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Industrial application of green chromatography—I. Separation and analysis of niacinamide in skincare creams using pure water as the mobile phase

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ABSTRACT

In this work, chromatographic separation of niacin and niacinamide using pure water as the sole component in the mobile phase has been investigated. The separation and analysis of niacinamide have been optimized using three columns at different temperatures and various flow rates. Our results clearly demonstrate that separation and analysis of niacinamide from skincare products can be achieved using pure water as the eluent at 60 °C on a Waters XTerra MS C18 column, a Waters XBridge C18 column, or at 80 °C on a Hamilton PRP-1 column. The separation efficiency, quantification quality, and analysis time of this new method are at least comparable with those of the traditional HPLC methods. Compared with traditional HPLC, the major advantage of this newly developed green chromatography technique is the elimination of organic solvents required in the HPLC mobile phase. In addition, the pure water chromatography separations described in this work can be directly applied in industrial plant settings without further modification of the existing HPLC equipment.

1. Introduction

Niacinamide is a chemical compound belonging to the vitamin B group that is commonly found in foods and used in cosmetic skincare products. Niacinamide is an amide derivative of niacin and is found in bound forms in nicotinamide adenine dinucleotide (NAD), its phosphorylated derivative NAD(P), and their reduced forms NAD(H) and NAD(PH), which are coenzymes important for cellular redox reactions [1,2]. These cofactors have many antioxidant properties and are involved in many enzymatic reactions in the skin [2]. Some of the cosmetic effects of niacinamide include improved skin barrier, reduced skin pore size, facial blotchiness, hyperpigmentation, and skin yellowing, antiwrinkle, and antiacne properties [2]. Niacinamide is also used as a bleaching agent in bleaching cosmetics and can control the transfer of melanin from melanocytes [3]. In addition, niacinamide is widely used as a color fixative in meats to maintain fresh color [4,5]. Its antioxidant properties help to reduce the speed of myoglobine oxidation and prolong the red fresh color [5].

It is important to be able to accurately analyze niacinamide levels in various products. Quantitative analysis of niacinamide in cosmetics is required for quality control, product release, and regulatory purposes. HPLC with UV or fluorometric detection has been widely used for the determination of niacinamide in pharmaceuticals, biological fluids, food, and cosmetics [1,3–6]. However, these traditional HPLC methods require organic solvents in the mobile phase that are hazardous and expensive.

With growing awareness about the environment and the increased initiative for the development of "green" technologies throughout the world, reversed-phase liquid chromatography using water as the sole component in the eluent has received increased attention. The ability to eliminate the enormous amounts of hazardous organic solvents that are consumed everyday worldwide and replace them with an efficient separation method that utilizes environmentally benign water offers many benefits both environmentally and economically.

Water is too polar to serve as a reversed-phase eluent at ambient temperature using normal RPLC stationary phases such as silica-based C18 and polymer PRP-1 columns. Therefore, there are two ways to achieve LC separation of organics using pure water as the sole mobile phase component. One way is to modify the stationary phase and the other is to heat the water mobile phase. Synovec

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and co-workers developed a special type of packing material by coating the stationary phase on non-porous glass beads or silica that allowed LC separation of organics using pure water at room temperature to occur [7–10].

Although ambient water is very polar, it can act like an organic solvent at elevated temperatures and has widely tunable properties such as dielectric constant, surface tension, viscosity, and dissociation constant that can be achieved by simply adjusting the temperature [11,12]. Pressure has little effect on the properties of the water but it is needed to keep the water in the liquid state at high temperatures [11–13].

Because of the unique properties of the high-temperature water, it has been used as the sole mobile phase component in reversed-phase liquid chromatography to achieve separation of many classes of organic compounds [11,13]. For over a decade, high-temperature (subcritical) water has been used to achieve chromatographic separation of polar, moderately polar, and even some non-polar solutes [11–22]. Most of the findings indicate that high-temperature water can be used to replace hazardous and expensive organic solvents required in the traditional RPLC to achieve comparable separation using water-only mobile phase [11,23–35].

