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Microdialysis of salicylic acid from viscous emulsion samples prior to high-performance liquid chromatographic determination

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Abstract

A micro-dialysis method was developed to isolate aqueous salicylic acid from viscous emulsion samples prior to HPLC determination. The optimal conditions for obtaining dialysis efficiency of salicylic acid as well as chromatographic conditions were investigated. Experimental results indicated that the dialysis achieved at pH 2.0 (0.025 *M* phosphate solution), 0.5 *M* NaCl addition, and 50-μl/min flow-rate of perfusion stream offered an optimal result. The proposed method provided a simple procedure for isolating salicylic acid from viscous emulsion samples. Application was illustrated by the analysis of salicylic acid in cosmetic products. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Microdialysis; Salicylic acid

1. Introduction

Liquid–liquid extraction is a conventional pretreatment step in the determination of interested species in samples. However, it usually is time-consuming and requires a large quantity of organic solvents, which is a potential health risk and may cause environmental pollution. Besides, in the extraction of viscous/emulsion samples, the unclear interfacial layer often influences the separation efficiency.

For complicated matrix samples, membrane-based separations have been applied as useful tools for sample pretreatment or sampling [1]. Thus, on-line dialysis has been widely used in on-line bioprocess monitoring [2–5]. A commercial Gilson ASTED system on-line combined with an HPLC instrument was thus developed and widely used. In order to increase the membrane area available for diffusion, hollow-fiber membranes was used instead of planar ones. Recently, micro-dialysis has been extensively applied in biotechnological and biomedical research [6–9]. Compared to the conventional extraction protocol, micro-dialysis separation has the advantages of easy operation, rapid isolation of interest components from turbid and complicate matrix samples, and free or less-use organic solvents. From the application of on-line dialysis in the sampling of bovine serum, muscle tissue, plasma, and for pharmacokinetic studies [6–14], dialysis has the potential to be the alternative to conventional separation techniques for non-biological samples. However, except for the on-line dialysis system used for the analysis of liquid food samples [15–19], micro-

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dialysis has seldom been approached in consumer products with a complex matrix.

Salicylic acid is used as preservative of food products, paste, glue, and hides. Recently, it has been widely used as a whitening agent in cosmetic products. In these viscous emulsion samples, it is difficult to use conventional separation techniques, such as liquid–liquid extraction, solid-phase extraction, and solid-phase micro-extraction, to achieve the isolation. In this paper, with the described advantages, the micro-dialysis technique is systematically investigated in order to develop a simple pretreatment process for viscous/emulsion samples prior to HPLC determination.

2. Experimental section

2.1. Apparatus

The HPLC used in this work was a Shimadzu LC-9A system (Shimadzu, Kyoto, Japan), and a Waters 484 tunable absorbance detector (Waters, Milford, MA, USA) with a 20-μl flow cell. The detection wavelength was set at 230 nm. A reversed-phase C_{18} column (25 cm×4.6 mm I.D. 5-μm particle size) (Supelco, Bellefonte, PA, USA) was used for separation. A Rheodyne 7125 injector (Cotati, CA, USA) with a 20-μl external loop was used for sample introduction. A Chromatocorder 12 Integrator (SIC, Tokyo, Japan) was used to obtain the chromatogram, and perform data calculations. The micro-dialysis system comprises a baby bee syringe pump, a worker bee controller, and a 1-ml syringe (Bio-analytical System, IN, USA)

A home-assembled linear cellular membrane probe (cellulose acetate, 5000 Da, I.D. 220 μm, O.D. 310 μm) micro-dialysis sampling system was prepared and set-up. The syringe pump containing perfusate was connected to the inlet of the probe with PE tubing. The outlet of the micro-dialysis probe was connected to a collected-vial with PE tubing (I.D. 380 μm, O.D. 1090 μm) for chromatographic determination.

2.2. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Thermolyne, Dubuque, IA, USA) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. Standard stock solutions (1000 μg/ml) of salicylic acid (Riedel-deHaën, Hanover, Germany) was prepared by dissolving 0.100 g in 50 ml water (added 1 ml 0.1 *M* NaOH) and then adding water to adjust the volume to 100 ml. The solutions were stored in brown glass bottles, and kept at 4°C for a maximum of 3 months. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. Phosphoric acid, sodium hydroxide, and sodium di-hydrogen phosphate were used to prepare buffer solutions for eluent preparation and pH adjustments. The HPLC eluent was prepared as 25% (v/v) of acetonitrile (Baker, Phillipsburg, NJ, USA) in pH 2.3, 0.013 *M* phosphate buffer. All eluent were filtered through a 0.45-μm poly(vinylidene difluoride) (PVDF) membrane filter and degassed ultrasonically. A skin lotion sample and a sun-proof cream sample were purchased from local department stores.

