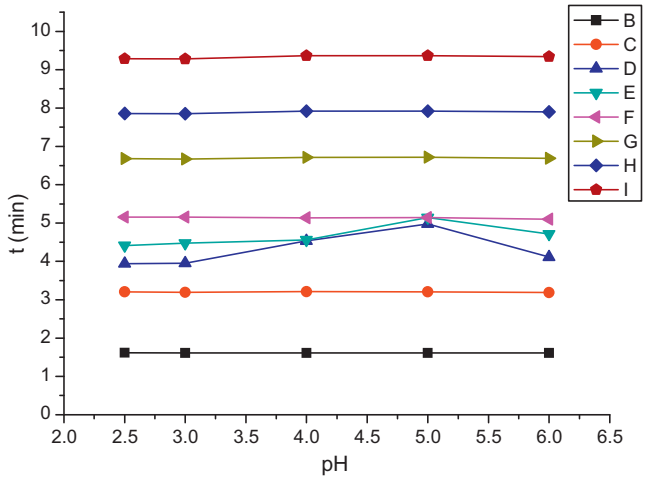

Fig. 2. The influence of buffer pH on the retention time of the eight components B. HQ, C. PO, D. SA, E. BA, F. MP, G. EP, H. PP, I. BP. under Section 2.3 experimental conditions, the injection volume: 2 μL the stock standard mixture solution including 20 g/mL for HQ and four parabens, 40 g/mL for PO, 8 g/mL for SA, 100 g/mL for BA.

Hence, the best resolution of the eight components was achieved under pH = 3.00. Optimal results are shown in Fig. 3 where the eight

Table 1
Linear equation, correlation coefficient and detection limit of the studied components.

Components	Linear equation	Correlation coefficient	Linear range/(μg/mL)	Detection limit/(μg/mL)
HQ	$y = 14.2x + 2.13$	1.0000	0.5–200	0.2
PO	$y = 8.15x - 1.77$	1.0000	1.0–200	0.5
SA	$y = 94.9x + 4.68$	0.9998	0.1–150	0.05
BA	$y = 5.90x - 4.13$	0.9999	2.0–200	1.0
MP	$y = 23.4x + 1.81$	0.9997	0.5–200	0.2
EP	$y = 22.9x + 0.762$	0.9999	0.5–200	0.2
PP	$y = 21.9x + 4.12$	0.9999	0.5–200	0.2
BP	$y = 21.0x + 0.992$	0.9999	0.5–200	0.2

Table 2
The recovery and precision of the studied component standard solution added to the cosmetics ($n = 6$).

Components	Amount added levels (μg)		Average recoveries (%)	RSD (%)
	Added	Found		
HQ	5	5.14–5.45	102.8–109.1	2.17–3.41
	50	51.6–57.9	103.3–115.9	1.15–3.02
PO	10	11.0–11.6	110.5–115.7	2.23–3.32
	100	100–105.2	100.0–105.2	1.74–2.84
SA	1	0.86–0.94	86.5–93.9	3.47–4.43
	10	9.55–9.96	95.5–99.6	1.21–2.86
BA	15	15.3–16.3	102.2–108.4	2.35–4.84
	150	140.6–154.4	93.7–102.9	1.88–3.16
MP	5	5.10–5.59	101.9–111.7	3.68–4.63
	50	47.8–51.5	95.5–103.0	1.73–3.43
EP	5	5.24–5.82	104.9–116.3	2.53–4.07
	50	49.2–52.8	98.5–105.6	1.98–3.46
PP	5	4.56–5.78	91.3–115.7	3.25–4.68
	50	51.6–54.9	103.1–109.9	1.53–3.78
BP	5	5.30–5.67	106.0–113.4	2.36–3.78
	50	51.2–53.8	102.4–107.6	1.92–3.69

studied components elute in less than 10 min and peak shapes are acceptable.

3.5. Linearity and limit of detection

The linearity was assessed using twelve standard solutions in the range of 0.05–200 μg/mL. The linear regression analysis for each compound was constructed by plotting the peak area against the standard concentration. As shown in Table 1, good correlation coefficients were observed for each component ($r > 0.9997$). The limits of detection were calculated according to signal to noise ratio equal to 3. These were as follows, for HQ, PO, SA, BA and four parabens were 0.2, 0.5, 0.05, 1.0 and 0.2 μg/mL respectively (Table 2). Limits of detection for SA and parabens were found to be lower than those reported previously [12].

3.6. Recovery and precision

The recovery and precision of the method were tested by adding the component standard solution in cosmetics at high and low concentration. The concentration levels were 1 g/mL and 10 g/mL for HQ and four parabens, 2 g/mL and 20 g/mL for PO, 0.2 g/mL and 2 g/mL for SA, 3 g/mL and 30 g/mL for BA. The results are shown in Table 2, the recovery and relative standard deviation (RSD%) values were in the range of 85.6–116.3% and 1.15–4.84%, respectively.

