SP79020

Experiments in Liquid Chromatography — Separations of FD&C Dyes

4-5-2020

This experiment is one in a series prepared by Waters Associates, Inc. to introduce liquid chromatography to undergraduates.

You are encouraged to copy the inside pages for your students use.

Identification of FD&C Dyes

- Isolation by a SEP-PAK C_{18} Cartridge
- Separation by Thin Layer Chromatography (TLC)

Objectives

To provide an introduction to both "normal phase" and "reverse phase" chromatography and to demonstrate applications in the isolation and identification of Food, Drug, and Cosmetic (FD&C) dyes.

Introduction

Within the past few years, much interest has been generated over the safety of FD&C dyes. At present, seven of these dyes are allowed in foods: Reds 3 and 40, Yellow 5 and 6, Blues 1 and 2, and Green 3. (Two additional dyes, Reds 2 and 4, have been officially "delisted" although foods packaged before the latter part of 1976 may contain them). Information on these dyes may be found in references 1-7. Because of continuing concern over dye safety and the fact that present law does not require listing of specific dyes on labels, there is genuine interest in dye separation and identification. This experiment involves the isolation of these dyes by SEP-PAK C_{18} Cartridge and subsequent analysis by TLC.

Choosing a Food

For ease of sample preparation, the following "foods" are ideal substrates for this experiment: a certified food color, soft drinks, pickle juice, juice from maraschino cherries, colored cough medicines, mouthwashes, or gelatin type desserts. Solid foods require dissolution and filtering before analysis. The food should indicate "artificial color" on the label.

Preparing Standard Dye Solutions

Stock solutions of the dyes are prepared by dissolving trace amounts of the dye in distilled water.

Equipment and Supplies

- Standard dyes (Note 1)
- Colored foods
- SEP-PAK C_{18} Cartridges (one/student)
- TLC developing solution: n-butanol/ethanol/water/concentrated aqueous ammonia (50:50:25:10)
- Silica TLC plates
- 2-propanol
- 1% acetic acid

Procedure: Dye Extraction

For reproducible results, it is necessary to separate the dye from interfering substances such as water, alcohol, flavorings, etc. before TLC analysis. Standard methods for this separation have traditionally involved either tedious ion exchange chromatography or time-consuming white wool extractions (8).

In this experiment, a SEP-PAK C_{18} Cartridge is used to isolate and "trace enrich" the dye in seconds. The dyes are adsorbed onto the C_{18} packing, allowing their concentration (trace enrichment) on the cartridge head while numerous interfering compounds (sugars, flavorings, preservatives, etc.) are eluted. The specific procedure is as follows:

Refer to Water Publication "SEP-PAK Cartridges for

Information for Instructor continued on page 4

Lab Work Sheet

Identification of FD&C Dyes

- Isolation by a SEP-PAK C_{18} Cartridge
- Separation by Thin Layer Chromatography (TLC)

Objectives

To introduce the theory of liquid chromatography and to demonstrate applications in the isolation and identification of FD&C dyes.

Introduction

Within the past few years much interest has been generated over the safety of Food, Drug and Cosmetic (FD&C) dyes.

Presently, seven dyes are allowed in foods: Reds 3 and 40, Yellow 5 and 6, Blues 1 and 2, and Green 3. (Two other dyes, Reds 2 and 4, have been officially "delisted" although foods packaged before the latter part of 1976 may contain them.) In this experiment, you will isolate dyes from a food with a SEP-PAK C_{18} Cartridge and then separate and idenfity them by TLC.

SEP-PAK C_{18} Cartridges are small chromatographic columns for rapid sample preparation. Their packing material is made by chemically bonding a C_{18} group to silica.

The procedure described here can be modified to analyze other food additives such as preservatives, artificial sweetners. etc.

Preparation of Food Sample

For ease of sample preparation, liquids or easily liquified foods are recommended for analysis. Acidic foods, i.e. soft drinks, pickle brine, and juices may be used without special preparation. Gelatin products should be dissolved in water (0.1 g in 3 ml), acidified (1 drop of 1% acetic acid), and filtered before analysis. Alcohol containing materials (mouthwashes, cough medicines, etc.) should be diluted 1:1 with water and acidified (1 drop of 1% acetic acid) before analysis to enhance dye retention on the SEP-PAK C_{18} Cartridge.

