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Sample Preparation for Hand Lotion Analysis

Leslie Hersh, Delta College, University Center, MI

INTRODUCTION

Description

In this experiment, students prepare a sample of a commercial hand lotion for analysis by HPLC to determine the percent composition of two common cosmetic preservatives, methylparaben and propylparaben. The product will be inspected for uniformity, standards will be prepared, the hand lotion sample will be prepared for analysis, the HPLC analysis will be performed, and the data will be evaluated. The significance and consequences of using syringe filters as a final sample purification step will be explored. In an optional pre-lab assignment, students are challenged to research their choice of syringe filter.

Student Audience

This activity is appropriate for students enrolled in a chemical technology program.

Goals for the Activity

The students are presented with an industrial problem-solving scenario. They gain experience with preparation of standard solutions, difficulties in dispersing/dissolving a relatively “stubborn” sample, use of a syringe filter for sample cleanup, and potential contamination from the syringe filter. Interferences are evaluated, and alternate sample preparation or test methods may be explored.

Recommended Placement in the Curriculum

This project can be used as part of a course on Chemical Analysis/Instrumentation, which is a final course in a two-year chemical technology program. The experiment could follow a previous laboratory experience using HPLC for a homogeneous sample, such as a caffeine analysis in drinks (coffee, tea, and cola).

STUDENT HANDOUT
Sample Preparation for Hand Lotion Analysis

Purpose
You will be presented with an industrial problem-solving scenario and will be expected to use outside resources to determine an appropriate method of sample preparation before analysis of the sample using HPLC. You will be expected to contact vendors either by Internet or telephone to aid in syringe filter selection and to evaluate the recommended/selected filters both for ability to perform a physical separation of the interfering waxes in a typical hand lotion and for non-contamination of the analyte. The "cleaned" sample will be analyzed using HPLC, and the results of the analysis will be evaluated.

Scenario
You are a chemical technician in a laboratory of a cosmetic company that manufactures hand lotion. There has been a problem with microbiological contamination of one lot of product, lot d1961a. This lot has been recalled. The plant manager needs to know whether there is a potential problem with plant sanitation that could affect future production or whether one of the preservative ingredients was not added to this particular lot in the required quantity.

To determine the level of preservative present in the recalled lot, the plant manager has asked you to develop a method to measure the quantity of each of the two preservatives, methylparaben and propylparaben, in the finished product from the lot. The analytical method should be simple enough to be used as an analysis performed prior to release of future lots of hand lotion for sale.

It is known that an HPLC C-18 column with a 55%/45% acetonitrile/water mixture can resolve the parabens with UV detection at 254 nanometers (nm). Because the emollients and waxes present in the hand lotion would gum up the HPLC column, it is necessary to remove these waxes before injecting the sample. The HPLC analysis is recognized to detect levels of 5–50 ppm of the parabens. The specified concentrations for the hand lotion you are testing are 0.15% methylparaben and 0.08% propylparaben.

Materials
Per class
- hand lotion sample
- methylparaben, NF grade
- propylparaben, NF grade
- acetonitrile, HPLC grade
- water, HPLC grade
- methanol
- 50- or 100-mL volumetric flasks
- 250-mL beakers
- syringes with Luer-Lok® fitting (acceptable range is 3–50 mL)
- syringe filters
- HPLC isocratic system with UV detection at 254 nm, C-18 column
- goggles
- (optional) glass stir rods, magnetic stirring apparatus, vortex mixer
- (optional) beaker or centrifuge tube

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

A hand lotion with microbiological contamination would require gloves when handling. (The hand lotion you will be asked to analyze will have no known microbiological contamination.)

Material safety data sheets (MSDSs) should be consulted on each of the chemicals to be used. The Internet location *www.phys.ksu.edu/~tipping/msds.html* may be used to search for MSDSs. Inhalation of acetonitrile and methanol should be minimized. A fume hood is required for standard and sample preparation. The acetonitrile vapors from the HPLC should be vented as well. Dispose of used reagents according to local ordinances.

Procedure

A. Pre-lab assignment

1. Review the MSDSs on all of the chemicals to be used.

2. HPLC columns are expensive and subject to contamination and/or inactivation by materials that can coat the column packing, such as the emollients found in hand lotions. Consider the use of syringe filters to perform a physical separation of the interfering waxes in a typical hand lotion from the analyte. Locate information on appropriate filters from filter vendors via catalog, Internet, or telephone. Possible sources of information are Millipore, Gelman, Supelco, Schleicher & Schuell (S & S). Use an Internet search engine if necessary to locate Internet information published by these companies or to contact a technical service representative. These filters cost about $1 each, so cost is a factor. Determine an appropriate syringe filter to use to prepare a solution of hand lotion in a 70% methanol solution for HPLC analysis.

