Isolation Of Vanillin From Vanilla Extract By A Combination Of Flash And Prep HPLC With The Gilson PLC 2250

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Abstract

Many phenolic flavonoids found in plants have shown promise as therapeutics for the treatment and management of many diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases. Vanillin, primarily known as the principal flavor component of vanilla extract, has been shown to exhibit some of these bioactive properties. Utilizing the dual flash and prep HPLC capabilities of the Gilson PLC 2250, vanillin was easily and rapidly isolated from all-natural vanilla extract. With the system configured for flash injection, 1 mL of all-natural vanilla extract (10x) was injected onto a 12 g Silicycle SiliaSep™ HP flash column. Using a hexane-ethyl acetate isocratic method, the vanillin-containing fraction was collected, with additional wavelength monitoring available using the four channel diode array detector. The vanillin-containing fraction was concentrated and the system was easily reconfigured for prep injection with a single valve switch. Using a 1mL injection on the preparatory configuration of the PLC system, the sample was further purified by injection onto a C18 preparatory HPLC column and isolated using a water-methanol gradient. Peak purity was subsequently confirmed by TLC using a hexane/ethyl acetate development system.

Purification of Vanillin using the Gilson PLC 2250 Purification System

Figure 1. Vanillin Purified from Crude Extract Using Flash and HPLC on the Gilson PLC 2250. Flash chromatography was used to clean up a sample of all natural vanilla extract (McCormick) prior to purification through preparative HPLC. Both chromatographic processes were completed on the Gilson PLC 2250, which allows quick and easy switching between flash HPLC. The Gilson Glider Prep software controls an Electronic Injection Valve, Quaternary Solvent Valve, and Column Switching Valve, along with an ECOM 06DAD 600 Four Wavelength UV-Vis (200 – 600 nm scan, 280 nm, 321 nm, and 347 nm) detector.

Step 1: Flash Clean up of Vanilla Extract

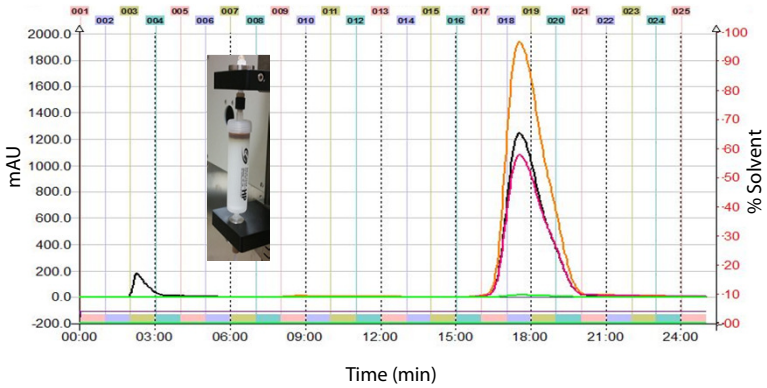

Figure 2. Clean up of Vanilla Extract by Flash Chromatography. Crude vanilla extract (1mL) was injected onto a flash column (Silicycle SiliaSep™ HP, 12g), using an isocratic method (84:16 Hexane:Ethyl Acetate) at 10mL/min. The flash column was equilibrated for 5 minutes prior to injection. Fractions were collected every minute (10 mL each) for 25 minutes. Fractions 17-20 were analyzed by Thin Layer Chromatography (TLC) for the presence of vanillin.

Fraction Analysis by Thin Layer Chromatography

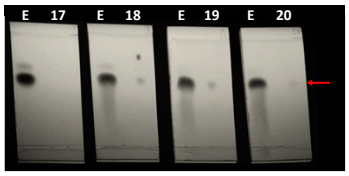

Figure 3. Thin Layer Chromatography used to detect Vanillin in Flash Fractions. TLC was employed throughout the experiment to test chromatography conditions and to monitor the presence of vanillin in fractions. In this figure four fractions (fractions 17 -20 from Figure 2) were each separated on a TLC plate next to the vanilla extract sample (E). Fractions 18-20 contained vanillin (red arrow) and were combined, evaporated, and subjected to preparative HPLC as shown in Figure 4.