Procedure: Dye Extraction

For reproducible results, the dye must be separated from interfering substances such as water, alcohol, flavorings, etc., before chromatographic analysis. A SEP-PAK C_{18} Cartridge isolates and concentrates ("trace enriches") the dye in seconds. The dyes are adsorbed onto the C_{18} packing while numerous, more polar compounds (sugars, flavorings, etc.) are eluted from this reverse phase cartridge. The specific procedure is as follows:

1) Use a syringe to prewet a SEP-PAK C_{18} Cartridge with 3 ml of 2-propanol.
2) Flush 5 ml of 1% acetic acid through the cartridge. This improves dye adsorbtion by insuring that the sulfonic acid dyes remain in their free acid form.
3) Flush 1 ml of sample through the cartridge, discard the eluate.
4) Flush with 2-3 ml of 13% 2-propanol, collecting only the colored portion (which may contain Blue 2, Yellow 5 and 6, and Red 40).
5) Flush with 2 ml of 50% 2-propanol, collecting only the colored portion (which may contain Red 3, Blue 1 and Green 3).
6) The eluates from Steps 4 and 5 will be used for chromatographic analysis.

Procedure: TLC Analysis

Spot a TLC silica plate with some of the dye extracted according to the directions of your instructor. Develop the plate with a solution of n-butanol/ethanol/water/concentrated aqueous ammonia (50:50:25:10). The R_f values of the seven standard dyes are found in Table I. Table II lists dyes found in common "foods."

Food for Thought

- Calculate R_f values for all colored components.
- Which dyes were in your food sample?
- Would a change in composition of the developing solution change the R_f values? Why or why not?
- Discuss how you might analyze a food for an artificial sweetner using a modification of this procedure.

Table 1. R_f Values for FD&C Dyes

Dye	R_f Value *	Comment
Red 3	0.77	Not used extensively
Red 40	0.65	Most common red dye
Yellow 5	0.47	Bright yellow
Yellow 6	0.57	Bright orange
Blue 1	0.57	Most common blue dye
Blue 2	0.61	Not commonly used — fades rapidly in light
Green 3	0.61	Not used extensively

*The R_f values may vary depending upon the activity of the silica, but their relative values should remain constant.

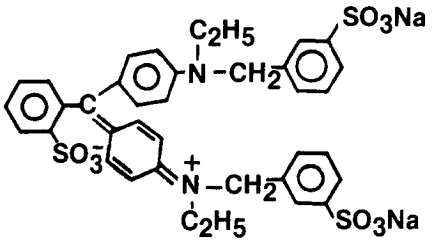
Lab Work Sheet

Table II. FD&C Dyes Present in Food Products

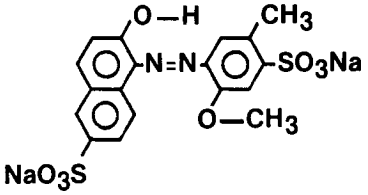
Food	FD&C Dye
Red cough medicine	Red 40, Yellow 6
Pickle brine	Yellow 5
Orange soda	Yellow 5 and 6
Grape soda	Red 40, Blue 1
Red mouthwash	Red 40
Green mouthwash	Yellow 5, Blue 2
Yellow food color	Yellow 5
Red maraschino cherry juice	Red 40

Structures of FD&C Dyes

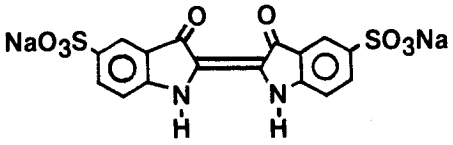
BLUE 1



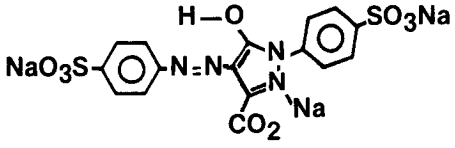
RED 40



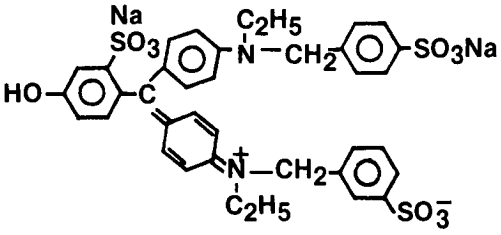
BLUE 2



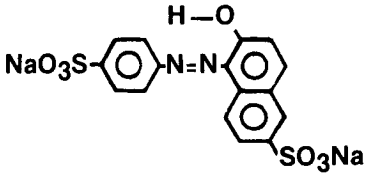
YELLOW 5



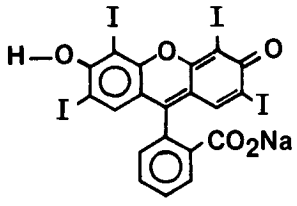
GREEN 3



YELLOW 6



RED 3



References

(1) "Separation and Identification of Some FD&C Dyes by TLC", McKone, H.T., Nelson, G. J., *J. Chem. Educ.*, **53**, 722, (1976).

