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Short communication

Microdialysis sampling coupled to on-line high-performance liquid chromatography for determination of arbutin in whitening cosmetics

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Abstract

We have developed a method that uses on-line microdialysis sampling coupled with high-performance liquid chromatography (HPLC) to determine arbutin in whitening cosmetics. The optimum analytical conditions for microdialysis sampling were a probe length of 10 mm and a dialysis flow-rate of 5 μl min^{-1}. The accuracy (% bias) for intra-day ($n = 6$) and inter-day ($n = 30$, five consecutive days) analyses ranged from −8.9 to 11.5%, with a precision below 7.64% R.S.D. The calibration curve was linear within the range from 0.1 to 20 mM ($R^2 = 0.9989$). The detection limit was 15 μM. By comparing the arbutin levels determined this way in the whitening cosmetics with the results obtained from the no-net-flux method, we conclude that our proposed on-line microdialysis–HPLC system displays good accuracy. We evaluated the robustness of our optimum conditions by means of a Plackett–Burman design. Apart from the effect of a low flow-rate of perfusate – an increase of 12.52 ± 2.31% – we observed no significant changes in the analyses upon changing the levels of any other parameter. Because this on-line method offers the advantages of simplicity, reliability, the lack of any tedious sample pretreatment process, and a reduced use of organic solvents, we believe that it is suitable for the routine analysis of commercial cosmetics.
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Keywords: Microdialysis sampling; High-performance liquid chromatography; Arbutin; Whitening cosmetics; Validation

1. Introduction

Arbutin can prevent serious sunburn caused by an accumulation of melanin in subcutaneous tissue produced through a tyrosinase-catalyzed metabolic pathway. Tyrosinase is the essential enzyme for melanin formation. The whitening effect of arbutin has been reported to reduce cellular tyrosinase activity without changing the cell's viability [1]. Because arbutin is a highly photosensitive compound [2], high concentrations of sunscreen agents are added in whitening cosmetics to protect the activity of arbutin against degradation. Because these sunscreen agents have an insidious effect on human skin, the regulatory authority in Taiwan has recommended a suitable level of arbutin for use in formulations. For whitening cosmetics, the maximum authorized concentration of arbutin is 7% [3]. To modify the dosing and to verify manufacturers' compliance with the regulation, it is important to discover an official assay for the analysis of arbutin in commercial whitening cosmetics.

Several methods exist for the quantitative analysis of arbutin in plant leaves, including spectrophotometry [4], capillary zone electrophoresis [5], and Nguyen–Hiep's chromatospectrophotometry [6] approaches. Furthermore, a high-performance liquid chromatography (HPLC) method has been developed to quantify arbutin in urine samples [7]. A square-wave voltammetry method using a clay-coated screen-printed electrode has been applied for the determination of arbutin in whitening cosmetics [8]. In each of these techniques, an appropriate pretreatment of the sample is required prior to analytical measurement. Isolation of arbutin from the matrices mentioned above, prior to quantitative analysis, requires several stages including sonication with acids, centrifugation, filtration, and solvent extraction. Because these processes are laborious, time-consuming, and involve large volumes of hazardous solvents, they are not suitable for routine analyses of commercial products.

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Microdialysis was developed initially for the release of neurotransmitters in the brain, but nowadays this technique is a standard one in neuroscience and is also applied in many other fields, including pharmacokinetics, toxicology, and bioprocess monitoring [9]. In recent years, microdialysis sample cleanup has become a powerful technique for studying biochemical events in the extracellular fluid of various tissues and in biological fluids [10]. Microdialysis has the additional advantage that the technique is readily automated and can be coupled on-line with many other analytical techniques, such as HPLC [11], capillary electrophoresis [12], and flow-injection analysis [13].

The aim of this study was to develop a simple, reliable and fast on-line microdialysis–HPLC method – one that uses a low amount of organic solvent – for quantitatively determining arbutin in whitening cosmetics. Our validation procedure followed the International Conference on Harmonization (ICH) guidelines [14] and we evaluated the validation parameters' selectivity towards the other components in the whitening cosmetics and the linearity, detection limit, precision, accuracy, and robustness of this approach [15].

