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Determination of phenolics in cosmetic creams and similar emulsions☆

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Abstract

A new method for the analysis of phenolics in cosmetic creams has been developed, based on a systematic study of the extractability of five phenolic compounds from such emulsions. A solid–liquid extraction using ultrasound was applied as a prior stage to the chromatographic determination of phenolics in the extracts. Three solvents, hexane, methanol and water, were used as extracting agents. These solvents permit both the de-emulsification of the creams and the extraction of phenolics. A factorial fractional experimental design was developed to analyse the influence in the extraction process of five different extraction variables: ultrasound horn, temperature, extracting volume, cycle and amplitude of ultrasounds. Graphic analysis of results revealed the variables with most influence in the extraction, as well as the interactions between the variables. Finally, the influence of the extraction time and the sample quantity were also studied. With this new method, phenolics can be extracted from silicone-based cosmetic creams in 10 min, using 50 °C as extraction temperature. RSDs ($n = 6$) calculated ranged from 1.5% for ferulic acid to 6.5% for epicatechin. Recoveries of between 88.9% for gallic acid and 98.4% for caffeic acid were obtained.

Keywords: Cosmetic cream; Emulsion; Phenolics; Extraction method; Experimental design

1. Introduction

The cosmetics manufacturing industry has recently become very interested in using phenolics as active constituents of cosmetic creams. However, a first consideration must to determine their stability in cosmetic preparations. There are no references in the literature to such determination. Moreover, a prior step to this must be the development of suitable analytical methods for the determination of phenolics in cosmetic creams.

A cosmetic cream is a matrix consisting of an emulsion of two different phases. In recent years, cosmetic creams have been evolving from oil-based to silicone-based creams, as the latter emulsions are more stable than the former. This is one of the main problems regarding the determination of phenolics in creams, for the first stage has to be de-emulsification to allow the phenolic compounds to pass to the aqueous phase.

There are reports in the literature describing the determination of active compounds in cosmetic creams. Some compounds similar to phenolics have been determined in creams like capsaicinoides using a liquid–liquid extraction before chromatographic analysis [1]. Some phenolic derivatives have been also determined in milk lotions by solid phase extraction (SPE) and HPLC [2]. Supercritical fluid extraction (SFE) has been used for determination of benzophenones in a cosmetic matrix [3]. For liquid pharmaceutical formulations no extraction are required in the determination of *p*-hydroxy benzoic acid preservatives [4]. Sunscreen agents have been determined in cosmetic products using microwave-assisted extraction before chromatographic analysis [5]. Sunscreen agents have been also determined after silylation by gas chromatography [6]. However, no reports were found related to the determination of phenolics in modern silicon based cosmetic creams. A new method based on the ultrasound assisted

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extraction of phenolics from cosmetic creams has been developed in this work.

2. Experimental

2.1. Sample

A silicone-based cream (200 g) was prepared by the method usually followed in the industrial process. Phenolic compounds incorporated to the cream were: gallic acid 50.3 mg, catechin 50.2 mg, caffeic acid 50.6 mg, epicatechin 50.4 mg and ferulic acid 50.9 mg.

2.2. Chemicals and solvents

The MeOH (Merck, Darmstadt, Germany) used was HPLC grade. Water was supplied by a Milli-Q water purifier system from Milipore (Bedford, MA, USA). The phenolic standards and 2,5-dihydroxybenzaldehyde were obtained from Sigma (St. Luis, MO, USA).

2.3. Extraction of phenolics

Extractions were carried out in a high-intensity ultrasound probe system of 200 W and 24 kHz (model UP 200S, Dr. Hielscher, Germany) with a horn fitted with two sizes of microtip: 2 and 7 mm. Amplitude of ultrasonic vibrations can be varied from 20% to 100% of nominal power. The extractions were performed at constant temperature ensured by a temperature controller coupled to the ultrasonic bath. All experiments were performed in duplicate.