However, much of the high-temperature water chromatography work was limited to academic studies. There are numerous industrial HPLC methods where a low percentage of organic solvents such as ~33% methanol are required in the mobile phase. Although one may argue that the methanol consumption is relatively low, the HPLC waste generated by such methods is threefold the volume of the methanol consumed and needs to be disposed of. For example, if an HPLC system is running continuously at 1 mL/min in an QC lab for a year, a total of 525 L of HPLC waste are generated for 175 L of methanol consumed using such an HPLC method. The industry has to pay not only for purchasing the 175 L of methanol but also for disposing of the 525 L of the methanol-containing waste. Fortunately, the organic solvents required in these HPLC methods can be replaced by heating pure water with only mild temperatures. Because the current commercial HPLC systems are equipped with column ovens capable of achieving 80 °C, the pure water chromatography separations may be directly applied in industrial plant settings without any further additions or modifications.

In this work the original Procter & Gamble (P&G) HPLC niacinamide method was identified as one of the existing industrial methods that may be replaced by a green method employing pure water chromatography. Therefore, the goal of this study was to develop a green chromatography method using pure water as the eluent for the analysis of niacinamide in skincare products. Waters XTerra MS C18 and XBridge C18 as well as Hamilton PRP-1 columns were used in this work. These columns were chosen because of their good stability at elevated temperatures [11]. Pure water chromatography separation has been optimized at different temperatures and flow rates. HPLC separation of niacinamide using methanol in the mobile phase was also performed for comparison purposes. Because niacin is often associated with niacinamide, we also included niacin in the last part of this study to broaden the application of this pure water chromatography method in other areas where the analysis of niacin is a must.

2. Experimental

2.1. Reagents

Niacin, niacinamide, ammonium acetate, and 4-acetamidophenol were purchased from Sigma–Aldrich Chemical (Milwaukee, WI, USA). HPLC-grade methanol and formic acid (90%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water (18 MΩ cm) was prepared in our laboratory. Olay skincare creams were purchased at a local store. The niacinamide placebo sample was received from Procter and Gamble (Cincinnati, OH, USA).

2.2. Preparation of diluent and standard solutions

The diluent was prepared by combining 200-mL 50 mM formic acid with 300-mL 50 mM ammonium acetate and mixed well. Internal standard solution was prepared by adding 0.10 ± 0.01 g of 4-acetamidophenol to a 100 mL volumetric flask and then diluting to the mark with methanol. Stock solution was prepared by adding 0.1 ± 0.05 g of niacinamide to a 10-mL volumetric flask and diluting to the mark with diluent. The working standard solution was prepared by transferring 1.00 mL of stock solution and 1.00 mL of internal standard to a suitable container and adding 8.00 mL diluent.

2.3. Preparation of samples

Each skincare cream sample was mixed well before sampling to ensure a homogeneous mixture. 0.2 ± 0.05 g of sample were weighed directly into a tared 25-mL glass vial (Supelco, Park Bellefonte, PA, USA). 1.00 mL of internal standard solution and 5 mL of methanol were added to the vials. The mixture was vortexed to completely disperse the cream. Once dispersed, approximately 5 mL of diluent was added to each vial and shaken to mix thoroughly. The solution was then filtered through a 0.45 μm Whatman GD/X filter (VWR, West Chester, PA, USA) into a 2-mL sample vial for chromatographic analysis.

The placebo sample obtained from P&G was spiked with 1.00 mL of the stock solution for niacinamide recovery study. The spiked placebo samples were then treated in the same manner as other regular cream samples prior to chromatographic separation and analysis.

