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Laboratory Experiment

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Determination of Niacinamide in Lotions and Creams Using Liquid–Liquid Extraction and High-Performance Liquid Chromatography

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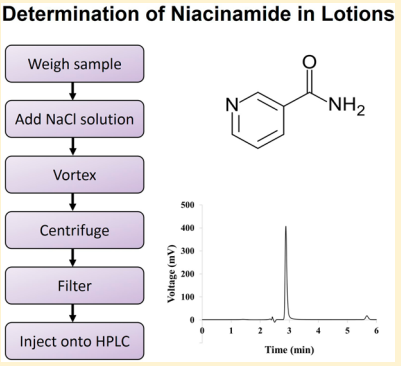
Supporting Information

ABSTRACT: Chemical separations are an important part of an undergraduate chemistry curriculum. Sophomore students often get experience with liquid–liquid extraction in organic chemistry classes, but liquid–liquid extraction is not as often introduced as a quantitative sample preparation method in honors general chemistry or quantitative analysis classes. This experiment offers a relevant, interesting way to use liquid–liquid extraction and high-performance liquid chromatography (HPLC) to analyze popular lotions and creams for niacinamide content. After completing the experiment, students will have a better understanding of the extraction technique, Beer–Lambert law, and HPLC. The percent recovery from a spiked sample of Olay Original Active Hydrating Beauty Fluid was found to be 99.22% with a standard deviation of 0.01%.



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INTRODUCTION

Niacinamide, a water-soluble vitamin shown in Figure 1, is often used in commercial beauty products as a whitening agent to lighten the skin and give a more even complexion. A 2002 study showed that a niacinamide moisturizer significantly lightened skin when compared to the moisturizer alone.[1] A 2007 study showed that there are potential benefits to using lotions containing 2% niacinamide to repair and protect against UV-induced skin damage.[2] Lotions and creams containing whitening agents are widely used around the world for both medical and aesthetic reasons. In the U.S., niacinamide is commonly used in eye creams and rejuvenating creams to reduce wrinkles and make skin look younger and healthier. The packaging for many niacinamide containing lotions and creams lists niacinamide in the ingredients but does not explicitly state the concentration used. In this experiment, a spike and recovery study for niacinamide in Olay Original Active Hydrating Beauty Fluid will be performed. The validated method will then be applied to determine the niacinamide concentration in a variety of commercially available Olay lotions that contain niacinamide.

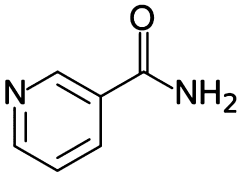

Figure 1. Structure of niacinamide (also commonly known as nicotinamide).

The laboratory experiment described in this paper is suitable for use in a wide range of undergraduate laboratory courses. The experiment was utilized by the authors in both an upper-division analytical chemistry course and in an honors general chemistry course with appropriate modifications as described in the Supporting Information. This experiment utilizes a liquid–liquid extraction for the extraction of niacinamide from lotions. Following this step, high-performance liquid chromatography (HPLC) is used for separation and quantification of the niacinamide. Liquid–liquid extraction is a sample preparation technique that has been used in chemistry laboratories for many years.[3] In the late 1960s, Robert V. Dilts and L. B. Rogers separately wrote about the importance of including separation techniques in the undergraduate analytical chemistry curriculum.[4,5] Rogers states that the principles for optimizing separations are appropriate topics for undergraduate analytical courses.[5] This idea, that separation, spectrophotometry, and sample treatment are important parts of undergraduate chemistry curriculum, has been repeated over the years.[6] These opinions have been well accepted, and many experiments involving HPLC have been designed for undergraduate laboratory courses. Some examples have been the analysis of analgesics,[7,8] nucleosides,[8] taurine in sports drinks,[9] and