The initial extraction protocol used 0.1 g of cream in 25–50 mL of the extraction solvent for 10 min. This protocol was further studied to optimize the extraction method. At the beginning of the extraction, 1 mL of 2,5-dihydroxybenzaldehyde (240 mg/L) was used as internal standard. The extracts were then filtered though a 0.45 μm nylon syringe filter (Millex-HN, Ireland) before chromatographic analysis.

2.4. High-performance liquid chromatography (HPLC)

The analyses of the extracts were performed by HPLC in a Waters system consisting of an auto sampler (717 plus), pump controller (600S), pump (616), and a photodiode array detector (996), using a RP-18 column (LiChrospher 100, 5 μm, Merck, Germany) and a gradient of acidified water (2% acetic acid) (solvent A) and methanol (2% acetic acid) (solvent B) at a flow rate of 0.3 mL/min. The gradient was as follows: 0 min, 15% B; 10 min, 30% B; 20 min, 30% B; 25 min, 100% B; 35 min 100% B. The UV absorbance was monitored from 200 to 400 nm. UV spectra were recorded and each phenolic peak area was quantified at its maximum wavelength. The sample volume injected was 10 μL. The analyzed phenolics were gallic acid, caffeic acid, catechin, ferulic acid and epicatechin. The identification of each phenolic was made by comparison of retention times with pure standards, as well as by UV–vis spectra.

3. Results and discussion

Five different phenolic compounds were selected as target compounds in the present study: gallic acid, caffeic acid, ferulic acid, catechin and epicatechin. These compounds have a wide range of polarity, as well as representing three different phenolic families, i.e. benzoic acids, cinnamic acids and flavan-3-ols. Their structures are shown in Fig. 1.

First of all, before extracting the polyphenols from the cream, it was necessary to determine how to de-emulsify the cosmetic cream so as to produce extracts without fat, to prevent any subsequent chromatographic problems.

Different solvents and mixtures were assayed on a cosmetic cream without phenolics. Using 100% methanol, water or methanol/water mixtures, sufficiently clear separation was not obtained and de-emulsification was not achieved. However, using methanol/water/hexane mixtures, a clear separation between the organic and aqueous phases was obtained.

Then, extractions from a cream containing phenolics were performed using three different methanol/water/hexane mixtures, with an ultrasonic horn at full cycle and 70% of amplitude. Temperature was set at 25 °C by a re-circulating water bath. These conditions were used as they had produced no degradation of phenolics in previous studies [7]. After the extraction, organic and aqueous phases were separated in a separation funnel and phenolics determined in the aqueous phase.

Resulting relative recoveries are shown in Table 1. Recoveries were calculated relative to the maximum recovery obtained for each phenolic compound. As can be seen the best mixture was methanol/water/hexane (40:40:20). Hence this solvent mixture was used in the method development.

3.1. Experimental design

No methods have previously been described for extracting phenolics from creams by ultrasound-assisted extraction. In a previous work an ultrasonic horn was used for enhancing the extraction of phenolics from plants. Obviously the matrix is rather different in this study, so a full evaluation of the influence of extraction variables on the recovery was needed,

Table 1
Relative recovery of phenolics using different extracting mixtures (hexane/methanol/water)

	60/20/20	40/40/20	30/50/20
Gallic acid	85.71	100.00	90.89
Catechin	85.63	100.00	90.13
Caffeic acid	85.35	100.00	97.58
Epicatechin	87.43	100.00	93.94
Ferulic acid	88.26	100.00	98.98