2.4. Instrumentation

A homemade chromatography system was mainly used in this study. A Hitachi L-7100 HPLC pump (Hitachi, Ltd., Tokyo, Japan) was used to deliver the mobile phase. A Valco injector (Valco Instruments Company Inc., Houston, TX, USA) with a 5-μL loop was connected to the outlet of the Hitachi pump using a stainless steel tubing that included a preheating coil. The tubing passed into a GC oven (HP 5890 Series 2, Hewlett Packard, Avondale, PA, USA) that was used to control the column temperature. The GC oven was allowed to heat up to the set temperature for the experiment and the first injection was made ~20 min after the set temperature was reached. The column was located inside the GC oven and connected to the outlet of the injector with a stainless steel tubing. A Hamilton PRP-1 column with 3-μm particles (150 mm × 4.1 mm, Reno, NV, USA) and a Waters XTerra MS C18 column (2.1 mm × 100 mm, 3.5 μm, Waters Corporation, Milford, MA, USA) were used in this work. The tubing then exited the GC oven and passed through an iced water bath before entering the Hitachi L-7400 UV detector set at 245 nm. After exiting the UV detector flow cell, the eluent passed through a back pressure regulator (Restek, Bellefonte, PA, USA) and then was collected in a waste container. Please note that the chromatography system used in this study was constructed to also allow high temperature applications. It is likely that both the backpressure regulator and the iced water bath could be eliminated for applications at lower temperatures such as the ones used in this work, provided that the UV detector precision is maintained. The UV detector was connected to a computer via an interface of PC/Chrom (H&A Scientific, Greenville, NC, USA). Data acquisition and analysis were made available by the PC/Chrom software.

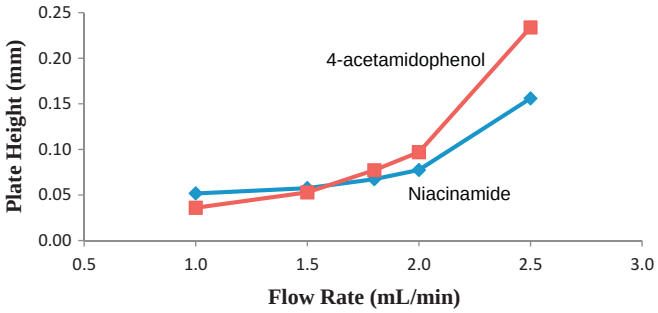

Fig. 1. Van Deemter plots obtained by pure water chromatography on the PRP-1 column at 80 °C.

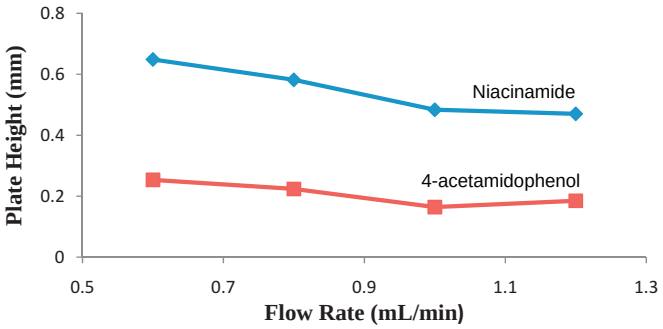

Fig. 3. Van Deemter plots obtained by pure water chromatography on the XTerra MS C18 column at 60 °C.

Additional experiments were performed using a Shimadzu Nexera UFLC system (Shimadzu Corporation, Tokyo, Japan). A Waters XBridge C18 column (4.6 mm × 100 mm, 3.5 μm, Waters Corporation) was used in this part of the work. Since niacin is normally associated with niacinamide, we also included niacin in this part of the study even though only trace amount of niacin is contained in the cream samples analyzed in this work.

3. Results and discussion

3.1. Hamilton PRP-1 column

3.1.1. Effects of temperature and flow rate on separation

The Hamilton PRP-1 column was tested for niacinamide separation using 100% water as the mobile phase. After initial evaluation of temperature effect on separation, the remainder of the pure water chromatography experiments on this column was carried out at 80 °C due to the efficient separation of niacinamide at this temperature. Please note that this temperature is within the manufacturer's temperature limit. Many reports show that PRP-1 columns are stable at much higher temperatures [11]. Another reason for the selection of 80 °C is that many commercially available HPLC systems are equipped with column ovens capable of operating at temperatures up to 80 °C. The HPLC experiments were performed at 25 °C.