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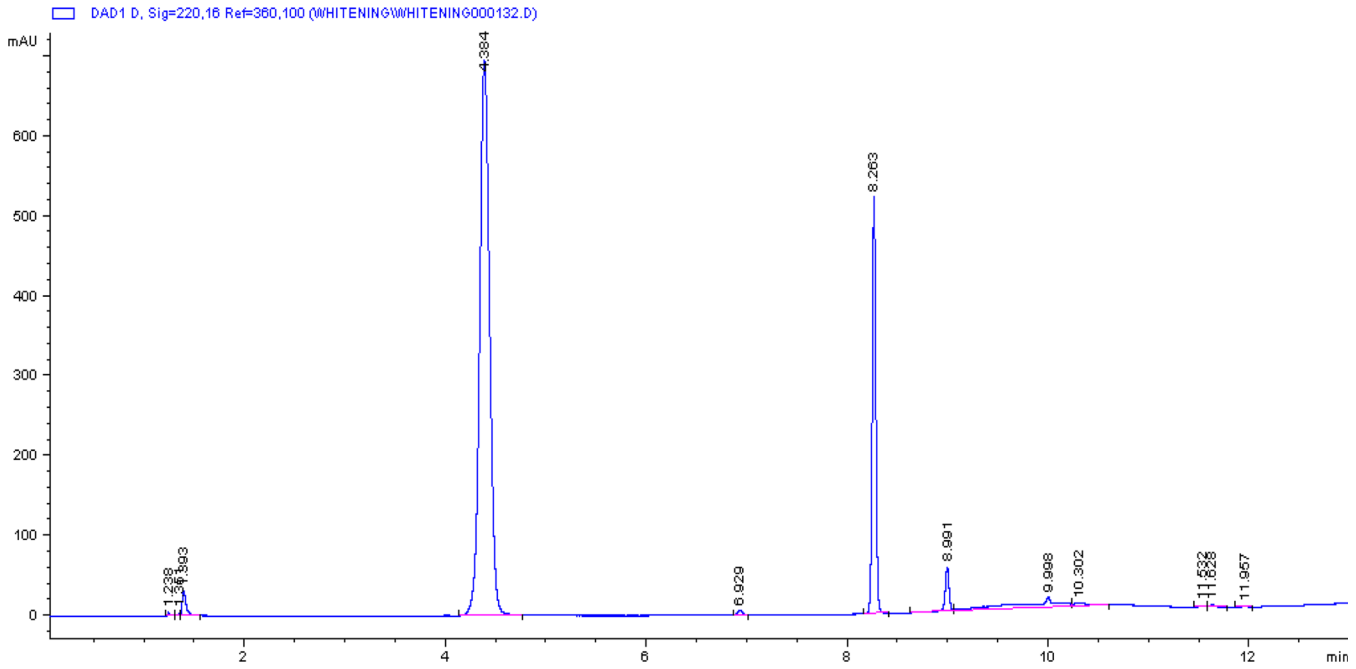

Figure 2. Chromatogram of the extract from Olay ProX Eye Restoration Lotion. Niacinamide peak is at ~4.3 min.

caffeine and vitamin B6 in energy drinks.[10] Undergraduate students often gain experience with liquid–liquid extraction in organic chemistry classes but not always in introductory analytical chemistry classes. This experiment offers a relevant, interesting way to use liquid–liquid extraction and HPLC to analyze popular lotions and creams for niacinamide content.

The following topics are emphasized in this experiment: (1) sample preparation (liquid–liquid extraction), (2) chromatography, (3) spectrophotometry (Beer–Lambert law), (4) proper dilution technique, (5) preparation of calibration curves, and (6) recovery studies. The experiment can be used to introduce students to some aspects of method validation. The lotions used in this experiment are readily available at many retail locations and are fairly low cost.

EXPERIMENTAL DEVELOPMENT

Experiments have been published that use liquid–liquid extraction in the undergraduate curriculum,[11,12] but there are no publications that describe the extraction of niacinamide from lotions using a simple liquid–liquid extraction as described in this paper. A previously published method for determination of niacin in cosmetics used online microdialysis for sampling followed by HPLC analysis.[13] This method requires a more complex apparatus than is typical for general chemistry or quantitative analysis undergraduate laboratories. The experiment presented is simple, rugged, and can be easily performed by students at various levels in their undergraduate career. This experiment is relevant because it uses well established techniques like liquid–liquid extraction and HPLC, techniques that chemistry students should be exposed to during the course of their undergraduate education. The experiment carries an additional level of interest for students due to the ubiquitous nature of commercial moisturizers and their myriad contents, many of which have benefits unknown to consumers. Because the amount of niacinamide in each product is not listed on the lotion packaging, students will find it interesting to calculate these results using the data from their experiments.

