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Caffeine in Hair Care and Anticellulite Cosmetics: Sample Preparation, Solid-Phase Extraction and HPLC Determination

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Synopsis

Caffeine is extensively used in cellulite and hair growth cosmetic products. Regulations in the field of cosmetics require manufacturers to list caffeine in the ingredient list on product labels, but its exact content in these products is not declared. On the other hand, daily exposure to caffeine from all sources may approach health reference values. For that reason, it is important to know the exact caffeine content in products for skin and hair care. Cosmetics are often viscous or semisolid products of very complex chemical composition. To analyze caffeine in these complex sample matrices by liquid chromatographic methods, an extraction step is often necessary. This article presents the applicability of the solid-phase extraction (SPE) procedure for the caffeine extraction and high-performance liquid chromatography (HPLC) determination in anticellulite gels, shampoos, and hair balsam. The samples of gels were centrifuged after ammonia addition to precipitate carbomer. In cellulite reduction, gel caffeine content was found to be in the range of 0.7–1.7%, whereas in the hair-care products, it was about 1.0%.

INTRODUCTION

Caffeine, an organic nitrogenous base, is a naturally occurring purine alkaloid of the methylxanthine class (Figure 1). Caffeine is being increasingly used in cosmetics because of its high biological activity and ability to penetrate the skin barrier (1). Based on the research studies that have been conducted in the last three decades, caffeine is used as an ingredient in hair growth products and cellulite reduction products.

Cellulite deposition is one of the most common aesthetic problems associated with the female population throughout the world, especially at the age greater than 35 years. Caffeine is seen as a potential way to prevent excessive accumulation of fat and promotes the lipolysis process. According to Hexsel et al. (2), it inhibits phosphodiesterases (PDEs), which lead to lipogenesis reduction. Caffeine also stimulates the draining lymph systems

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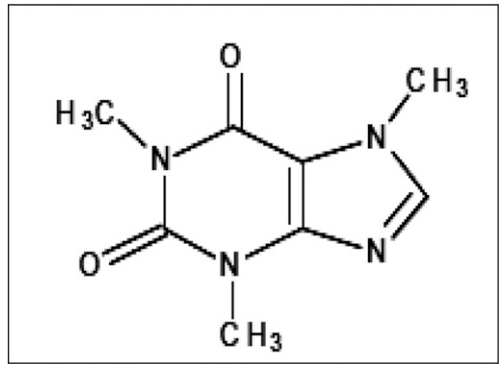

Figure 1. Chemical structure of caffeine (1,3,7-trimethylxanthine).

in fatty tissues by removing accumulated fat and toxins, which can improve the microcirculation of blood vessels (1). The commercially available topical anticellulite formulations usually contain 1–2% of caffeine, although some products may have up to 3% of this ingredient (3). In addition, hair products containing caffeine have recently been used to prevent and treat alopecia. 5-α-reductase is an enzyme that converts testosterone into the more active dihydrotestosterone, which is responsible for baldness (1). Earlier investigations have shown that caffeine in concentrations of 0.001% and 0.005% inhibits the activity of 5-α-reductase (4) and phosphodiesterase. This increases the intracellular concentration of cyclic adenosine monophosphate (5), stimulating microcirculation, cellular metabolism, and delivery of nutrients to the hair follicle, thereby contributing to strengthened and more rapid human hair growth (1,6).

It has been documented that the application of caffeine on the skin leads to its fast absorption and occurrence in the blood. Caffeine is detected in blood samples only 5 min after topical application of formulation containing 2.5% caffeine, reaching the highest value after 1 h (7,8). It is known that a high dose of caffeine can cause insomnia, anxiety, elevated blood pressure, and tachycardia (9). Because of this fact, information on the exact caffeine content in a cosmetic product may be important for some consumer groups. Caffeine is also frequently daily administered through beverages (coffee, soft and energy drinks, tea, and cocoa) or certain analgesic and nonsteroidal anti-inflammatory drug mixtures, which is another reason for increased attention when using cosmetics with caffeine. On the cosmetic product label, caffeine is listed according to the International Nomenclature of Cosmetic Ingredients. The precise content is not declared; therefore, the exact concentration of this substance is unknown to the consumers. According to the U.S. Food and Drug Administration, thigh creams may more appropriately be classified as drugs under the Food, Drug, and Cosmetic Act because removal or reduction of cellulite affects the "structure or function" of the body (10). All of these points increase the need to develop test methods to assess the presence and the content of caffeine in different products for human use.