2. Experimental

2.1. Equipment

The microdialysis sampling system was purchased from the Carnegic Medicine Associate (CMA, Stockholm, Sweden). The sampling system used consists of a microinjection pump (CMA/100), an on-line injector (CMA/160), a microdialysis probe (CMA/20 14/10), and a CMA 1.0 ml syringe. Microdialysis sampling was performed by perfusing the probe with either deionized water or different concentrations of standard arbutin solutions (0, 1, 5, 10, 15, and 20 mM) at a flow rate of 5 μl/min while stirring efficiently with a magnetic stirrer at room temperature.

The HPLC system used consisted of a Jasco 980-PU pump, a Jasco 2075 plus UV–vis detector (Tokyo, Japan), and a Rheodyne 7725i injector (Cotati, USA) equipped with a 20 μl sample loop. Separation was achieved on a 5 μm, 250 mm × 4.6 mm i.d. Hypersil Fluophase PFP column (Cheshire, England). The analyte was detected by UV absorption at 254 nm. Integration of peak areas and determination of retention times were performed using SISC Chromatography Data Station v.1.0 software (Taipei, Taiwan).

2.2. Chemicals and reagents

All chemicals and reagents were of analytical or HPLC grade. High-purity water was prepared using a Barnstead Nanopure water system (Iowa, USA). Methanol, potassium dihydrogen phosphate, phosphoric acid and potassium hydroxide (Merck, Darmstadt, Germany) were used to prepare the eluents and adjust their values of pH. A standard stock solution (20 mM) of arbutin (Sigma, Steinhein, Germany) was prepared by dissolving arbutin with deionized water. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The HPLC eluent was 40% (v/v) methanol in 0.020 M phosphate buffer.

2.3. Method validation

We validated the method of determining arbutin through monitoring the selectivity towards the other components in the whitening cosmetics, linearity, detection limit, precision, accuracy, and robustness of the analyses. To determine the intra- and inter-day precision and accuracy of our proposed on-line microdialysis–HPLC method, we analysed – over six runs performed on five consecutive days – four different arbutin standards (0.1, 1.0, 5.0, and 10.0 mM) that we added into a cosmetic lotion that initially did not contain arbutin. Because no certified values for the arbutin contents in cosmetics were available, we examined the accuracy of our proposed on-line microdialysis–HPLC system by comparing arbutin in the cosmetics with the results obtained from the no-net-flux method [16]. The concentrations of the arbutin standards that we added to the perfusate were 0.1, 1.0, 5.0, 10.0, 15.0, and 20.0 mM. To evaluate the robustness of our optimal experimental conditions, we performed a Plackett–Burman design [17] by methodically varying the parameters of the two main processes: (i) the microdialysis sample cleanup and (ii) the HPLC–UV analysis. These variations, with respect to their nominal level, were ±5% [18].

2.4. Analysis of real samples

To examine the applicability of the proposed method, three kinds of real whitening cosmetic samples (including a whitening lotion, cream, and essence), which were purchased from local drugstores, were analyzed. Aliquots of the cosmetic products were weighed and then diluted with deionized water to 20 ml in a volumetric flask. After vortex mixing thoroughly, the sample solution was poured into a 4 ml dialysis vial. The determination of arbutin was performed under conditions of thorough stirring and constant temperature.

3. Results and discussion

3.1. Optimization of chromatographic conditions

For reverse-phase chromatography, the retention time decreases when the percentage of methanol increases. Considering the solubility of potassium dihydrogen phosphate in methanol, we found that 0.020 M phosphate-buffer solution in 40% (v/v) methanol was the optimal eluent. Because, on the one hand, arbutin is readily hydrolyzed to D-glucose and hydroquinone in the presence of dilute acid and, on the other, the tolerance of the Hypersil Fluophase PFP column to acid is lowest at pH 2.0, we studied the chromatographic separation over the pH range 4.0–7.0. We observed no differences in retention times and peak areas within this range. For convenience, we chose pH 5.5 for adjustment of the eluent in subsequent analyses.