Five different flow rates ranging from 1.0 to 2.5 mL/min were tested in optimizing the separation of niacinamide and 4-acetamidophenol. As shown in Fig. 1, the plate height for niacinamide stays relative flat in the range of 1–2 mL/min. However, the

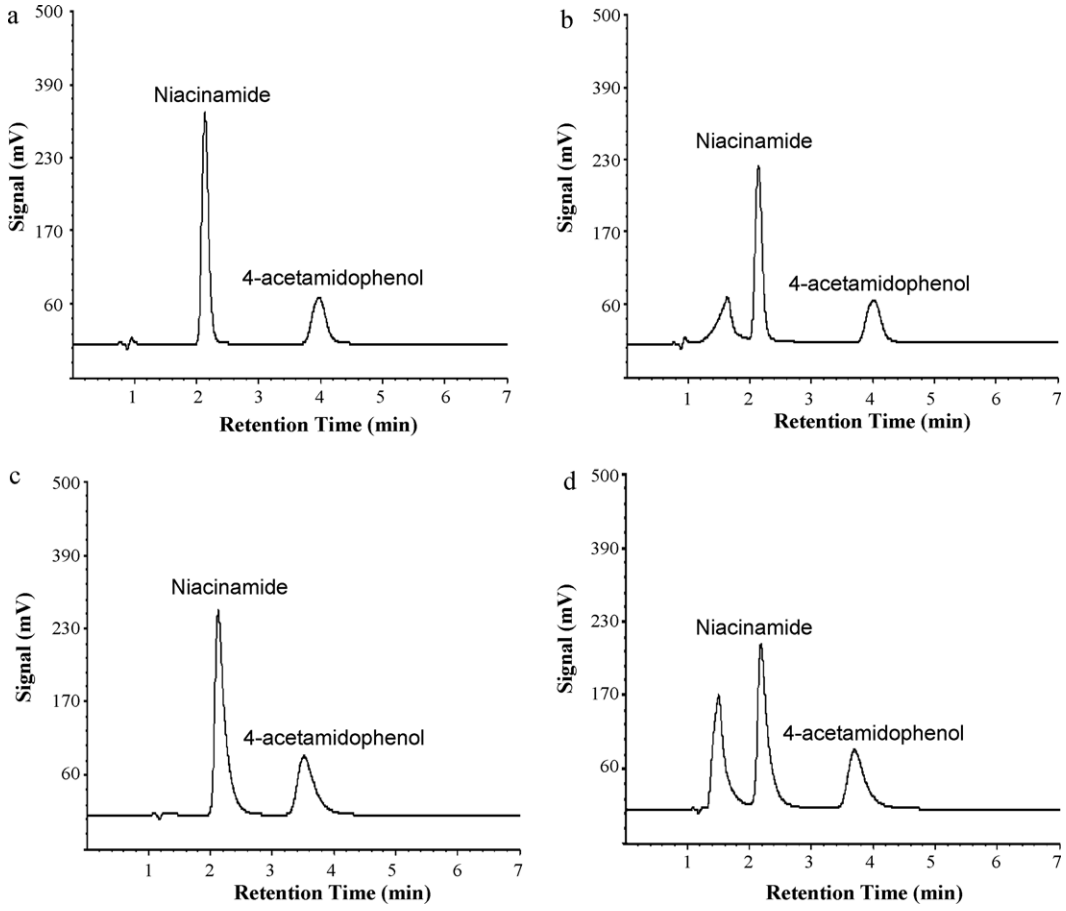

Fig. 2. Chromatograms of skincare cream samples on the PRP-1 column. (a) Pure water chromatography at 80 °C with 1.8 mL/min for SC-EC1; (b) pure water chromatography at 80 °C with 1.8 mL/min for SC-EC2; (c) HPLC at 25 °C with 1.2 mL/min for SC-EC1; and (d) HPLC at 25 °C with 1.2 mL/min for SC-EC2.

Table 1
Niacinamide recovery obtained by pure water chromatography compared with HPLC at 25 °C using 30% methanol in the mobile phase.