So far, the interest in determining caffeine has been mainly focused on foods and beverages. Limited number of articles has described caffeine quantification in cosmetics (11–13). Because cosmetics are complex semisolid or high viscous emulsions, for their analysis, different and lengthy sample pretreatments may be required (14). Sample preparation in the cosmetic analysis is a crucial step as the complex matrices may seriously interfere with the determination of target analytes (15). Sample preparation and clean-

up are important for the high-performance liquid chromatography (HPLC) analysis, where the sample solutions are directly injected into an HPLC column. The complexity of some cosmetics may obstruct their direct analysis. Thus, in many cases, cosmetic samples cannot be analyzed only by "dilute and shoot" approach because direct injection of untreated samples may cause a large increase in column pressure. Also, injecting excipient molecules of higher molecular weights can lead to a decrease in column quality, shortening its lifetime. In addition, retention of excipients may require a long column rinse after measurements. Therefore, a sample pretreatment step may be required to eliminate proteins, emulsifying agents, and many other viscous constituents. Also, anticellulite gels and creams contain a wide variety of synthetic and natural (plant-derived) substances which may interfere in the caffeine determination. Solid-phase extraction (SPE) is useful for sample preparation and clean-up in the analysis of pharmaceutical creams. It can be more rapid and efficient than liquid–liquid extraction and yields quantitative extractions without using large volumes of harmful organic solvents. In this work, a solid-phase extraction method followed by HPLC with ultraviolet detection was developed and applied to caffeine determination in anticellulite gels and hair-care products.

EXPERIMENTAL

CHEMICALS AND REAGENTS

Caffeine (1,3,7-trimethylxanthine) standard solution of LC-MS grade was obtained from Thermo Scientific (Waltham, MA), (Lot ER01081601). Caffeine powder of analytical grade (serial number 0030810) was supplied from NRK Inženjering (Beograd, Serbia). Methanol was of HPLC grade (Sigma-Aldrich, Eschenstrasse, Germany, Lot STBG4373V). For the sample preparation, 96% ethanol Ph. Eur. grade (Zorka-Pharma, Šabac, Serbia, serial number 57/226) was used.

STOCK SOLUTIONS AND CALIBRATION CURVE

The stock solution of caffeine with a concentration of 1.0 mg/mL was prepared in deionized water. This solution was stored for up to 1 week at 4°C. Working standard solutions were prepared by diluting the stock with deionized water.

COSMETIC PRODUCTS

Two caffeine-enriched shampoos, one caffeine-enriched hair balsam, one caffeine-free shampoo, and two caffeine-enriched cellulite reduction products (in the form of gels) were obtained from the local cosmetic shops. According to the manufacturers' declaration, both cellulite reduction products and hair-care products contained caffeine, plant extracts, and a large number of other substances. In all the examined cosmetics, caffeine was one of the major components as it was one of the first seven ingredients in the ingredient list. The exact concentration of caffeine in any of the examined products was not declared.

SAMPLE PREPARATION

After optimization of the sample preparation procedure (see discussion), the following protocol was adopted: 0.5 g of the anticellulite gel was accurately weighed and dissolved in 10 mL of 96% ethanol. After that, NH_4OH was added to obtain pH 7–8. Insoluble excipients were separated by centrifugation for 10 min at 4,000 rpm at 20°C. Then, 1 mL of the supernatant was loaded on the cartridge, and the SPE procedure was performed. 0.5 g of the hair-care product was dissolved in 10 mL of distilled water and heated at 50°C in a water bath for 45 min. The obtained pH of the samples was 5–6. After cooling and centrifugation for 10 min at 4,000 rpm at 20°C, 1 mL of the supernatant was loaded on the SPE cartridge.