EXPERIMENTAL OVERVIEW

Procedure

In this experiment, niacinamide was extracted from the lotions and creams using a classic liquid–liquid extraction. Approximately 0.2 g of lotion was measured directly into a centrifuge tube, 5 mL of 20% (w/v) solution of sodium chloride was added, and the mixture was shaken and centrifuged. The niacinamide was extracted into the water layer, which was then collected and injected into the HPLC instrument. The HPLC method, which utilized a methanol/water mobile phase, was developed to separate the niacinamide peak from other chemicals that were also extracted from the lotions and creams. This step was necessary because of the nonspecific nature of a liquid–liquid extraction. In the extraction step, any chemicals in the lotion that have similar polarity to niacinamide were coextracted. Figure 2 is an example of a chromatogram that was collected in this experiment. The resulting HPLC data were then used to determine the percent of niacinamide recovered from an intentionally spiked sample of lotion that originally contained no niacinamide and the mass percent of niacinamide in the lotions containing niacinamide.

A stock solution of niacinamide (certified USP) was prepared and used to create calibration standards by serial dilution. The HPLC instruments utilized in the two class applications featured in this article used different sample injection techniques (the quantitative analysis course used an autosampler, and the honors general chemistry course used manual injection); therefore, the range of standard solution concentrations varied. For the quantitative analysis class procedure, the standards ranged from 40–400 μg/mL. For the honors general chemistry procedure, these standards ranged from 50–200 μg/mL.

The instrument used by the quantitative analysis class was an Agilent Technologies 1200 SL HPLC with diode array detector. The column was an Agilent Zorbax Eclipse Plus C18 column. The chromatographic conditions that were used to collect the author's data are given in Table 1. Different instruments may

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Table 1. HPLC Conditions for Determination of Niacinamide in Olay Lotions

Temperature	Ambient
Spectrum	Full
Wavelength	220 nm
Injection volume	1.70 μL
Flow rate	1.00 mL/min
Response time	0.02 s
Gradient	
Time	%B
0.00 min	10%
4.00 min	10%
9.00 min	90%
Solvents	
A	H_2O (Millipore)
B	Methanol

require modifications to these conditions. The retention time for the niacinamide peak was 4.3 min. Run time was 15 min with a re-equilibration time of 3 min.

Equipment

To perform this experiment, an HPLC instrument is needed. The experiment may be performed with or without an autosampler, although the time required to complete the experiment will be longer if there is a large number of groups and an autosampler is not used. Syringe filters (0.1 μm) are required for filtration of the water layer extract prior to injection into the HPLC. A centrifuge is highly recommended to aid in the removal of the water layer during extraction. The full list of supplies and equipment is given in the Supporting Information.

HAZARDS

Hazards involved with this experiment are associated with the use of methanol in the HPLC mobile phase. Methanol (CAS 67−56−1, PN: A 452−4, Fisher Scientific, Fairlawn, NJ) is a flammable liquid. If methanol gets in contact with eyes, flush eyes thoroughly with water. Solutions including methanol must be placed in an organic waste container and disposed of properly. Students should avoid inhalation of the methanol fumes and should wear gloves to avoid skin contact. Safety glasses should be worn at all times during this experiment.

RESULTS

The original experiment was performed by all seven students (three groups) enrolled in a quantitative analysis course during a 3 h lab period. The class average for percent recovery of the niacinamide spiked into the Olay Original Active Hydrating Beauty Fluid was 99.98% with a standard deviation of 3.77% when all results are included. The class average for the mass percent in the extraction portion of the experiment using Olay Age Defying Anti-Wrinkle Night Cream was 2.08% with a standard deviation of 0.12%. The experiment was then modified for use in an honors general chemistry course. The modified experiment was performed by all 15 students (four groups) enrolled in the honors general chemistry course during a series of 3 h class periods. The class average for the recovery experiment was 93.4% with a standard deviation of 20.7%. The large standard deviation was due to one group's result, which was only 62.9%. The results for each group can be viewed in the Supporting Information. The class average for the mass percent in the extraction portion of the experiment was 1.70% with a standard deviation of 0.40%. Author results for six different types of Olay lotions and creams that contain niacinamide following the original procedure used in the quantitative analysis class are included in Table 2.