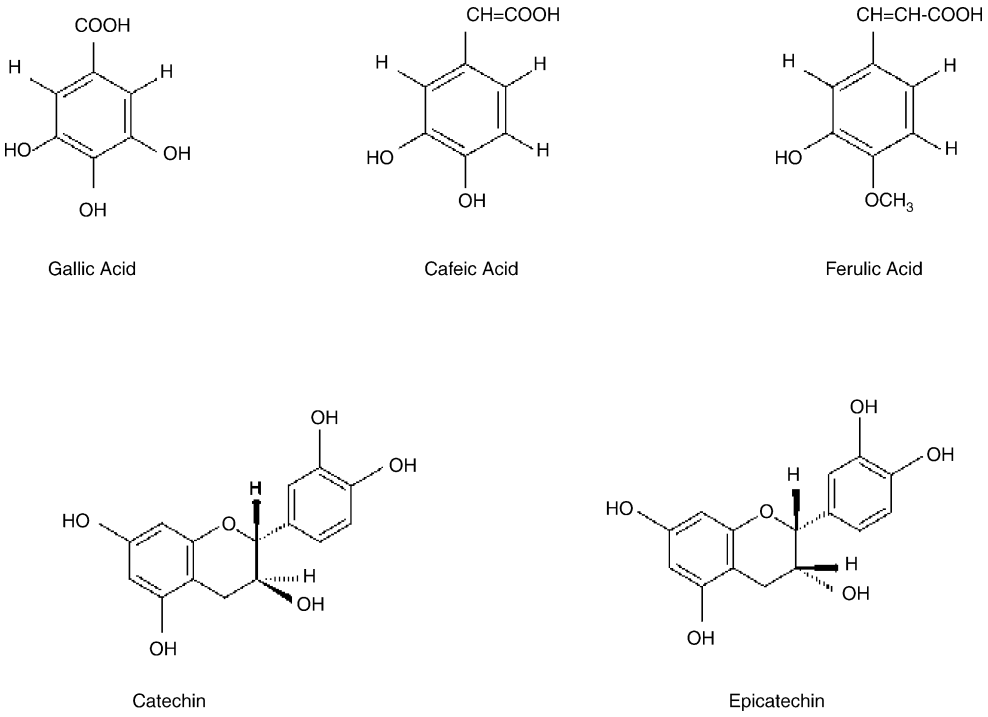

Fig. 1. Chemical structures of phenolics in the cream.

as well as the determination of their interactions. Because there are several variables which could affect the extraction of phenolics in an ultrasound-assisted extraction, an experimental protocol was designed to evaluate their influences. The variables in the experimental design were: (1) type of ultrasonic horn, (2) volume of extracting solvent, (3) temperature, (4) ultrasonic cycle and (5) amplitude. Other very important extraction variables like the extraction time and the sample quantity would be evaluated separately after the experimental design.

A factorial design was used since this enables both the influence and the interactions of variables to be determined. The experimental design was fractional in order to reduce the number of experiments needed to evaluate the influence of the variables. Thus, only 16 experiments were performed instead of the 32 (2^5) that would otherwise be needed for the full evaluation of five variables.

Graphical analysis of the results by comparing the main effects of each variable and the graphs of the interactions of each pair of variables allowed the influence of each variable on the recovery of the phenolics to be determined. This type of analysis has been applied previously, giving good results for developing extraction methods [8].

The experiments were performed over several days (almost a full month was needed to complete all the extractions). Since no information was available on the stability of phenolics in this type of cream, a reference extraction was performed. This meant that every day that extraction in the experimental design was done; another three extractions under fixed conditions were done on the same day. Afterwards the recoveries obtained for phenolics in the extractions in the experimental design were calculated relative to the average recovery in extractions under the fixed conditions. By this means corrections are made of errors due to degradation of the phenolics, should such degradation be occurring.

Table 2 shows the recoveries of phenolics found in the extracts obtained. All the concentrations are shown relative to the amount found using the most effective conditions (100%). This means that recovery was not calculated relative to the total amount of phenolics present in the samples, but relative to the highest concentration found in the extracts.

One of the most time-consuming steps in the extraction process was the separation of the two phases in the separation funnel. Therefore, in order to avoid this step, 2,5-dihydroxybenxaldehyde was used as internal standard in the extraction. It was thus necessary to determine the distribution of this compound in both phases before running the extractions in the experimental design. Three extractions were performed using around 1 g of cream, and 1 mL of a solution 2,5-dihydroxybenxaldehyde (245 mg/L) was added before starting the ultrasound-assisted extraction. The aqueous phase was obtained using a separation funnel. Pure water was used to reach 25 mL and the 2,5-dihydroxybenxaldehyde was determined chromatographically. It was found that 97.6 % (±1.5) of the 2,5-dihydroxybenxaldehyde was found in the aqueous phase. Therefore, this compound can reliably be used as internal standard for the extraction process.