SOLID-PHASE EXTRACTION

Visiprep™ SPE vacuum manifold Supelco 57030-U (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) was used for the SPE. Five different SPE sorbents (C_{18}, C_{18}ec, C_{18} Hydra, C_8, and HR-X) of the same volume and capacity (1 mL/100 mg) from the same producer (Macherey-Nagel, GmbH, Düren, Germany, REF 730 207, LOT 60.007) were tested. The cartridge C_{18}ec was octadecyl modified endcapped silica, C_{18} Hydra was a special octadecyl phase for polar analytes, and HR-X was a hydrophobic polystyrene-divinylbenzene copolymer.

SPE cartridges were conditioned with 3 mL of methanol and 3 mL of deionized water. One milliliter of the prepared sample was loaded on the cartridge at a flow rate of 1 mL/min. The elution of caffeine was achieved with 4×1 mL of methanol.

HPLC CONDITIONS

HPLC analyses were performed on Agilent Technologies 1200 Series apparatus (Santa Clara, CA) with an diode array detector (DAD) and a fluorescence detector. The separation was carried out on the Restek Ultra IBD C_{18} column (150 × 3 mm, 3 µm, Lot 13122OP, Ser 14020153J) (Bellefonte, PA) at 30°C. The mobile phase consisting of a methanol–water mixture (40:60, v/v) was pumped in an isocratic mode at a flow rate of 0.4 mL/min. Two microlitre of the final eluate was injected into the HPLC column. UV detection set at 274 nm was used as the optimal wavelength for caffeine determination.

RESULTS AND DISCUSSION

OPTIMIZATION OF THE CHROMATOGRAPHIC CONDITIONS

Chromatographic conditions for the determination of caffeine in food matrices are well established and have been published in many articles (16–20). Because the C_{18} analytical column has been used for caffeine separation in most of the articles, this column was also selected in our work. To optimize HPLC conditions for the caffeine analysis of cosmetic products, the methanol-to-water volume ratio was varied from 20:80 (v/v) to 50:50 (v/v). The most suitable mobile phase composed of 40% methanol and 60% water, giving well-