(2) "Identification of FD&C Dyes by Visible Spectroscopy", McKone, H.T.,*J. Chem. Educ.*, **54**, 376, (1977).

(3) "Red Food Coloring — How Safe Is It?" *Consumer Reports*, **38**, 131 (Feb. 1973).

(4) "Countdown on Color Additives", Hopkins, H., *FDA Consumer*, **10**, 5, (1976).

(5) "Behavioral Disturbances Learning Disabilities and Food Additives", Feingold, B., *Chemtech.*, **5**, 264, (1975).

(6) "Handbook of Food Additives", 2nd ed, Furia, T.E. (editor), The Chemical Rubber Co., Cleveland, OH, (1972).

(7) "Additives for Eye Appeal", Damon, G. E. and Janssen, W.F., *FDA Consumer*, 7, 15, (1973).

(8) "High Pressure Liquid and Thin Layer Chromatography of Synthetic Acid Fast Dyes in Alcoholic Products", Martin G.E., *et al., J. Assoc. Off. Anal. Chem.*, **61**, 908, (1978).

Instructor's Notes (con't.)

Rapid Sample Preparation", pages 4 and 5 before using a cartridge.

1) Use a syringe to prewet a SEP-PAK C_{18} Cartridge with 3 ml of 2-propanol.

2) Flush 5 ml of 1% acetic acid through the cartridge to improve adsorption of the dye on the C_{18} packing.

3) Flush 1 ml of acidified food sample through the cartridge — discard the eluate (see Note 2).

4) Flush with 2-3 ml of 13% 2-propanol, collecting only the colored portion (which may contain Blue 2, Yellow 5 and 6, and Red 40).

5) Flush with 2 ml of 50% 2-propanol, collecting only the colored portion (which may contain Red 3, Blue 1, and Green 3).

6) The eluates from Steps 4 and 5 will be used for chromatographic analysis.

TLC Analysis

Spot a TLC silica plate with some of the extracted dye and develop with a solution of n-butanol/ethanol/water/concentrated aqueous ammonia (50:50:25:10).

Results

Table I lists the R_f values for standard dyes.

Summary

The identification of FD&C dyes by SEP-PAK Cartridge isolation and TLC analysis is an instructive means of introducing the theory of chromatography to students. The SEP-PAK C_{18} Cartridge isolation shortens the preparative procedure considerably, uses fewer chemicals, and is less hazardous than standard methods. This experiment can be extended to include High Performance Liquid Chromatographic (LC) analysis. Information on the LC analysis of FD&C dyes may be obtained from Waters Associates, Inc., University Marketing Division, Maple Street, Milford, MA 01757.

Note 1: Dyes may be purchased from chemical supply houses or easily extracted by a SEP-PAK C_{18} Cartridge from "foods" (particularly mouthwashes) that list specific colors on their labels.

Note 2: To insure retention of the dye on the column, the food sample should be acidified (one drop of 1% acetic acid) before analysis. In this way, the sodium sulfonate dyes are converted to the free acid forms which are adsorbed more strongly on the "reverse phase" C_{18} packing.

- For certified food colors, dilute 3 drops of dye with 1 ml of water; for solids, such as gelatin products, dissolve approximately 0.1 g of sample in 3 ml warm water and filter if necessary.

- The dyes in alcohol-based matrices such as mouthwashes are retained better on the SEP-PAK Cartridge if the liquid is acidified and diluted 1:1 with water before isolation.

Note 3: The R_f values may vary depending upon the activity of the silica; however, the relative values should remain constant. Other developing solutions-*iso*-butyl alcohol/methanol/water/concentrated aqueous ammonia (75:25:25:10) result in a separation similar to that illustrated in Table 1.

Note 4: If you have a valuable teaching experiment that could incorporate liquid chromatography, and would like to share it with others, contact the University Marketing Div. at Waters Associates, Inc.

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