B. Sample evaluation

1. Transfer the sample to a glass container in order to observe the homogeneity of the sample. Does the sample appear to be uniform? If the sample is not uniform, how will you make it uniform before taking a sample? Do not proceed until you have a uniform sample.

2. Calculate how much you will have to dilute a portion of your sample if the expected concentration of methylparaben in the product is 0.15% and the expected concentration of propylparaben is 0.08%. The detection range for the method is expected to be 5–50 ppm for each of the parabens.

C. Standard preparation and evaluation

1. This experiment will use the following HPLC conditions:
   - 55% acetonitrile/45% water mobile phase at appropriate flow rate to achieve separation
   - C-18 column
   - UV detection at 254 nm

2. Prepare a standard solution that contains both 15 ppm methylparaben and 8 ppm propylparaben. The sample will be dispersed in a solution that is 70% methanol, so this should also be used for the standards. To determine the expected order of elution for these two chemicals when they are in the combined standard solution, prepare a small amount (25 mL) of about 15 ppm methylparaben in the 70% methanol. Inject a sample of this solution and record the elution time. Also prepare 25 mL of about 8 ppm propylparaben in 70% methanol, inject, and record its elution time.

3. Filter the combined standard solution, recording the identity of the filter. Inject a sample of this filtered standard solution. Are there extra peaks when the two chemicals are injected together? If present, are the extra peaks a problem? What do you think could be the source of any extra peaks? How would you minimize the extra peaks if they were a problem? If they are a problem, how can this be resolved?

D. Sample preparation and method exploration

1. Determine the most effective way to disperse the desired quantity of the sample in the 70% methanol. Depending on volumetric glassware available, plan to prepare either 50 or 100 mL of dispersed sample solution.

2. Filter sufficient quantity of the solution for injection by syringe or autosampler. Record the identity of the filter used. What difficulties did you have in filtering the sample?

3. Inject the filtered sample. Compare peaks to elution times for standards.

E. Analysis

1. You now know the expected elution times for methylparaben and propylparaben and have an expected appearance for the chromatogram of your sample. Devise a plan to produce an appropriate number of chromatograms so that you may compare the peak height of the methylparaben and propylparaben in your sample to that of the standard solutions and thus determine the concentration of the methylparaben and propylparaben in your sample. Discuss your plan with your instructor before proceeding.

2. Complete your HPLC analysis and calculate the percent methylparaben and percent propylparaben in the product by comparison to chromatograms for the standard solution. Note that this calculation assumes a linear response for the methylparaben and propylparaben.

F. Implications of the analysis

Did your sample contain less than the specified concentration of the parabens? Prepare a report for the plant manager indicating your results and an evaluation of whether your results would imply that less than the specified quantity of either of the parabens was added to the batch.

Questions

1. How could you improve the dissolution of the lotion in the solvent system?

2. What problems did you have in filtering the sample? How did you address them?

3. What observations do you have about the precision of the analysis?

4. How would you refine this method to provide a method that could be validated to comply with FDA requirements for active ingredient analysis; that is, how would you show that the method analyzes for all of each of the parabens and nothing else?

5. What suggestions would you have for improving this method for routine quality control use?

Reference

Mayer, M. "Selecting Filters for Chromatographic Applications," *LC*GC (The Magazine of Separation Science).* 1996, *14* (10).

INSTRUCTOR NOTES
Sample Preparation for Hand Lotion Analysis

Time Required
Part A, the pre-lab assignment, requires up to 2 hours.

Parts B and C can be done in laboratory session 1. Part B requires up to 1 hour. (The instructor should start and stabilize the HPLC during this time.) Part C requires up to 2 hours.

Do parts D and E in laboratory session 2. Part D could require up to 1-1/2 hours depending on student creativity and equipment available. Samples may be difficult to disperse. Part E could require up to about 10 minutes per run depending on column size and flow rate. Each group should run up to three sample and three standard runs to average results. If class size is large, possible techniques to use are as follows:

- Do only one injection for each group's sample and standard solution, although additional injections are preferred for data analysis.
- Do three injections each for a sample and standard solution and have the entire class do the calculations on this data set.
- Have an autosampler or dedicated students continue to make injections over an extended period of time.

Part F, preparing a report for the Plant Manager, could take 1 to 2 hours depending on mathematical rigor employed.