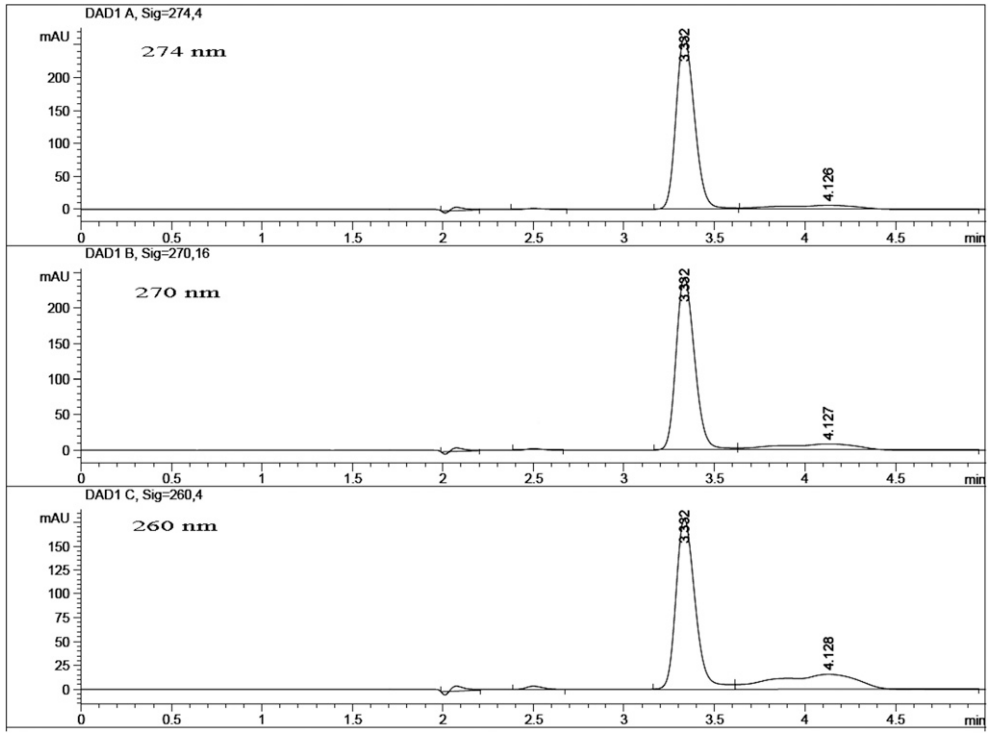

Figure 2. Chromatogram of shampoo 1 extract on the HR-X cartridge at 260, 270, and 274 nm; caffeine retention time 3.332 min.

separated and good shaped peaks, with a run time less than 5 min. Thus, the retention time of caffeine was about 3.3 min. The low retention time enabled a fast chromatographic analysis but also very good selectivity of the method. As can be seen in Figure 2, caffeine-enriched shampoos had a compound that appeared at 4.1 min with a low resolved peak, which gave an absorbance in a similar UV region as caffeine (260–280 nm) (Figure 3). The resolution between peaks of caffeine and this ingredient was 0.98 recorded at 260 nm, whereas at 274 nm, this potentially interfering peak gave a very low signal. Adequate selection of working detector wavelength eliminated the signal of an unknown substance. Thus, the unknown substance at 4.1 min does not interfere in the caffeine analysis when a detector was set at 274 nm because at this wavelength, this substance has very low absorption. On the other hand, the chromatograms of gel after SPE (Figure 4) were very clean when the detector was set at 260 nm, 270 nm and 274 nm. For these reasons, isocratic elution was adopted, and by selecting the detection at 274 nm, good peak area, peak width, and selectivity for caffeine were achieved (Table I).

The examination of the system suitability was conducted in terms of retention time repeatability, peak symmetry, peak width, number of theoretical plates, capacity factor (k'), resolution, and selectivity (Table I). The capacity factor (k') is optimal as reference values are between 1 and 5. The values of k' larger than 5 lead to longer retention and analysis time, whereas values less than 1 are unreliable and cause low resolution. The number of theoretical plates higher than 5,000 indicates a good separation of caffeine. Also, the se-

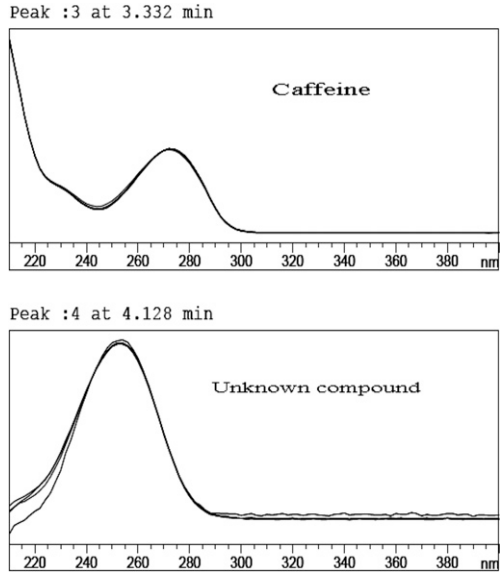

Figure 3. UV spectrum of the caffeine peak and peak of unknown compound from shampoo 1 extract on the HR-X cartridge.

lectivity factor and resolution had excellent values demonstrating satisfactory overall column efficiency in the caffeine analysis.