Samples	Column	Temperature (°C)	Flow rate (mL/min)	Niacinamide recovery (%)	%RSD
SC-EC1	PRP-1	80	1.8	101.0[a]	1.2[b]
SC-EC1	PRP-1	80	1.8	100.1[a]	1.3[c]
SC-EC2	PRP-1	80	1.8	100.2[a]	1.5[b]
SC-E	XTerra	60	1.0	100.4[d]	2.5[b]
SC-EC2	XBridge	60	2.0	100.5[a]	1.3[b]

[a] Recovery% = $\frac{\text{Weight\% obtained by this method}}{\text{Weight\% obtained by HPLC}} \times 100\%$.
[b] Based on five replicate sample preparations analyzed by both pure water chromatography and HPLC.
[c] Based on 21 replicate injections of one sample preparation.
[d] Recovery% = $\frac{\text{Mass of nicacinamide recovered by this method}}{\text{Mass of niacinamide added to the placebo}} \times 100\%$.

plate height increases significantly at 2.5 mL/min. Considering both separation efficiency and speed, 1.8 mL/min was chosen as the flow rate of pure water for the remainder of this study with the PRP-1 column.

3.1.2. Comparison of pure water chromatography and HPLC separations

HPLC separation of niacinamide was conducted at 25 °C using a mobile phase of 70% deionized water:30% methanol. For fair comparison purposes, similar retention times were obtained by both HPLC and pure water chromatography. Under the condition of similar retention times for each solute, the plate height obtained by HPLC is 0.154 and 0.180 mm for niacinamide and 4-acetamidophenol, respectively. These plate height values are about 2.3 times higher than that obtained by pure water chromatography with 1.8 mL/min as demonstrated in Fig. 1. In this case, pure water chromatography not only eliminates the use of methanol in the mobile phase but also yields better efficiency than HPLC with methanol involved in the mobile phase. The improvement in pure water chromatography separation efficiency can also be seen by comparing the chromatograms of both pure water chromatography and HPLC shown in Fig. 2.

3.1.3. Separation and analysis of niacinamide in skincare creams

Fig. 2a and b shows the chromatograms of two skincare cream samples, SC-EC1 and SC-EC2, obtained by pure water chromatography at 80 °C with 100% water as the mobile phase. The chromatograms of the same two cream samples obtained by HPLC

