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A Green Approach To Separate Spinach Pigments by Column Chromatography

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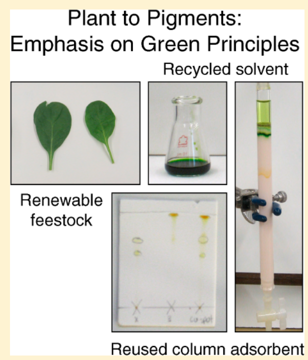
Supporting Information



ABSTRACT: Chromatography has been a fundamental technique used for chemical separation that dates back to the 1850s. Specifically, column chromatography, typically taught in introductory organic chemistry laboratories, traditionally involves the use of halogenated or harmful solvents, which novice students often overuse. This situation runs contrary to the principles of responsible chemical and waste management emphasized by the green chemistry movement. Since this movement began, conventional means of separation using harmful solvents have been modified to emphasize the need for safer, less hazardous materials and the generation of such waste. The current experiment emphasizes the green chemical principles of renewable feedstocks and recycling to minimize waste, while simultaneously introducing or reinforcing common organic techniques, including solvent extraction, column chromatography, and thin-layer chromatography for the isolation and identification of photosynthetic pigments from spinach leaves. Students gain practical experience processing plant material to isolate and identify the pigments, β-carotene, xanthophylls, and chlorophyll *a*, using the solvents hexane and acetone. This experiment was designed for use as a standalone single-session lab or, alternatively, it can be coupled with an experiment to recycle waste acetone to further emphasize sustainable practices.

KEYWORDS: *Second-Year Undergraduate, Organic Chemistry, Environmental Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Chromatography, Green Chemistry, Separation Science, Thin Layer Chromatography*

An undergraduate organic chemistry laboratory exercise is described that incorporates conventional chromatography with an emphasis on renewable feedstocks and recycling to minimize waste. Prior literature reports address the topic of pigment extraction from spinach, separation by micro- or macroscale column chromatography, and identification of pigments by either thin-layer chromatography (TLC) or ultraviolet–visible spectrophotometry.[1−5] The current work incorporates best practices from prior reports and specifically addresses laboratory activities, solvents, and practices that raise student consciousness of green chemistry principles at an early point in their organic chemistry laboratory training. The rising costs of raw materials, chemical waste disposal, and associated student fees reinforce the need to educate and demonstrate to students that chemistry can be done in a safe, economic, and sustainable manner.[6]

Students use solvent extraction followed by column chromatography to isolate β-carotene, xanthophylls, and chlorophyll *a* from spinach leaves. These components are identified by comparison to standards using TLC. Spinach chromatography is presented as a standalone exercise; however, the experiment may be performed immediately following "Recycling of Waste Acetone by Fractional Distillation."[7] In the first lab, students use fractional distillation to recycle waste acetone to achieve 85−90% purity. In the second lab, students use their purified acetone to extract spinach pigments and isolate those pigments by column chromatography.

Spinach is a renewable feedstock that serves as the raw material for extraction, isolation, and characterization of pigments. Upon drying, the residual waste spinach is a nonhazardous solid that offers inexpensive disposal and safe handling. Spinach is affordable and the pigments are readily extractable using acetone, a common, cost-effective, non-halogenated organic solvent.

The use, exposure, and disposal of hazardous organic solvents has long been a concern for organic chemists.[8] Most labs currently use a green approach to column chromatography to separate pigments, but examples of halogenated solvents can still be found. A survey of the solvents used to extract, separate, and identify pigments from spinach ranges from hazardous (chloroform and methylene chloride)[9,10] to moderately hazardous (cyclohexane, isooctane, petroleum ether, diethyl ether, hexanes, and pentane),[1−5] to less hazardous (ethyl acetate, isopropanol, *n*-propanol, methanol, and acetone).[1−5] Hazards include flammability, volatility, neurotoxicity, and, in the case of the halogenated solvents, carcinogenicity. To minimize exposure to and disposal of hazardous solvents, this experiment and others like it limit use to hexanes and acetone.[11−13]

The green principle of recycling may be introduced by instructing students to use acetone they purified the prior week by fractional distillation to extract the pigments from spinach.[7] Alternatively, the alumina adsorbent used for pigment

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separation by column chromatography may be collected and recycled for reuse.

■ EXPERIMENT

This laboratory experiment is performed using Williamson microscale glassware to minimize student exposure to chemicals and limit waste.[14] The basic steps of the lab are shown in Figure 1: (A) boiling spinach leaves in a water bath, (B) drying the leaves, (C) acetone extraction of plant pigments, and (D) column chromatography separation of pigments. The verification of pigment identity is performed by comparison to pigment standards using TLC and R_f comparison. At the end of the laboratory experiment, the acetone and alumina are collected and recycled.