OPTIMIZATION OF SAMPLE PREPARATION PROCEDURE

In cosmetic products, caffeine is dispersed in complex emulsion matrices. Before the analysis, cosmetic emulsions are usually dissolved in water, methanol, or buffers by heating in a water bath. As the hair-care products and anticellulite gels are highly viscous, the first step in the optimization of the preparation procedure was the selection of the dissolution solvent. The optimal solvent should fulfill two requirements: to provide a solution that is not viscous and that can easily flow through SPE cartridges, and to ensure good solubility and release of the caffeine from the sample matrix. The latter is actually a classic single-stage solid–liquid extraction that is desirable to perform before the application of SPE purification. Considering that caffeine is sparingly soluble in water at room temperature, but freely soluble in boiling water (21), we selected the sample preparation which included dissolution in deionized water and heating at 40°–50°C in a water bath. This avoids the use of solvents such as methanol or acetonitrile which are harmful to health and the environment. In addition, phosphate buffer (pH 7) was also tried, but the samples dissolved in phosphate buffer clogged the SPE cartridges as some insoluble phosphate compounds were precipitated.

The additional procedure was applied to anticellulite gels. They were first dissolved in warm deionized water, but viscous samples that could not pass through the cartridge were obtained. One of the main problems in these samples was the presence of carbomer. Carbomer is a synthetic high–molecular weight polymer of acrylic acid which is soluble in water. It is widely used in anticellulite gels as a vehicle for incorporation of liposomes

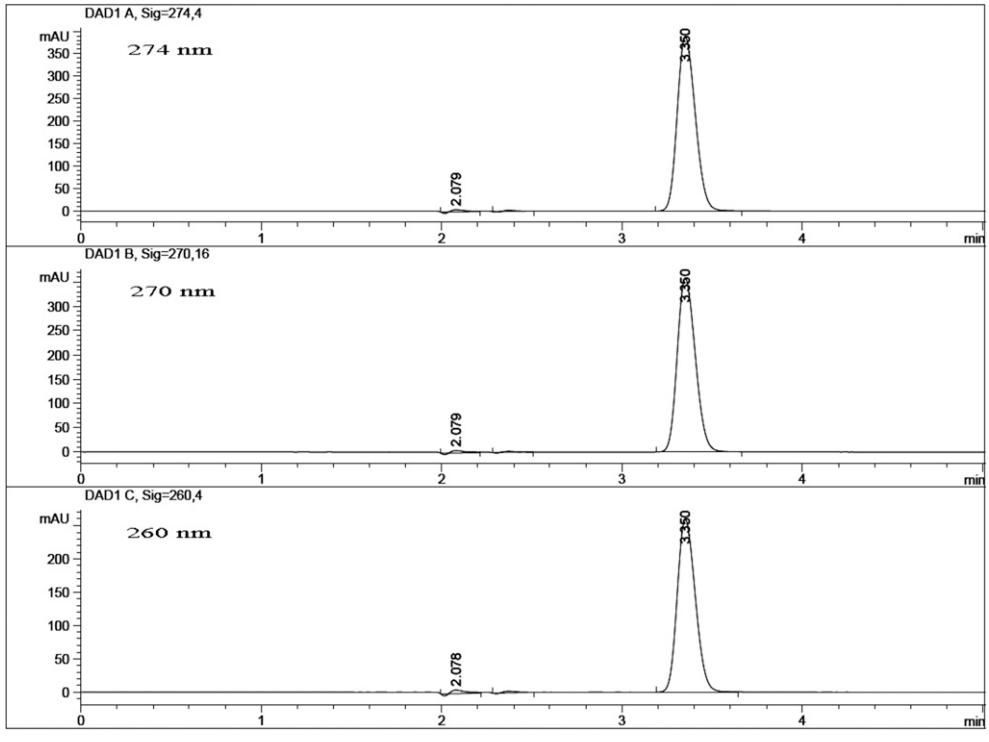

Figure 4. Chromatogram of anticellulite gel 1 extract on the HR-X cartridge at 260, 270, and 274 nm; caffeine retention time 3.35 min.