3.2. Effect of perfusate flow rate

The perfusate flow rate is an important factor that defines the performance of a microdialysis probe and has a direct influence on the value of dialysis recovery (D_R) of the probe. To determine

an acceptable value of D_R within a reasonable operating time, we examined the influences that both the perfusate flow rate and various arbutin standards (0.1, 1.0, and 10 mM) have on D_R. Our results indicate that the value of D_R of the probe was independent of the concentration of the analyte. For an optimal analytical protocol including microdialysis, we chose a flow rate of 5 μl/min for this on-line microdialysis–HPLC system and used a no-net-flux method.

3.3. Analytical performance and applications

Fig. 1a–c display the chromatograms of arbutin in a whitening cosmetic lotion, cream, and essence, respectively. Under our proposed chromatographic conditions, the retention time of the 1 mM arbutin standard was 3.05 min and the reproducibility was high (R.S.D. below 0.38% from six runs over five consecutive days).

A correlation coefficient of 0.995 generally is considered as evidence of an acceptable fit of data to a regression line.

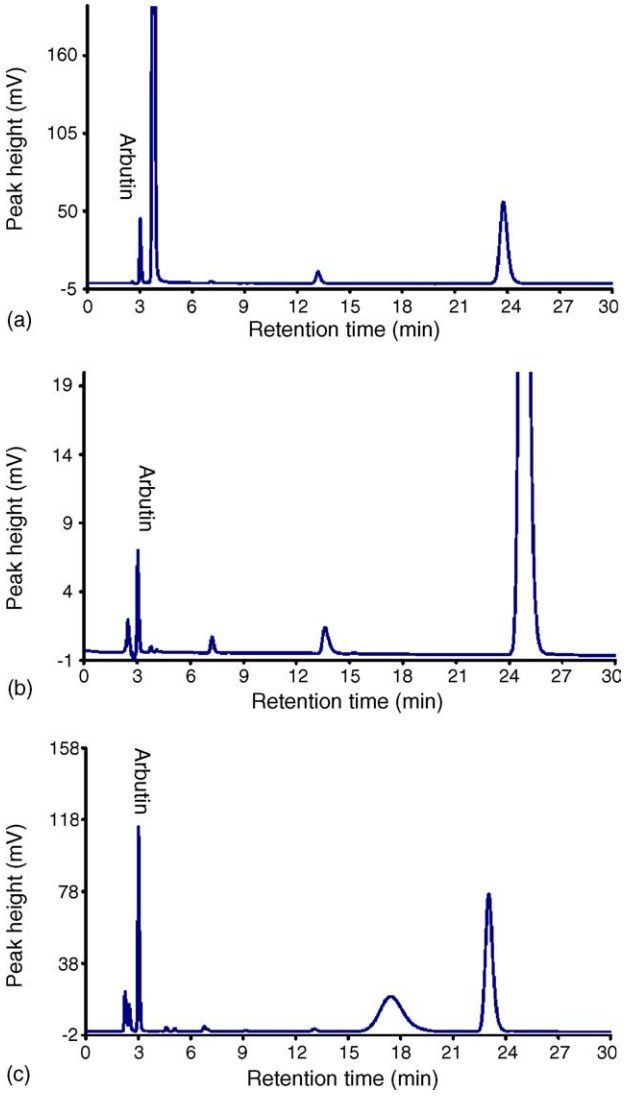

Fig. 1. Typical chromatograms obtained from samples of (a) a cosmetic lotion, (b) a cosmetic cream, and (c) a cosmetic essence.

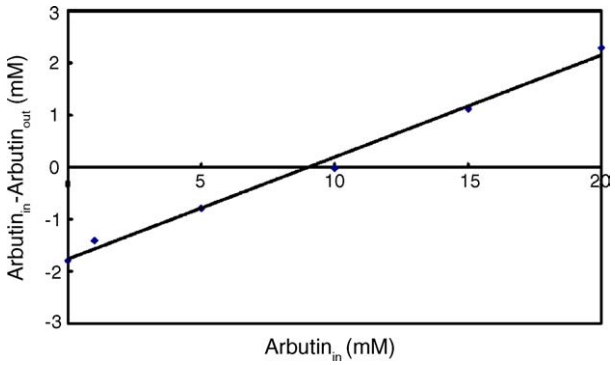

Fig. 2. Analysis of the arbutin microdialysis of a cosmetic lotion solution using the no-net-flux method. A positive number on the *y*-axis indicates dialysis to the test solution (gain to the test solution), whereas a negative number indicates dialysis from the test solution (loss from the test solution). The basal concentration of the test solution was 9.01 ± 0.18 mM, with a dialysis recovery of 16.0 ± 1.5%.