Step 2: Purification of Vanillin by Preparative HPLC

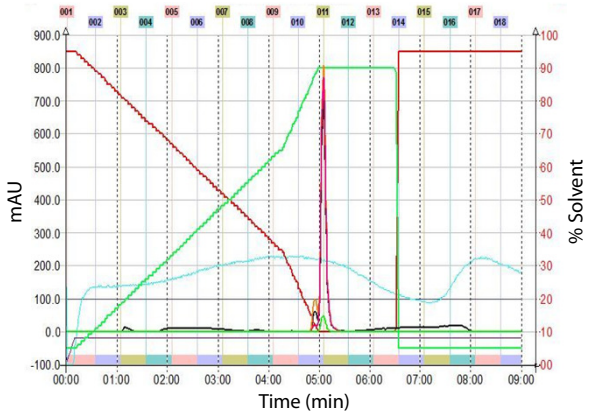

Figure 4. Purification of Vanillin by Preparative HPLC. Vanillin containing flash fractions (confirmed by TLC, Figure 2) were concentrated with nitrogen, resuspended in 5mL mobile phase, and injected onto the prep column (Phenomenex Luna C18(2), 21.2 x 50 mm, 5 micron, 100 A, Axia-packed) at 20 mL/min. Mobile Phase: A = Water + 0.1% Acetic Acid; B = Methanol. The prep column was equilibrated for 5 minutes (5%B) prior to injection. A gradient (0-4.25min: 5%-65%B, 4.25-5min: 65-90% B, 5-6.5min: 90% B, 6.5-6.6min: 90-5% B, 6.6-9min: 5% B) was used to retain and elute the vanillin. Fractions were collected every 30 seconds (10 mL each) for 9 minutes. Fraction 11 contained purified vanillin. Peak Spectra: Orange = 280 nm, 321 nm = Magenta, and 347 nm = Green)

UV-vis Scan of Vanillin Peak During Preparative HPLC

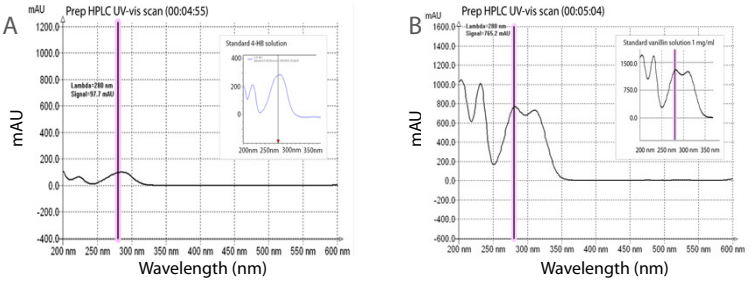

Figure 5 . Prep HPLC UV-vis scan. Flash fractions 18-20 were pooled and concentrated prior to prep HPLC. UV-vis scans (200 – 600 nm) are shown. A) Taken at 4:55 min which is toward the end of the collection of fraction 10. The inset shows a UV-vis of a standard solution of the common contaminant 4-Hydroxybenzaldehyde. B) Taken at 5:04 min which is toward the beginning of fraction 11. The inset shows a UV-vis of a standard vanillin solution.

Summary

- Gilson Glider Prep software allows the user to quickly create flash and preparative HPLC methods, monitor run-time conditions, and generate reports for easy and complete recordkeeping.
- Vanillin was isolated from all-natural vanilla extract using the Gilson PLC 2250, demonstrating the utility of the Gilson PLC 2250 in natural product purification with potential for product scale-up.
- The combination of flash and preparative chromatography available on the Gilson PLC 2250 enables bulk flash purification and refined preparative HPLC on the same platform.

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