The average relative recovery for all phenolic compounds was the target value in this experimental design study. Due

Table 2
Fractional factorial experimental design for the determination of significant variables and relative recovery for each phenolic ($n = 2$)[a]

	Probe (mm)	Volume (mL)	Temperature (°C)	Amplitude (%)	Cycle (s)	Gallic acid (%)	Catechin (%)	Caffeic (%)	Epicatechin (%)	Ferulic (%)
1	0.2	25	10	20	0.7	76.33	62.84	82.69	70.09	92.21
2	0.7	25	10	20	0.3	81.77	56.75	85.05	71.23	90.36
3	0.2	50	10	20	0.3	80.56	62.59	80.67	78.78	78.35
4	0.7	50	10	20	0.7	83.41	68.80	85.19	82.6	78.86
5	0.2	25	50	20	0.3	87.09	74.71	93.57	84.46	97.23
6	0.7	25	50	20	0.7	91.15	85.38	89.13	88.09	87.59
7	0.2	50	50	20	0.7	91.89	86.28	94.93	95.48	89.16
8	0.7	50	50	20	0.3	81.84	66.85	80.76	84.48	78.22
9	0.2	25	10	80	0.3	90.69	76.28	95.16	86.07	100.00
10	0.7	25	10	80	0.7	90.46	66.78	88.15	81.37	87.16
11	0.2	50	10	80	0.7	84.75	81.18	90.45	98.20	87.45
12	0.7	50	10	80	0.3	79.11	65.80	79.78	79.95	75.64
13	0.2	25	50	80	0.7	85.61	79.40	87.58	80.47	86.72
14	0.7	25	50	80	0.3	100.00	100.00	100.00	100.00	96.95
15	0.2	50	50	80	0.3	91.73	92.80	95.34	95.14	87.07
16	0.7	50	50	80	0.7	90.37	81.62	92.51	94.20	84.93

[a] Probe: diameter of probe used; volume: volume of extracting liquid; temperature: extraction temperature; amplitude: amplitude of ultrasounds (percentage of maximum ultrasonic power); cycle: pulse of ultrasound in fractions of second.

to the large volume of data collected here, it was decided to use graphical analysis. First the main effects were plotted, in the graph presented as Fig. 2.

The variables with most influence on average relative recovery were extraction temperature, ultrasonic amplitude and the type of ultrasonic horn. The other two variables had less influence on recovery. It can be seen that the higher the extraction temperature and the higher the ultrasonic amplitude, the higher the relative recovery obtained. The 2 mm ultrasonic horn tip produced a higher relative recovery than the 7 mm one.

In order to determine possible interactions, their effects on relative recoveries and the best values for extraction volume and ultrasonic cycle, graphs of the following data were constructed: average relative recovery of experiments with (1) the highest value for two variables, (2) the lowest value for two variables and (3) the highest and the lowest values for each pair of variables.

In this way, interaction graphs for all pairs of variables were produced. The most important interactions for extraction volume and ultrasonic cycle are described in the following paragraphs.

Interaction graph (Fig. 3) obtained for the ultrasonic horn dimension and the extraction volume showed that, when the narrow ultrasonic horn tip was used, 50 mL of extraction volume would produce a higher relative recovery than 25 mL.

Regarding the ultrasonic cycle, the most important interaction was found with the extraction volume (Fig. 4). When 50 mL of extraction volume is used, a cycle of 0.7 will produce higher relative recoveries than one of 0.3.