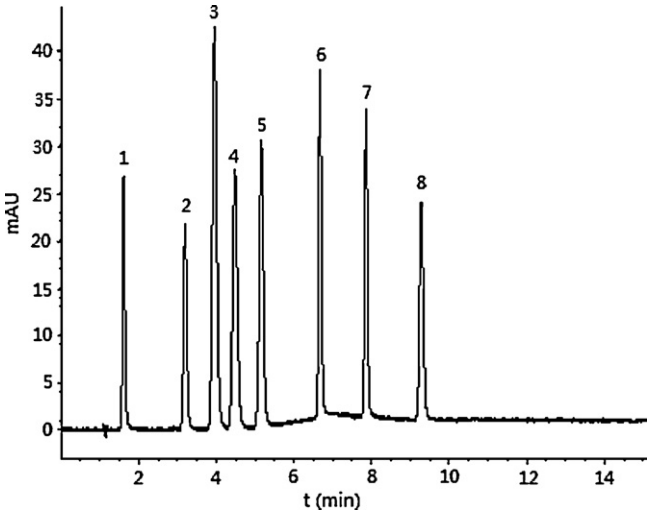

Fig. 3. Chromatogram of the eight studied components 1. HQ, 2. PO, 3. SA, 4. BA, 5. MP, 6. EP, 7. PP, 8. BP under 2.3 experimental conditions, the injection volume: 2 μL the stock standard mixture solution including 20 g/mL for HQ and four parabens, 40 g/mL for PO, 8 g/mL for SA, 100 g/mL for BA gr3.

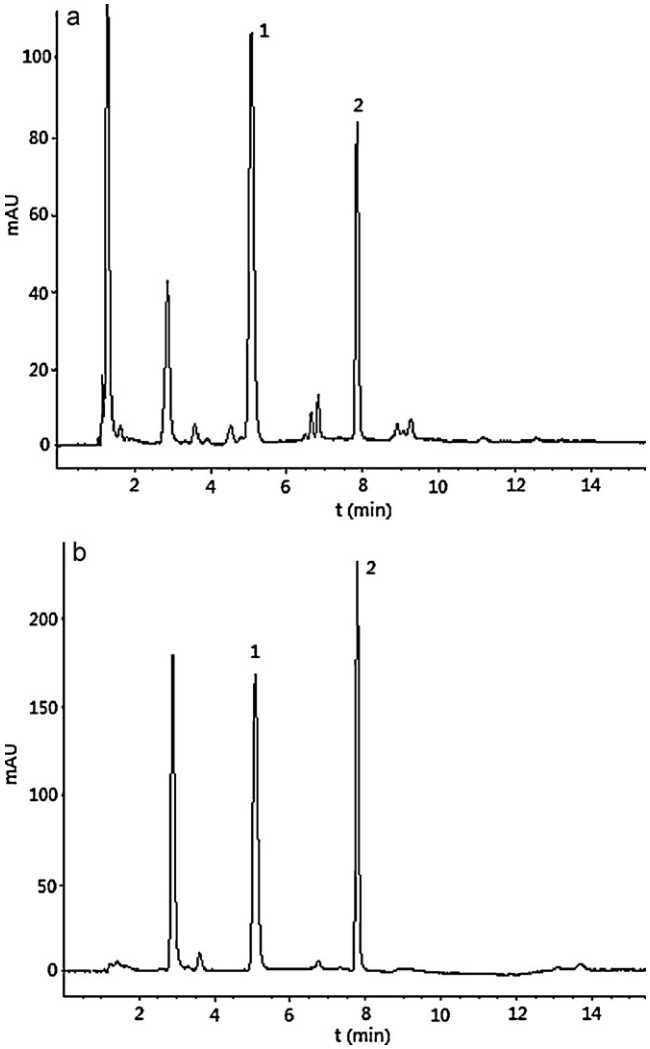

Fig. 4. Chromatograms of two typical samples a. cream, b. lotion; under Sections 2.3 and 2.5 experimental conditions.

3.7. Determination of real samples

In order to examine the studied compound residues in cosmetic products, 10 samples, including creams and lotions, were randomly purchased in five different countries. HQ, PO, SA, BA, and four parabens, i.e. MP, EP, PP, BP, were determined using the method. Two typical chromatograms are shown in Fig. 4. The 10 samples were found to contain at least one or more of the eight studied components. The components detected in the studied samples were MP, EP, PP and BP and their concentrations were found in the range of 189.6–2608 mg/kg. The remaining 4 components, HQ, PO, SA and BA were not detected in the studied samples.

4. Conclusion

In summary, a new fast and sensitive reversed-phase liquid chromatography method for separation of HQ, PO and six preservatives was developed. After optimization of the sample preparation conditions and the composition of the mobile phase, pH and buffer, baseline separation of all of the studied components was obtained in less than 10 min. In addition, this method showed good results in terms of linearity, accuracy, repeatability, and limits of detection. The HPLC method can be applied to determine residue of the eight studied components in cosmetics and to analyze residues of these components in food, drug or environmental samples.

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