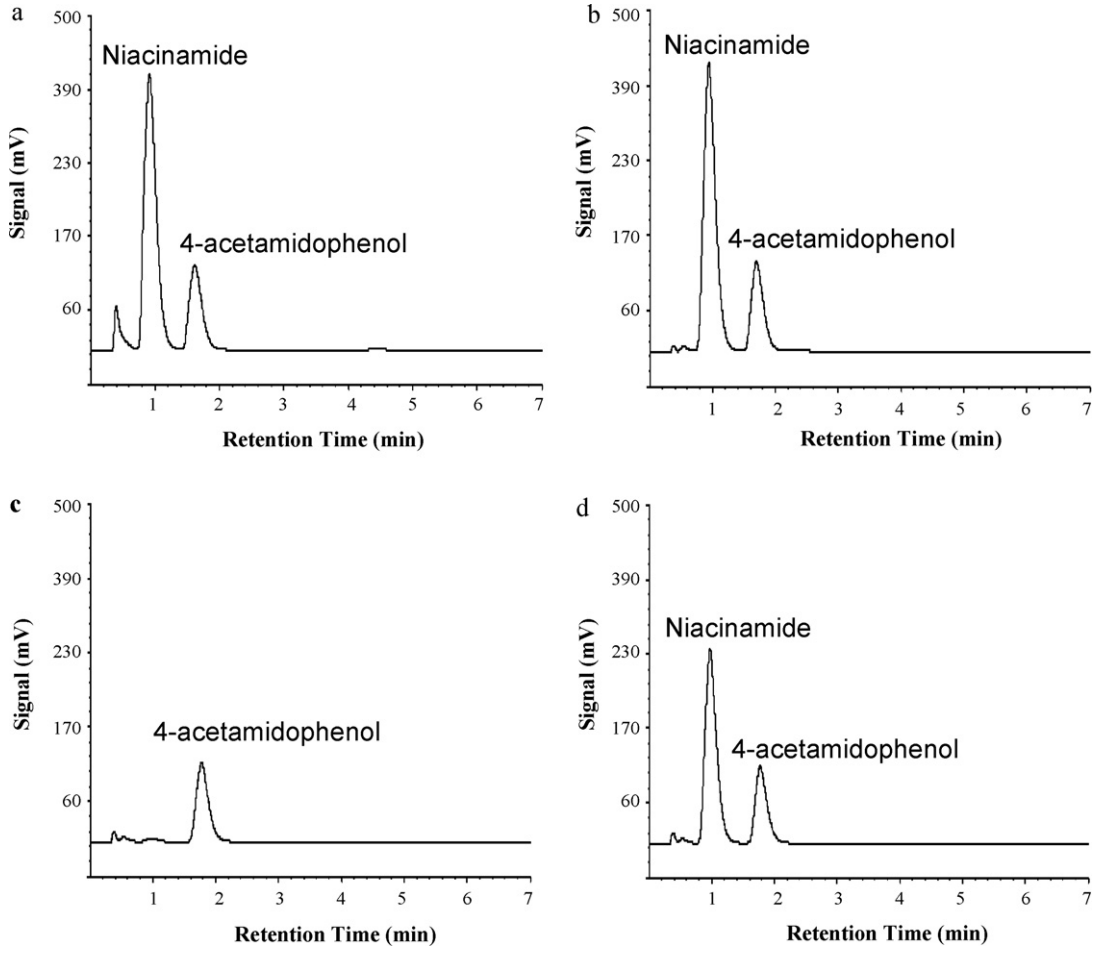

Fig. 4. Chromatograms on the XTerra MS C18 column using pure water as the mobile phase at 60 °C with 1 mL/min. (a) SC-EC1 cream sample; (b) SC-E cream sample; (c) SC-E placebo; and (d) SC-E placebo spiked with 9.6 mg of niacinamide.

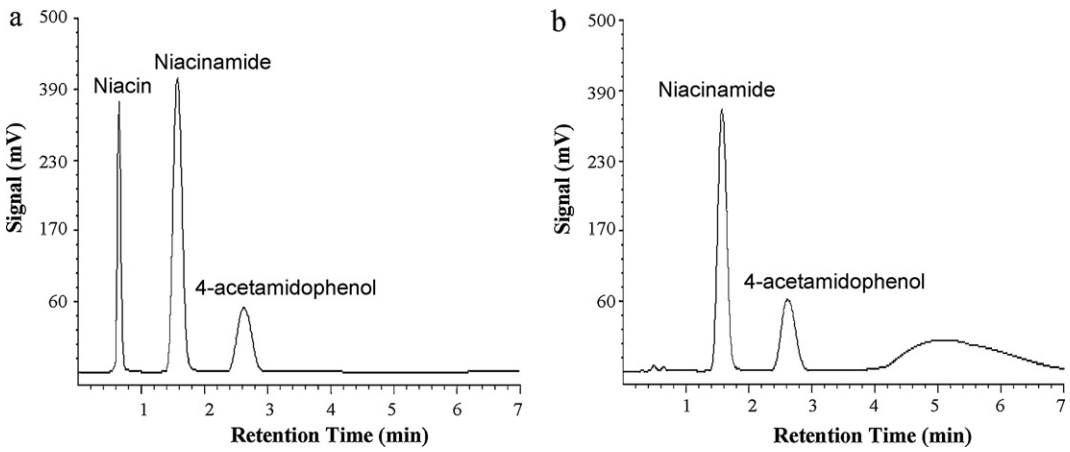

Fig. 5. Chromatograms on the XBridge C18 column using pure water as the mobile phase at 60 °C with 2 mL/min. (a) Standard mixture containing niacin and (b) SC-EC2 cream sample.

with 30% methanol in the mobile phase are given in Fig. 2c and d for comparison. The extra peak eluted before niacinamide as shown in Fig. 2b and d is from the sample matrix.

In order to evaluate the pure water chromatography method, the percent weight niacinamide determined by this green chromatography method was compared with that obtained by HPLC. As shown in Table 1, the recoveries for skin care creams SC-EC1 and SC-EC2 were 101.0% (RSD = 1.2%) and 100.2% (RSD = 1.5%), respectively. The percent recovery and RSD values were based on five replicate experiments. These recoveries are not significantly different from 100%, indicating that this new method provides the same results as the traditional HPLC method. In addition, the relative standard deviation obtained by pure water chromatography is below 2%, meaning that this green method is very reproducible.