2.3. Procedure

A 200-μl real sample was diluted to 100 ml in a measured flask with pH 2.0 phosphate solution (0.025 *M*) containing 0.5 *M* NaCl. After mixed thoroughly, the sample solution was poured into the dialysis cell to achieve the dialysis. The dialysate was collected for HPLC analysis.

3. Results and discussions

In order to verify the applicability of the proposed micro-dialysis isolation method, factors that affect the dialysis efficiency such as the flow-rate of perfusion, the pH and salt addition in sample solution, as well as the chromatographic conditions were studied thoroughly to optimize the sampling and analytical conditions.

3.1. Optimization of chromatographic conditions

Chromatographic condition was optimized and built-up prior to investigation of micro-dialysis conditions. Referred to the literature [20], a reversed-phase C_{18} column has potential to resolute salicylic

acid or salicylate from other species very well, and was thus used. In order to obtain an acceptable resolution of peaks within a time-interval, an appropriate quantity of organic modifier is generally added in the eluent and the pH of eluent is also adjusted appropriately. After series tests, the eluent prepared as 25% (v/v) of acetonitrile in 0.013 *M* phosphate aqueous solution (pH 2.30) offered an acceptable resolution. By the way, the peak of salicylic acid was kept from the base-line interference of solvent front, phosphate and sodium chloride. Fig. 1a,b shows the chromatograms of salicylic acid in real samples. The peak in retention time of 16.0 min relates to salicylic acid; it is well separated from other species.

From the UV-spectrum, salicylate (as in the eluent of pH>pK_a) has characteristic absorption at 226 and 296 nm, whereas salicylic acid (as in the eluent of pH<pK_a) has characteristic absorption at 230 and 302 nm. Because the characteristic absorption in the lower wavelength has a relative high molar absorbance coefficient, the detection wavelength was thus set at 230 nm.

Under these conditions, the retention time of salicylic acid is 16.0 min with 0.16% RSD (n=3), the reproducibility of quantitative detection for 40 μg/ml salicylic acid is 1.82% RSD (n=3).

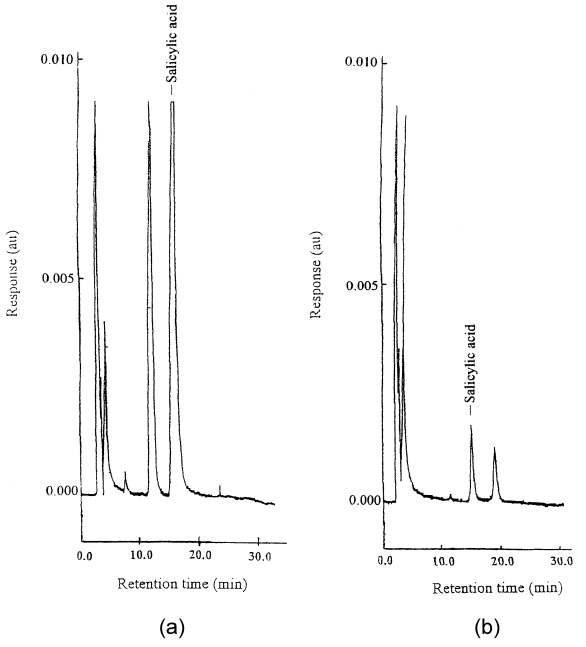

Fig. 1. Chromatograms of salicylic acid in real samples: (a) a skin lotion, (b) a sun-proof cream.

3.2. The pH effect on the dialysis of salicylic acid

In the application of micro-dialysis in biological and biomedical studies, the pH is always kept at the biological pH (7.4). However, the recovery of species from a dialysis system depends on the sample pH [21–23]. Therefore, the effect of pH on the dialysis of salicylic acid was studied. Fig. 2 shows the dialysis efficiency under varied pH. It can be seen the dialysis efficiency is nearly constant when the pH is far from pK_a (2.97) of salicylic acid, and there is a sharp variation near the pK_a. At lower pH (pH ≪ pK_a) the diffusion efficiency is much larger than that at pH ≫ pK_a. Obviously, the salicylate is un-favored to diffuse through the cellular fiber due to its negatively charged, and the salicylic acid (in its neutral molecular form) diffuses the cellular fiber more easily. Therefore, the pH of sample matrix for micro-dialysis of salicylic acid is recommended at pH 2.0.