Group Size
Students could work in groups of two or three to prepare standards and sample solutions.

Materials
Standards: Methylparaben and propylparaben, both NF grade, may be purchased from a specialty chemical supplier such as Sigma, or it may be possible to get evaluation samples from a manufacturer. Manufacturers and their telephone numbers are listed in *Soap Cosmetics Chemical Specialties*, April 1997 Blue Book.

Syringe filters: Syringe filter suppliers often provide free evaluation samples. Suggested suppliers of syringe filters to contact for samples are as follows:

- Supelco: www.sigma-aldrich.com/saws.nsf/SupProducts?OpenFrameset
- Gelman: www.pall.com/gelman
- Millipore: www.millipore.com
- Schleicher & Schuell: 800/245-4024

Suppliers will be happy to make recommendations. Possible choices of syringe for sample preparation are as follows:

- Schleicher & Schuell Uniflo Plus, 25 mm, nonsterile, 0.45 mm, ca membrane/glass fiber filter (contains a pre-filter)
- Gelman GHP Acrodisc GF syringe filter hydrophilic polypropylene with prefilter, P/N S4559,
- Gelman 0.45-mm GHP Acrodisc syringe filter, P/N S4560

Hand lotion: Use a hand lotion that contains both methylparaben and propylparaben as preservatives. Examples are Vaseline® Intensive Care/Dry Skin and Lubriderm® Skin Therapy. Hand lotions will

vary in viscosity, so the filter requirements will vary depending on the lotion used. The more viscous lotions are more likely to require the filters with prefilters included to prevent clogging. The shampoo Revlon Flex® also contains the paraben preservatives and could be used as an alternate sample for this technique if syringe filters are unavailable.

Safety, Handling, and Disposal

As the instructor, you are expected to provide students with access to SOPs, MSDSs, and other resources they need to safely work in the laboratory while meeting all regulatory requirements. Before doing this activity or activities from other sources, you should regularly review special handling issues with students, allow time for questions, and then assess student understanding of these issues.

Inhalation of acetonitrile and methanol should be minimized. A fume hood is required for standard and sample preparation. The acetonitrile vapors from the HPLC should be vented as well. Dispose of used reagents according to local ordinances.

Points to Cover in the Pre-Lab Discussion

For Parts B and C

1. Safety issues are described above.

2. Explain that the HPLC system requires at least an hour to stabilize, so you will be starting the instrument at the beginning of the lab so that it will be available when the students have a standard prepared for injection.

3. You may wish to discuss the preparation of the 70% methanol solution or even prepare a sufficient quantity of the solvent as part of a demonstration. (The quantity to be prepared is sufficient for one volumetric flask (50 or 100 mL) per sample or standard to be used for the class.) With careful volume measurements, it becomes apparent that if 300 mL water is added to 700 mL methanol, the total volume is something less than 1,000 mL. Preparation of vol/vol solutions is generally done by using 700 mL in a 1,000-mL graduated cylinder, which is then diluted with distilled or deionized water to 1,000 mL. A discussion of “to deliver” vs. “to contain” glassware may be appropriate at this time in relation to how to measure the 700 mL. Alternatively, the solution could be prepared by delivering 700 mL in a volumetric flask and bringing to volume. The exact dilution is not critical if all samples and standards are prepared using the same solution.

For Parts D and E

1. Discuss possible ways of dispersing a difficult sample. Indicate equipment available to aid in sample dissolution: vortex mixer, ultrasonic bath, magnetic stirrers. You may wish to advise the students to disperse the lotion first in a container such as a beaker or centrifuge tube (depending on the method used) and then quantitatively transferring this material to a volumetric flask for final dilution. Suggest that the students “play” with different methods first to determine an effective method before doing their analytical preparation.

   Each member of a group could be encouraged to try a different technique. One of the most effective techniques is to give the mixture a good stir and then allow it to sit for a period of time. After perhaps one hour, the lotions will often rapidly disperse. Heating the solution might also accelerate the dispersal and could be used as a basis for discussing how to maintain sample

integrity. There will be visible particulate matter. Brands of hand lotion will vary in the appearance of the dispersed particulate. Some brands will have a cloudy precipitate. Once a means of dispersing the lotion has been decided upon, the students can plan the actual dilution step.

2. Discuss stability of the HPLC system and feasibility/desirability of doing replicate samples. Develop a plan for the students to be able to have the instrument time they need to get sufficient data. Typical analytical work would require averaging three sample runs and three standard runs all done as close together in time as the instrument permits.