3.1.4. Study of potential building-up problem

The reproducibility of this method was further evaluated by performing 21 replicate injections of one sample preparation using the SC-EC1 cream to check for building-up in the chromatography system resulting from numerous injections of real samples. As shown in Table 1, the niacinamide recovery is 100.1% with RSD of 1.3%, indicating that no sample building-up occurred. The excellent accuracy and precision determined from multiple injections of real samples suggests that pure water chromatography could be used in real industrial applications.

3.2. Waters XTerra MS C18 column

3.2.1. Effects of temperature and flow rate

Further investigation of separations using 100% water was conducted on the Waters XTerra MS C18 hybrid column. Temperatures of 60 and 80 °C were tested. Because the separation at 80 °C was not significantly better than that at 60 °C, the latter temperature was used for pure water chromatography separation on this Waters column. Another reason for choosing 60 °C is that it is within the manufacturer's recommended temperature limit for this column. It should be noted that other researchers found this column's actual temperature limit is much higher than the recommended limit of 60 °C [11].

Different flow rates ranging from 0.6 to 1.2 mL/min were evaluated to optimize the pure water chromatography separation. Fig. 3 shows that 1.0 mL/min flow rate yields the best separation. Therefore, this flow rate was used for the remaining experiments using this Waters XTerra column.

3.2.2. Separation and analysis of niacinamide in skincare creams

The optimized temperature (60 °C) and flow rate (1 mL/min) conditions for separations using pure water were applied to skincare cream and placebo samples. Fig. 4 shows the chromatograms of (a) SC-EC1 (skincare cream), (b) SC-E (skincare cream), and (c) SC-E placebo (skincare cream containing no niacinamide). As expected, no niacinamide peak appeared in the placebo sample (Fig. 4c). The niacinamide concentrations obtained by pure water chromatography was quantitative and the RSD is again below 2%.

3.2.3. Recovery study using a spiked placebo sample

To further test the reliability and validity of this green method, the placebo sample was spiked with 9.06 mg of niacinamide. The spiked placebo sample was treated in the same manner as a real skincare cream sample and analyzed using this new method. The chromatogram of the spiked placebo sample is shown in Fig. 4d. The percent recovery of niacinamide is 100.4% (RSD = 2.5%). The good recovery and the low %RSD value again demonstrate that this green separation method is accurate and precise.

3.3. Separation of niacin and niacinamide on the Waters XBridge C18 column

To further explore the industrial application of this green method, a commercial system, Shimadzu Nexera was employed in this part of the study. As mentioned earlier, niacin often co-exists in niacinamide-containing samples. To broaden the application of this green chromatography method, we also included niacin in this part of the study.

The conditions used here for separation using pure water were 60 °C and 2 mL/min. As shown in Fig. 5a, niacin was well separated from niacinamide using only pure water as the eluent. Fig. 5b shows the chromatogram of SC-EC2 cream sample. Only trace amount of niacin can be seen in the chromatogram. The last peak is from the sample matrix. The quantification shown in Table 1 reveals a niacinamide recovery of 100.5% with 1.3% RSD, again indicating the good accuracy and precision of this new method using a commercial system.

4. Conclusions

Our results clearly demonstrate that separation and analysis of niacin and niacinamide using only water as the eluent can be achieved at temperatures of 60–80 °C on either Waters XTerra MS C18 and XBridge C18 or Hamilton PRP-1 columns. The quantification quality and reproducibility of the organic-free methods

developed in this study are comparable with those of traditional HPLC methods. In addition, the separation efficiency obtained by the pure water chromatography methods is even higher than that of ambient HPLC methods with methanol required in the mobile phase.

Considering that most current commercial HPLC systems are equipped with column ovens capable of achieving 80 °C, the pure water chromatography separations described in this work can be directly applied in industrial plant settings without any modifications. There are numerous existing HPLC methods in industry where a fraction of organic solvents (e.g., 30% methanol) are required in the mobile phase. Although the methanol percentage is relatively low, the HPLC waste generated by such methods is threefold the volume of the methanol consumed. Fortunately, organic solvents are eliminated in pure water chromatography and a significant amount of money spent on HPLC solvents and waste disposal can be saved if a company is willing to adopt this green chromatography technique.

Acknowledgements

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