Therefore, the main plots and the interaction plots for combinations of the main variables indicate out that the optimum extraction conditions are: high values for temperature, ultrasonic amplitude, extraction volume, ultrasonic cycle and a narrow ultrasonic horn. Among these variables we were able to check some at higher values, i.e. temperature, amplitude and cycle. However, the extraction volume should not be increased as this would produce smaller chromatographic peaks, and there are no narrower ultrasonic horn tips available.

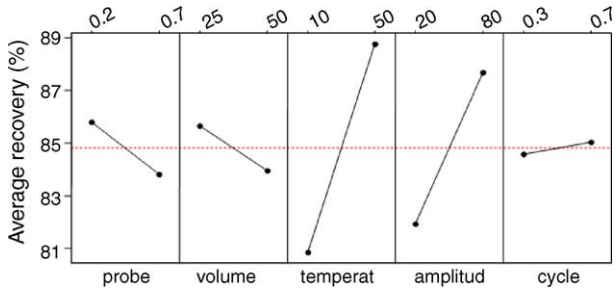

Fig. 2. Main effects plot of variables on the average relative recovery of phenolics. Probe: diameter of probe used; volume: volume of extracting liquid; temperature: extraction temperature; amplitude: amplitude of ultrasounds (percentage of maximum ultrasonic power); cycle: pulse of ultrasound in fractions of second.

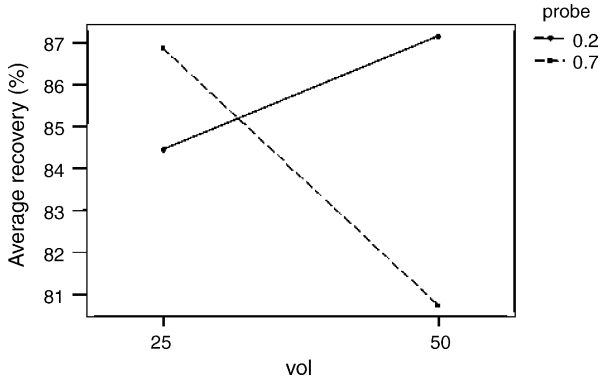

Fig. 3. Effects of interactions between extracting volume and ultrasonic probe over the average recovery phenolics.

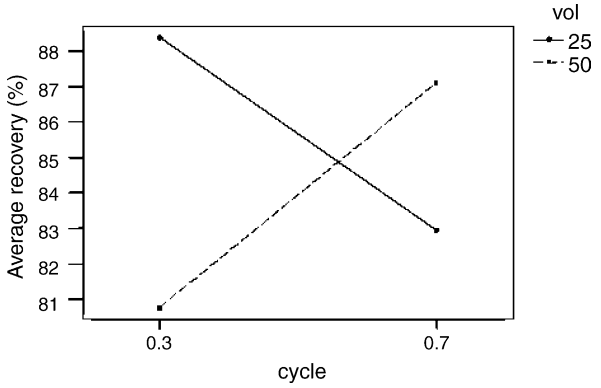

Fig. 4. Effects of interactions between extracting volume and ultrasonic cycle over the average recovery phenolics.

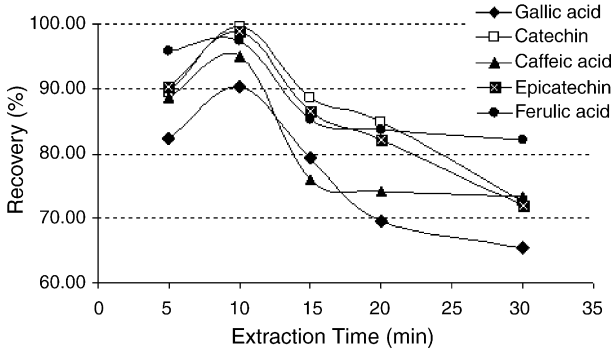

Fig. 5. Kinetics of extraction obtained for phenolics from a cosmetic cream.