for transdermal delivery. By heating, it gives a viscous solution which is unsuitable for application to the SPE cartridge. Therefore, to remove carbomer, an additional procedure must be applied to the preparation of anticellulite gels. Thus, the samples were dissolved in 96% ethanol, and after vortex-mixing, 0.5 mL of 0.1M NH_4OH was added to obtain

Table I
Chromatographic and Analytical parameters of the Method

Chromatographic parameters						
Retention time (min)	Capacity factor (k')	Peak symmetry	Peak width	Theoretical plates (N)	Resolution	Selectivity
3.34 ± 0.007	1.11	0.81	0.108	5,781	5.62	2.25
Analytical parameters						
Range (mg/mL)	Slope	Intercept	R^2	LOD S/N (1:3)[c]	LOQ S/N (1:10)[c]	
0.01–0.2[a]	13,971.6	20.04	0.9997	0.007%	0.02%	
0.08–1.6%[b]	–	–	–	–	–	

[a]Concentration in final extracted solutions.
[b]Assumed content in cosmetic products.
[c]S/N ratio for LOD and LOQ expressed as spiked caffeine content in shampoo sample.

pH 7–8. In this weakly alkaline solution, carbomer was precipitated as a white solid clod which was further separated by centrifugation (22).

OPTIMIZATION OF SPE PROCEDURE

Optimization of the SPE procedure included cartridge and solvent selection, as well as extraction recovery evaluation. Caffeine is soluble in methanol; therefore, this solvent was selected for elution. The cartridge was selected based on the extraction yields obtained for standard solutions and real samples on each type of the tested sorbents. A known amount of caffeine standard solution, at three different concentration levels (0.01 mg/mL, 0.02 mg/mL, and 0.1 mg/mL) which corresponded to caffeine content of 0.02%, 0.04%, and 0.2% in cosmetic product was extracted on different cartridges, and the extraction yield was calculated as the ratio of the loaded and eluted caffeine. The percentage extraction yield was found to be 84–90% for HR-X cartridges, whereas for the other types of cartridges, these values were lower. Thus, for examined cartridges, recoveries were 70–77% on C_{18}, 68–75% on C_{18}ec, 75–86% on C_{18} Hydra, and 80–90% on C_8. However, the final cartridge selection also depended on the sample matrix; therefore, the extraction yield test was made for all real samples on five cartridges. Also, the chromatographic parameters obtained for these extracts were analyzed. The results showed that the highest chromatographic peak purity, peak area, and concentrations of caffeine from all real samples except hair balsam were obtained on HR-X cartridges. The octadecyl modified silica endcapped cartridge (C_{18}ec) was eliminated because an opalescent solution was obtained after eluting with methanol. Also, some ingredients of the viscous shampoos clogged C_{18}ec and C_{18} cartridges, and the flow of the sample was difficult. A similar situation was found for the hair balsam extraction on C_{18}ec, $C_{18,}$ and C_{18} Hydra cartridges. In Table II, chromatographic peak areas obtained for the examined cosmetics on different cartridges were presented. An HR-X cartridge was selected for the extraction and clean-up because it allows fast sample flow and high extraction yields. Only for hair balsam, the C_8 cartridge was used because of a significantly higher peak area for caffeine.

ANALYTICAL PARAMETERS

The calibration line for caffeine determination was constructed by plotting the peak areas versus the concentration of the standard solution. A least-squares linear regression analy-

Table II
Average Caffeine Peak Area Obtained on Different Cartridges

	Peak areas ± SD[a]		
Product	HR-X	C_{18} Hydra	C_8
Anticellulite gel 1	2,702.7 ± 75.4	715.6 ± 46.1	594.9 ± 42.8
Anticellulite gel 2	1,195.2 ± 80.3	442.1 ± 52.7	180.6 ± 20.6
Shampoo 1	1858.5 ± 182.6	911.4 ± 38.4	450.3 ± 40.9
Shampoo 2	1772.0 ± 130.4	1730.8 ± 105.9	604.9 ± 51.4
Hair balsam	1,375.1 ± 95.2	–	1748.2 ± 78.5

