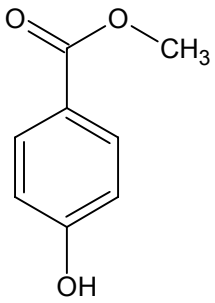
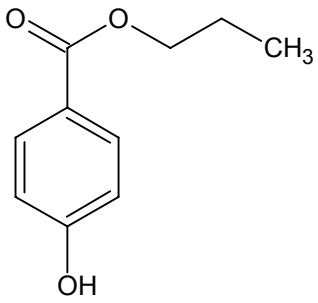
The Determination of Parabens in Hand Lotion by
High Performance Liquid Chromatography (HPLC)
Last Modified: October 6, 2006

Introduction:

This experiment is designed to introduce the basic use of an HPLC for performing a separation and quantitative determination by tackling a realistic chemical problem. You will assume the role a chemist working in a laboratory that specializes in deformulations. A cosmetics company has contracted with a problem. It seems that one of their competitors has a hand lotion whose shelf-life is far superior to their product. They have hired your company to determine the quantities of two preservatives, methylparaben and propylparaben, in the competitor's product so that they can determine if adjusting the composition of their lotion will make their product more competitive. The senior chemist in your group has assigned you the task of developing an HPLC procedure to determine the paraben content in the lotion.



Methylparaben



Propylparaben

Required Chemicals and Solutions:

1. Hand lotion sample.
2. Methylparaben stock standard solution.
3. Propylparaben stock standard solution.
4. Methanol
5. HPLC Grade acetonitrile (for mobile phase)
6. 18 MΩ Resistive Water
7. 4 standards with paraben concentrations between 5-50 ppm (for each paraben).

Sample Preparation:

Typical paraben concentrations are 0.05-0.2% in commercial products. Assuming this, determine an appropriate means to disperse the lotion sample into a 70% methanol/water solvent to prepare a solution that has a paraben concentration in the range of 5-50ppm. Realize that the lotion may not dissolve completely, but efficient extraction of the parabens, which are soluble in methanol/water is key. Next, the sample must be filtered to remove particulates using a syringe filter with no larger than 0.45 μm pore size. One should prepare 3 replicate samples.

Analysis:

You will be doing a reverse-phase separation using a C_{18} bonded-phase resin as the stationary phase and an acetonitrile/water mixture as the stationary phase. Using the standard paraben solutions that have been prepared for you, you will need to determine a set of optimum

conditions to use for your analysis. These conditions include: mobile phase composition, flow rate, and detection wavelength. It would be wise to prepare a solution that contains both parabens and use this solution as you optimize the method. Use a UV-Vis spectrophotometer to collect a spectrum of the paraben solution and determine the optimum wavelength for the LC detector. As you optimize the method, be sure to note how changes you make impact the quality of the separation in terms of retention time and column efficiency. Sample sizes should be consistent and should be within the range of 5-20 µL.

In order to quantify the amount of paraben present in the lotion, you will need to prepare the appropriate standards and develop a calibration curve. Use the relative sizes of the paraben peaks in the stock solution and in your sample to determine the appropriate dilutions needed to prepare four standards whose concentrations bracket your sample. To save time, it may be advised to prepare a single set of standards that contains both parabens. Once the solutions are prepared, run injections of your standards and sample, as well as a blank to prepare your calibration curve. Run at least three injections of your sample to determine the precision of the method. Prepare a running calibration curve to be sure that things are proceeding as expected.

Results:

Using calibration curves created by both peak areas and peak height, determine the concentration of each paraben in the lotion sample. (Remember, this requires you to back-calculate to the original concentration of your sample; thus knowing your dilution trail precisely becomes critical).

It is also crucial to report the precision of the analysis and ultimately the confidence limits. Whenever possible, you should report the % error by comparison of your calculated analyte concentration to that expected for your sample. Finally, with knowledge of your precision and the calibration curve you obtained, you should be able to determine an approximate limit of detection for the method of analysis, under the experimental conditions you used for the measurement.

What determinate errors might have played a role in impacting the accuracy of your results? What factors might have been the greatest contributors to the precision you obtained during this analysis.