Table 3
Optimization of main variables in the extraction process

	Temperature	Amplitude	Cycle	Average recovery (%)
Extraction 1	50	80	0.7	95.4
Extraction 2	60	80	0.7	88.6
Extraction 3	50	100	0.7	104.2
Extraction 4	50	80	1	97.6

3.2. Fine tuning for temperature and solvent

Studying the graphical analysis, it was found that high values for temperature, amplitude and cycle produced the highest recoveries, so these variables were studied separately from the other variables, since higher values for these could be assayed. Additional extractions and their results are shown in Table 3.

Extraction 1 was carried out under the optimum conditions resulting from the experimental design. As can be seen in extraction 2, when temperature was increased further, the recovery of phenolics decreased, so temperatures higher than 50 °C should not be used. On the other hand, if amplitude is increased from 80% up to 100% (extraction 3), the resulting recovery increases. Finally, few differences were obtained applying a ultrasonic cycle of either 0.7 (extraction 1) or 1.0 (extraction 4).

3.3. Optimization of extraction time

The extraction time must be adjusted to obtain quantitative recoveries of phenolics. To determine the time needed, different extractions were performed using increasing extraction times to establish the kinetics of the extraction. All the extractions were done in duplicate. The resulting graphs for each individual phenolic compound are shown in Fig. 5. As can be seen, 10 min is the optimum extraction time for all phenolics. A dramatic decrease was found by increasing time from 10 to 15 min. Using 5 min for the extraction, recoveries of more than 90% were obtained for most phenolics. Lower recoveries found using longer extraction time are most likely due to oxidation of phenolics.

3.4. Determination of sample quantity

All the extractions described above were performed using 0.1 g of cream. It is interesting to knowing if larger quantities

Table 4
Optimization of sample quantity in the extraction process

Sample quantity (g)	Gallic acid (%)	Catechin (%)	Caffeic acid (%)	Epicatechin (%)	Ferulic acid (%)
0.1	89.63	95.73	101.30	99.58	97.17
0.2	77.14	78.10	99.89	82.13	88.51
0.3	64.35	67.52	84.61	71.15	75.85
0.5	55.47	57.87	74.10	61.18	68.20

Table 5
Repeatability obtained for phenolics using the final conditions

	Recovery (%)						
	Extraction 1	Extraction 2	Extraction 3	Extraction 4	Extraction 5	Mean	SD
Gallic acid	96.46	85.13	86.27	87.31	89.12	88.86	4.5
Cathechin	103.66	92.54	89.46	91.00	92.11	93.76	5.7
Caffeic acid	108.95	95.08	93.57	99.88	94.40	98.38	6.4
Epicathechin	109.32	95.32	94.07	94.10	97.22	98.01	6.5
Ferulic acid	96.99	95.43	95.61	99.10	96.31	96.69	1.5

of sample could be used, since if higher chromatographic peaks could be obtained, this would reduce quantification errors in the chromatographic analyses. Table 4 shows results obtained using larger quantities of sample.

As can be seen, recoveries of less than 90% were obtained for most phenolics when sample quantities above 0.1 g are used. It is thought that if larger sample quantities were used, longer extraction time would need to be applied. But, as explained before, degradation of phenolics starts rapidly after 10 min, so a longer extraction time should be avoided.

3.5. Repeatability

To evaluate the repeatability of the extraction procedure, a series of five replicated extractions of the same cream were performed on the same day. The results obtained for all phenolics revealed a RSD lower than 7%. Results are shown in Table 5.

4. Conclusions

Ultrasound-assisted extraction is an adequate method for de-emulsifying silicone-based creams, as well as for extracting their phenolic constituents to the aqueous phase.

A systematic evaluation of variables influencing the ultrasound-assisted extraction of phenolics from cosmetic cream demonstrated that the extracting temperature, ultrasound amplitude and extraction volume are the most influential variables.

The optimization of the extraction method, based on the graphical analyses of the experimental design, enables an extraction method with high repeatability (RSD < 7%) to be devised.

Therefore, a fast (10 min) and reliable analytical method has been developed, using fractional factorial experimental design as a way to minimize the number of experiments required to develop the method.

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