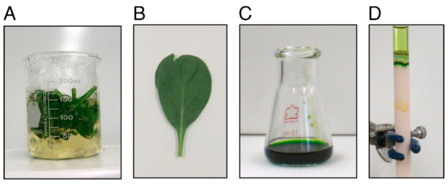

Figure 1. Preparation of spinach for column chromatography: (A) spinach leaves in boiling water, (B) dried leaves after water bath, (C) extracted pigments in acetone, and (D) pigment separation by column chromatography.

■ HAZARDS

Acetone and hexanes, or mixtures thereof, are skin and lung irritants. Hexane is a neurotoxin. Usage of these solvents should be restricted to a hood to avoid exposure to harmful vapors, and protective gloves and goggles should be worn at all times. All organic solvents and vapors are extremely flammable; no open flames should be used in the laboratory when this experiment is performed. Caution should be taken when handling alumina. Alumina is harmful if inhaled and may cause irritation to skin, eyes, and respiratory tract. Alumina should be handled with proper personal protection including safety goggles and gloves. Caution is recommended when dealing with the TLC spotters as they may cause lacerations to the skin and eyes. TLC spotters can also cause contamination of the skin and eyes. All glass material should be handled with proper personal protection including safety goggles and gloves.

■ DISCUSSION

This experiment was done as the second experiment in the organic laboratory curriculum. The first experiment was the "Recycling of Waste Acetone by Fractional Distillation."[7] In the first experiment, students learned the green principles of minimizing chemical exposure, recycling, and reuse of waste solvent. In the second experiment, the recycled waste acetone was used for spinach pigment extraction. The alumina waste (with sand) was collected in a bin in the hood. The alumina was washed with recycled acetone. The acetone was collected for purification by distillation. The alumina/sand mixture was dried in an oven overnight followed by separation from the course, larger particle-size sand by filtration through an 80-mesh sieve. The hexane/acetone mixed solvent, used for chromatography, was collected in a nonhalogenated waste container in the hood. This waste solvent was also retained for recycling by distillation.

The extraction and identification of pigments from spinach experiment was completed, by over half of the 144 students surveyed, within 2 h, whereas a small minority of students required the full laboratory period (3 h 40 min). The pigments extracted from spinach provided a visual means to monitor separation and elution by column chromatography. Using a mobile phase of 100% hexane, the first band to pass through the column was β-carotene, which had a yellow to orange color. Once the β-carotene band had been collected, the eluting solvent was changed to 90:10 hexane/acetone (v/v) to elute the pigment consisting of a yellow band of xanthophylls. Upon collection of the second band, the eluting solvent was changed to 80:20 hexane/acetone (v/v) to elute the remaining polar pigments consisting of a first band for blue-green chlorophyll *a* and a second band of bright green chlorophyll *b*. Chlorophyll *b* was typically not collected for analysis, although it could be collected if an instructor chose to have students do so. Figure 1D shows the appearance of the column approximately halfway through the elution of β-carotene and xanthophylls (yellow bands, just above the clamp) and toward the beginning of the blue-green chlorophyll *a* elution (top of column).

Spinach pigment TLC R_f results for the three spinach pigments isolated by column chromatography and identified by TLC using a 60:40 hexanes/acetone solvent system in the developing chamber are given in Table 1. The data showed sufficient difference in R_f values between β-carotene, xanthophylls, and chlorophyll *a* for students to unambiguously identify each pigment. Confirmation of pigment identity was easily performed by use of β-carotene and xanthophyll standards and color recognition (see the instructor notes in the Supporting Information).

Table 1. Average R_f Values and Observed Color for Spinach Pigment Extracts Collected by Column Chromatography and Identified by TLC

Spinach Pigment	R_f[a]	Color[a]
β-Carotene	0.94–0.98	Yellow to orange
Xanthophyll	0.80–0.86	Yellow
Chlorophyll *a*	0.43–0.48	Blue-green

[a]Data obtained from 48 students in two sections of organic lab.

■ SUMMARY

This experiment used solvent extraction, column chromatography, and thin-layer chromatography to extract, isolate, and identify plant pigments, while simultaneously introducing the green principles of renewable feedstocks and recycling to minimize waste.

■ ASSOCIATED CONTENT

Supporting Information

Student laboratory procedure; instructor notes; teaching assistant checklist; a PowerPoint presentation containing two video demonstrations of column chromatography and thin-layer chromatography. 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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