Table 2. Author Results for Mass Percent of Niacinamide Extracted from Six Types of Olay Lotions Using the Original Method with an Autosampler

Sample	Mass % Niacinamide	STD DEV
Olay Professional ProX Eye Restoration Complex	4.835	0.040
Olay Regenerist Regenerating Serum	1.080	0.011
Olay Regenerist Night Recovery Cream	3.317	0.029
Olay Age Defying Antiwrinkle Day Lotion Sunscreen	1.763	0.023
Olay Total Effects 7-in-1 Antiaging Eye Treatment	1.727	0.045
Olay Age Defying Antiwrinkle Night Cream	2.107	0.072

DISCUSSION

The experiment was performed by students enrolled in a quantitative analysis course at one university, and then a modified experiment was performed by students enrolled in an honors general chemistry course at a second university. The two courses were taught by different faculty members and used different models and brands of equipment. Both procedures allowed the students to first validate the method with a percent recovery study and then to determine the niacinamide content present in an Olay lotion. The percent recovery was determined by spiking the Olay Original Active Hydrating Beauty Fluid with a known amount of niacinamide, then performing the extraction and HPLC determination. Once the amount of recovered niacinamide was calculated, it was compared to the spiked amount to determine the percent recovery. The procedure used for the quantitative analysis class allowed for replicates, increased precision, and more points in the calibration curves as would be expected for a course at this level.

Students enrolled in the quantitative analysis laboratory had already learned the basics of liquid chromatography and were familiar with liquid−liquid extraction. The instructor started the class with a 30 min discussion that included an overview of the experiment, a quick review of liquid chromatography including discussion of the instrument modules, and a complete discussion of laboratory safety that was relevant to this experiment. The students found the procedures easy to follow and were able to complete them in the 3 h period. It is likely that students could complete the procedure individually in a 4 h laboratory session. The students commented that it was nice to see how techniques they had used in other classes (they had performed liquid−liquid extractions in organic chemistry) could be used along with more advanced analytical techniques. Students also commented that they were unaware that niacinamide could be used as a whitening agent and that popular creams and lotions included this ingredient.

Students enrolled in the honors general chemistry laboratory had no working knowledge or exposure to liquid chromatography or liquid−liquid extractions. A few members of the class had performed dilutions and standard solution preparation in previous coursework, and all members of the class had utilized calibration curves to solve for unknown variables in previous

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experiments for this honors general chemistry laboratory course. The students were instructed to review online tutorials on HPLC as prelab preparation. It is highly advisable when performing this experiment with honors freshmen to include a prelab session with discussion on HPLC and liquid−liquid extraction. The HPLC instrument used by the honors general chemistry students did not have an autosampler, which increased the time of analysis and necessitated the use of separate 3 h lab periods for each group of four students. Although they were inexperienced with many of the techniques utilized in the experiment, the honors general chemistry students had little difficulty following the procedures for solution preparation and liquid−liquid extraction. As shown in the class results in the Supporting Information, the quality of the calibration curves (thus the quality of the solution preparation) for some of the groups suffered due to inexperience. Despite this, the students seemed to thoroughly enjoy the procedure and the exposure to techniques and instrumentation they otherwise would not have seen until upper-level courses. The data analysis proved somewhat challenging for the students, but with a little guidance from the instructor, the students all successfully completed the analysis.

CONCLUSION

This experiment was originally designed for students in a quantitative analysis course. As designed, the experiment allows students to learn and practice several analytical techniques including liquid−liquid extraction, pipetting, dilution, external standard calibration, and HPLC. In the first part of the experiment, students learn how to validate an extraction method using the percent recovery. In the second part of the experiment, students learn to use the extraction data to calculate the mass percent of analyte in a sample. The procedure was validated by the authors using the percent recovery from a spiked sample of Olay Original Active Hydrating Beauty Fluid, and the recovery was found to be 99.22% with a standard deviation of 0.01%. The student comments and results show that the procedures are easy to follow and give good results if students use the glassware and instrument properly.

ASSOCIATED CONTENT

Supporting Information

Notes for the instructor, equipment and supplies lists, prelaboratory lecture ideas, and sample chromatograms and tables of actual student results. Student procedures for the quantitative analysis course experiment and for the honors general chemistry experiment are also included in editable format. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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