[a]SD of three measurements.

sis of a seven-point calibration curve was used to calculate analytical parameters such as slope, intercept, linearity, limit of detection (LOD), and limit of quantification (LOQ). A regression curve was obtained for the caffeine concentration of 0.01–0.2 mg/mL in final extracted solutions, which corresponded to assumed caffeine amounts of 0.08–1.6% in cosmetic products (Table I). High sensitivity of the method was enabled by a high value of the slope. A good linear relationship with a regression coefficient $R^2 = 0.9997$ was found. The real LOD and LOQ were estimated by injecting different volumes of caffeine-free shampoo spiked to 0.08% caffeine until the signal-to-noise (S/N) ratio reached three for LOD and 10 for LOQ (Table I).

ACCURACY AND PRECISION OF THE METHOD

The overall accuracy and precision of the developed HPLC-SPE procedure were estimated by using the standard addition method. Caffeine-free shampoo (from the same producer as one of the shampoos with caffeine) was used as a blank. Ten millilitre of the dissolved blank shampoo was spiked at four concentration levels (0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.6 mg/mL) which corresponded to caffeine content of 0.1%, 0.2%, 0.4%, and 1.2% in the product, and three replicate measurements were conducted. The mean recoveries of the extraction, calculated as the ratio of found and added caffeine, were 78%, 85%, 90%, and 97% respectively, and relative SDs were from 3.5% to 10.7%. These results indicate satisfactory accuracy and precision of the method, having in mind the sample complexity and the necessity of performing the extraction step.

APPLICATION OF THE METHOD TO THE COSMETIC SAMPLES

After SPE treatment of the cosmetics, clear and nonviscous extracts were injected into the HPLC column. The chromatograms of shampoo 1 and anticellulite gel 1 are shown in Figures 2 and 4. Very clean chromatograms with no interfering peaks were obtained, indicating a good selectivity of the method. Identification of caffeine was achieved based on retention times and by UV spectrum of the chromatographic peaks obtained by using the DAD detector (Figures 3 and 5). The caffeine peaks exhibit a purity factor of 989.6 for shampoos and 999.2 for gels. The average retention time was 3.34 ± 0.007 min, with a relative SD of 0.21%. The caffeine content in the examined samples (Table III) was determined by using the calibration curve.

Table III
Content of Caffeine in Cosmetic Products

Cosmetic product	Caffeine (g/100 g ± SD)[a]	RSD[b] (%)	Caffeine percent in products (%)
Anticellulite gel 1	1.7 ± 0.05	2.9	1.7
Anticellulite gel 2	0.73 ± 0.05	6.9	0.73
Shampoo 1	1.08 ± 0.1	9.3	1.08
Shampoo 2	1.04 ± 0.08	7.7	1.04
Hair balsam	1.0 ± 0.07	7.0	1.0

[a]The average concentration of caffeine from three measurements expressed per 100 g of the products.
[b]The relative SD of three measurements.