Our calibration curves were linear within the range from 0.1 to 20 mM; the correlation coefficient from linear regression analysis was 0.9989. The detection limit, based on three times the standard deviation of the baseline noise, was 15 μM. The precision was less than 7.64% R.S.D. and the accuracy (% bias) ranged from −8.9 to 11.5%; note that a value to within ±15%, when covering the range of actual experimental concentrations, is considered acceptable [19]. The time required for the proposed on-line microdialysis–HPLC system was 26 min per sample.

Fig. 2 illustrates the results of our typical no-net-flux experiments; it demonstrates the gain or loss of arbutin detected from the cosmetic lotion as a function of the concentration of arbutin added to the perfusate. In Fig. 2 we observe that the value on the intersection with the *x*-axis is 9.01 (mM). From this measurement and calculations, we determine that the content of arbutin in the whitening cosmetic lotion is 4.91 ± 0.10% (Table 1). A comparison between the percentages of arbutin analyzed using the on-line microdialysis–HPLC system (4.95 ± 0.15%) and the no-net-flux method with that of its percentage formula constituent (5%), indicates that our results are in good agreement to within experimental error. We also applied this no-net-flux method to determine the levels of arbutin in whitening cosmetic cream and essence: these values are 2.96 ± 0.21% and 0.85 ± 0.07%, respectively, while those determined using our proposed on-line microdialysis–HPLC system are 3.04 ± 0.22% and 0.83 ± 0.06%, respectively. The percentage of arbutin in the formula constituent of the cosmetic cream is 3%; although the

Table 1
The percentage (w/w) of arbutin in three commercial whitening cosmetics as determined using the on-line microdialysis–HPLC system and the no-net-flux method

Sample	Label claim	On-line microdialysis–HPLC system	No-net-flux method
Cosmetic lotion	5	4.95 ± 0.15[a]	4.91 ± 0.10
Cosmetic cream	3	3.04 ± 0.22	2.96 ± 0.21
Cosmetic essence	[b]	0.83 ± 0.06	0.85 ± 0.07

[a] Each value is the mean of data from triplicated runs.
[b] Not declared on the product label.

Table 2
Effects (%) that the factor levels of the Placket–Burman design have on the robustness of the microdialysis sample cleanup and the HPLC–UV analytical procedures

Factor	High level (+)	Nominal level (0)	Low level (−)
Microdialysis sample cleanup			
Perfusate flow-rate	4.08 ± 0.85[a]		12.52 ± 2.31
Arbutin	5.13 ± 1.24		4.76 ± 1.65
Moment			1.25 ± 0.47
HPLC–UV			
Eluent flow-rate	3.42 ± 0.83		4.05 ± 0.51
Methanol	1.43 ± 0.22		2.16 ± 0.35
pH	1.20 ± 2.01		1.45 ± 0.37
Arbutin	4.08 ± 0.85		4.52 ± 1.31
$H_2PO_4^-$	2.13 ± 0.29		1.76 ± 0.46
Moment			2.41 ± 0.27

[a] Each value is the mean of data from triplicated runs.

content of arbutin was not stated on the label of the cosmetic essence, we believe that the similarity between the analytical results obtained from our proposed on-line microdialysis–HPLC system and those from the no-net-flux method suggests that the accuracy of former is high and acceptable. Apart from the effect (an increase of $12.52 \pm 2.31\%$) caused by a low perfusate flow-rate (4.75 μl/min), we observed no significant changes in the robustness upon changing any of the other levels (Table 2).

4. Conclusion

We have developed a microdialysis sample cleanup process coupled on-line with an HPLC–UV analysis and validated its use for determining the level of arbutin in whitening cosmetics. Our study demonstrates that the determination of arbutin in whitening cosmetics can occur with acceptable precision and accuracy when using a simple one-step procedure. Because this method offers a number of advantages – simplicity, reliability, the lack of any tedious sample pretreatment process, and low amounts of organic solvents – we believe that it is suitable for determination of arbutin in cosmetic products.

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