3. Discuss use of peak height as a means of comparing sample composition to standard composition for each of the components.

$$\text{mass detected, g} = \frac{\text{peak height of sample} \times \text{mass of standard, g}}{\text{peak height of standard}}$$

$$\text{paraben, \%} = \frac{\text{mass detected, g} \times \text{volume of diluted sample, mL}}{\text{injection volume, mL} \times \text{sample mass, g}} \times 100\%$$

Procedural Tips and Suggestions

Tested conditions that will work for this analysis

- use of Schleicher & Schuell filter with hand lotion of viscosity up to 30,000 cP
  - does not clog with up to about 10 mL of 1% hand lotion in 70% methanol
  - does not give peaks that overlap the parabens
- Fisher HPLC-grade solvents
- Perkin Elmer C-18 column, 25 cm
- 55% acetonitrile/45% water mobile phase at 2 mL/minute
- UV detection at 254 nm

For Part B

A 1:100 dilution is required. Note that 0.15% is 1,500 ppm. To dilute to 15 ppm requires using 1 part of sample in a total of 100 parts of sample solution. It is expected that students will plan to disperse 1.000 g of product in a 100-mL volumetric or 0.500 g in a 50-mL volumetric flask.

For Part C

DO NOT MAKE SCHLEICHER & SCHUELL SYRINGE FILTERS AVAILABLE TO THE STUDENTS AT THIS POINT. Students are instructed to inject individual samples of methylparaben solution followed by propylparaben solution and are not told to filter these solutions. They are then instructed to prepare a solution of the two together, filter this solution, and inject it. At this point, any additional peaks introduced by the syringe filter should become apparent. This step is intended to make students analyze where the extra peaks (if present) could have been introduced and lead them to consider the possibility of contamination by the filtering process. If the filter is the source of any extra peaks, this can be determined by injecting a sample of the 70% methanol solution which has not gone through the syringe filter. Students are to determine how such peaks could be eliminated. Possible remedies are rinsing the filter with solvent solution before use or changing brand/type of syringe filter. (The S & S filters do not generally introduce interfering peaks.)

For Part F

It is important to help students recognize that their job is not completed when the calculations are done. Rather, they must look at their results, be able to interpret them for nontechnical personnel, and present them in a coherent manner. At this point students may be able to conclude there is paraben present (parabens were not completely forgotten by the operator), and they may need to present the plant manager with the confidence level with which they can say there is 100% of the specified amount present.

Sample Results

Commercial hand lotions vary from 0.05–0.20% methylparaben and 0.05–0.15% propylparaben.

Plausible Answers to Questions

1. How could you improve the dissolution of the lotion in the solvent system?
   The lotion could be dispersed with agitation. (The method would depend on available equipment.) The lotion could be dispersed more quickly by heating the solution. The lotion could be dispersed by an initial mixing and then allowed to sit for a period of time before remixing.

2. What problems did you have in filtering the sample? How did you address them?
   The filter leaked when I put pressure on the plunger because I didn't have it tightened securely to the syringe, so I tightened the filter.

   The filter plugged up with the dispersion and I had to try a different filter to be able to take out the waxes without plugging the filter.

   The filter introduced an additional peak. I had to pre-rinse the filter with 70% methanol before use or use a different brand of filter.

3. What observations do you have about the precision of the analysis?
   The HPLC results varied but seemed to be more reproducible if I ran the samples close together in time. I was careful not to introduce any air bubbles in my injection so that the sample size was precise.

4. How would you refine this method to provide a method that could be validated to comply with FDA requirements for active ingredient analysis; that is, how would you show that the method analyzes for all of each of the parabens and nothing else?
   The validity of the procedure could be confirmed by determining that the test detects all of the parabens present and nothing else as an interference. A calibration curve could be run to confirm the linear response of the parabens over the expected range of paraben concentration. Blank samples (available only from the manufacturer, since the product formula is proprietary) would confirm the elimination of any interferences from within the product itself. An internal standard such as ethylparaben could be added to the sample solution to determine the actual recovery of the parabens by this sample preparation method.

5. What suggestions would you have for improving this method for routine quality control use?
   Student responses may vary.

Extensions and Variations

- Students could prepare a range of standard solutions for injections to establish a calibration curve and validate the linearity of response for the method.

- Students could evaluate the reproducibility of multiple runs over a period of time to get an understanding of the expected precision of the method and explore ways to improve the precision.

References

Gelman Sciences Technical Information Bulletins:

- The Cost of Not Filtering
- Filtering Analytical Samples
- Preventative Maintenance for HPLC
- Maintaining Analytical Integrity during Sample Preparation