3.3. Salting-out effect on the dialysis of salicylic acid

Salting-out effect is often applied to improve the recovery in conventional extraction processes. Because the salicylic acid is kept in its neutral form, the Donnan equilibrium effect was not in significant under the dialysis condition. In our studies, the

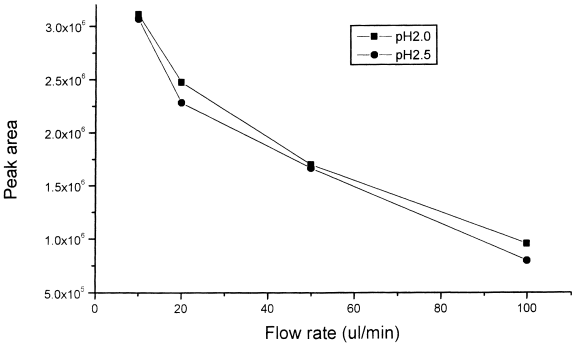

Fig. 2. The pH effect on the dialysis efficiency of salicylic acid. Micro-dialysis condition: sample, 50 μg/ml salicylic acid in 0.025 *M* phosphate solution or buffers under varied pH; perfusion solution, water.

diffusion efficiency in a 0.025 *M* phosphate solution was enhanced 4.6 times (referred to water). When 0.5 *M* NaCl was added into the phosphate solution, the diffusion efficiency increased 12.6%. Although a higher NaCl addition increased the diffusion efficiency, but it worse the chromatographic performance due to its increasing the pressure in HPLC system. Based on these results, the sample was dialyzed in the pH 2.0 aqueous solution of 0.025 *M* phosphate with 0.5 *M* NaCl. Because only 12.6% of the diffusion efficiency increased when adding 0.5 *M* NaCl in sample; if the salicylic acid in sample was high enough to detection, the addition of NaCl is recommended to omit to simplify the procedure.

3.4. Effect of perfusion flow-rate and membrane length

As reported in literature [24], the diffusion efficiency is a function of the perfusion flow-rate and length of the cellular dialysis membrane. As shown in Fig. 3, the recovery decreases with flow-rate increases. Although a low perfusion flow-rate increases the diffusion recovery, but it takes time to collect perfusate enough to be injected into the chromatographic system. Here, it is worthy to note that the detection sensitivity would decrease in a high perfusion flow-rate due to the dilution effect. Hence, the sampling time and detection sensitivity should be compromised in an analytical protocol including micro-dialysis. In general, 1.0-ml sample is required for five injections with a 20-μl sample loop. It takes 20 min to collect 1.0 ml with 50-μl/min flow-rate. This matches the time for one-run of chromatographic determination. Therefore, the perfusion flow-rate was selected at 50 μl/min. It is well known that the diffusion efficiency increases with the length of a cellular dialysis membrane. Because the cellular membrane obtained was only 15.5 cm, it was thus used.

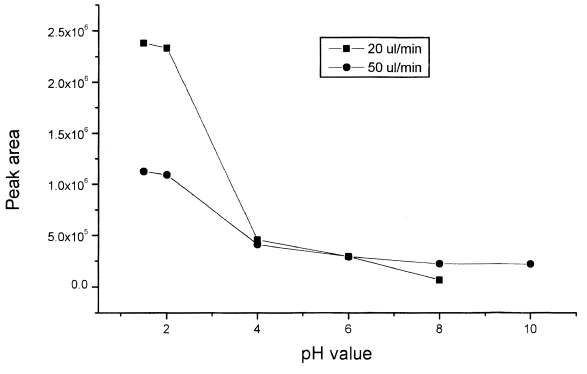

Fig. 3. The effect of flow-rate of perfusion on the dialysis efficiency of salicylic acid. Micro-dialysis condition: sample, 50 μg/ml salicylic acid in 0.025 *M* phosphate solution; perfusion solution, water.

3.5. Calibration plots of salicylic acid injected directly and posterior to dialysis

In order to realize the diffusion efficiency in varied concentration, calibration plots were built-up by injecting standard solutions of salicylic acid directly or the dialysates collected from standard solutions. The calibration plot was with equation of $Y=49519X+1128$ in the range of 0.1–50 μg/ml for direct injection, and $Y=46158X+169$ in the range of 0.1–100 μg/ml for posterior to dialysis. Both linear correlation coefficients are 0.9999. The detection limit is 14 μg/l derived from the 3 times of standard derivation with direct injection. The sample for direct injection is considered to have 100% recovery in concentration from dialysis. From the ratio of slopes for both linear regression equations, the average diffusion efficiency of salicylic acid in the concentration range under the optimal dialysis conditions is 93.21%.

3.6. Analysis of real samples

In order to test the applicability of the proposed method, a skin lotion sample and a sun-proof cream sample were diluted and dialyzed under the optimal conditions, and then determined by HPLC. Chromatograms are shown in Fig. 1a,b. From the measurement results and calculation, the contents of salicylic acid in the skin lotion and the sun-proof cream are 5.74 g/l (3.22% RSD) and 0.276 g/l (1.30% RSD), respectively. For a complicate viscous matrix sample, the life of cellular fiber is generally limited by the clogging of matrix. However, in our test samples, the viscous character of cosmetics comes from the emulsion phenomena; the performance of the micro-dialysis did not have significant variation over the test period.

4. Conclusion

This paper investigated the potential of micro-dialysis applied in the isolation of salicylic acid from viscous/emulsion samples such as cosmetics. The results indicate that the proposed micro-dialysis can be an alternative to conventional extraction step prior to HPLC determination with the advantages of easy operation, less time-expense, and free-use organic solvent.

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