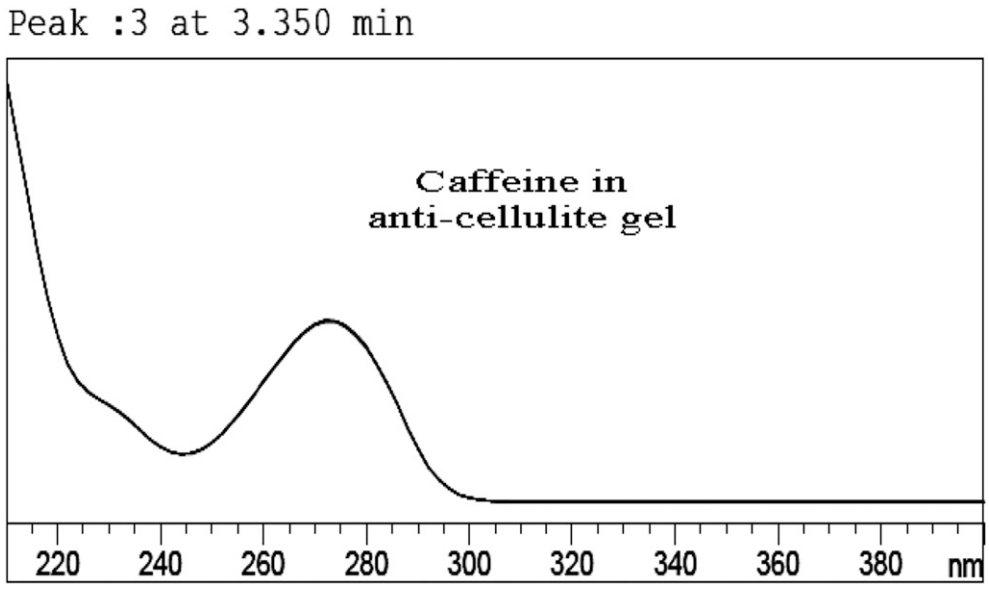

Figure 5. UV spectrum of the caffeine peak from anticellulite gel 1 extract on the HR-X cartridge.

Because of the lack of reference methods for caffeine determination in cosmetics, we could only compare our results with the expected recommended maximum values and with the results reported in other studies. Thus, the found contents in cellulite reduction cosmetics were in agreement within the recommended values of 1–3% of caffeine (3). In addition, the obtained results are comparable with those given by Injac et al. (1.2–1.6% of caffeine) (11) and Marchei et al. (0.03–5.6% of caffeine) (12). Also, the examined hair-care products contained caffeine in accordance with the usual amounts of 1.0–2.5% (7,8).

Because caffeine is considered as a stimulating bioactive compound that can be readily absorbed through the skin (7,8), its exact content in cosmetics is important, especially for caffeine-sensitive consumers. According to the European Food Safety Authority, daily intake of up to 400 mg of caffeine from all sources does not raise safety concerns for adults. On the other hand, the estimated proportion of the adult population exceeding daily intakes of 400 mg ranges from about 6% to almost 33% (23). All the examined cosmetics contained a significant amount of caffeine; however, none of them listed its precise content. Moreover, the anticellulite gel packaging does not indicate the presence of caffeine on the product`s principal display panel, whereas hair-care products have this information on the front panel.

CONCLUSIONS

Methods to monitor and control the caffeine content in cosmetics are of great importance to the cosmetic industry. Caffeine is dispersed in the complex cosmetic matrices; therefore, its extraction is difficult and depends on physicochemical properties of the sample and other excipients. Solid-phase extraction was an effective and useful method for pretreatment and clean-up of cosmetic formulations before HPLC analysis. By appropriate selection of cartridge sorbent, very good extraction of caffeine can be achieved. After SPE

preparation of anticellulite gels, shampoos, and hair balsam, clear and nonviscous solutions were obtained. Potentially interfering compounds were removed, and the caffeine content was determined with satisfactory accuracy and precision.

The selection of the cartridge sorbent is of great importance to obtain high extraction yields. Surprisingly, more polar octyl modified silica phase gave higher recoveries only for hair balsam, whereas for gels and shampoos, the most suitable sorbent was a polystyrene–divinylbenzene copolymer. These indicate that the choice of the cartridge sorbent depends not only on physical and chemical characteristics of the analyte but also depends on the sample matrix. Thus, different cartridges must be used for caffeine extraction from different cosmetic products.

This article contributes to the development and application of SPE in the analysis of cosmetic products. The SPE procedure optimization showed the importance of screening the performance of a range of cartridge types when applying SPE techniques to samples of the complexity often encountered in personal care products.

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