Information Rich Flash Chromatography I: Mass Directed Fractionation

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Chromatography Application Note AN93

Overview

Mass spectrometers (MS) have long been used for detection and collection when coupled with liquid chromatography. Nearly universal detection of compounds is achieved when MS is combined with UV detection. The introduction of lower-cost mass spectrometers allows automated flash chromatography to enjoy the advantages of mass directed purification. These include collection of only the desired product utilizing a targeted molecular weight as a trigger. For natural products, known compounds can be identified and ignored during purification allowing the chemist to isolate only those materials with molecular weights that would suggest they are novel. Mass-directed purification of synthesized compounds is also demonstrated.

Background

There is a need for a flash chromatography detector that allows researchers to identify compounds as they are purified so time is not wasted concentrating the product of a side reaction. Mass spectrometers are useful as detectors because the molecular weight of a synthesized compound is known. Used in conjunction with UV detection, specific compounds can be collected without additional confirmation of the compound identity after elution. Likewise, in natural products, certain species are known to produce certain compounds[1]. Knowledge of these molecular weights allows the user to screen potentially interesting compounds during elution.

The Combi*Flash*® Rf⁺ PurIon system allows Flash Chromatography- Mass Spectrometry (FCMS) to be performed on a routine basis. Typically, flash systems have been used with mass spectrometers "borrowed" from other LC systems[2]. This creates issues with correlating data to control the mass spectrometer, UV detector, and fraction collector at all flow rates delivered by the chromatography system. Flash purifications reported to date have used reverse-phase solvent systems[2]; we report purification of compounds using normal phase solvents.

1. Hou, Z.; Luo, J.; Kong, L. Medium-Pressure Liquid Chromatography Coupled to Electrospray Ionization Mass Spectrometry for Separation and On-Line Characterization of Flavonoids from Asparagus officinalis. *Chromatographia* 2009, 70, 1447-1450.
2. Strum, J.S.; Aldredge, D.; Barile, D.; Lebrilla, C.B. Coupling flash liquid chromatography with mass spectrometry for enrichment and isolation of milk oligosaccharides for functional studies. *Anal. Biochem.* 2012, 424, 87-96.

Experimental and Results

All experiments were run on a Combi*Flash* Rf⁺ PurIon system controlled by Peak*Trak*®. Pure chemicals were obtained from Sigma-Aldrich (St. Louis, MO); Solvents were ACS grade from VWR Scientific (Radnor, PA). The carrier solvent (used to dilute and deliver the sample to the mass spectrometer) was 0.1% formic acid in methanol.

Other details are described in each section below.

Figure 1: Combi*Flash* Rf⁺ PurIon

Single Ion Current (SIC)

Single ion current (SIC) is an experimental run where the mass spectrometer is programmed to generate a data trace from a narrow range of mass-to-charge ratio (m/z) values, typically 1 or 2 Daltons. This generates a trace specific for a molecular weight and allows purification of a single compound. As a demonstration, caffeine and theophylline were dissolved in methanol and adsorbed onto Celite 545 (1:9 w/w alkaloids: Celite) to make a 10% sample. To determine the appropriate molecular weight to collect caffeine, the Mass Spectrometer Method Development function in Peak*Trak* was used. The sample was made 0.05 mg/mL and injected into the system.

The mass spectrum from the method development screen verified that the [M+H]+ ion for caffeine was seen (as opposed to a major fragment or another adduct). Since the method development program allows a user to directly inject a sample into the mass spectrometer, it is also useful for monitoring the progress of a reaction.

The alkaloid/Celite mixture 0.3 g of (30 mg alkaloids, 0.12% column load each compound) was placed in a 5 g solid load cartridge (PN 69-3873-235) and run

on a 12 g Redi*Sep*® Rf silica column (PN 69-2203-312). The mass spectrometer was set to a m/z value of 195 (caffeine [M+H]+) and run as electrospray positive ion mode. The compounds were resolved with a gradient of 2 to 30% methanol in dichloromethane (Figure 2,3).

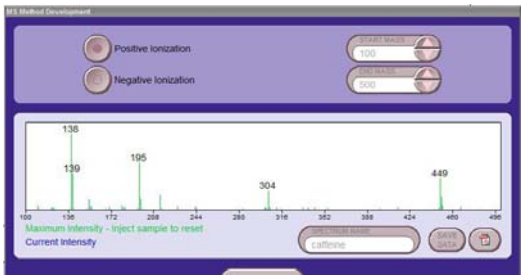

Figure 2: Method Development screen

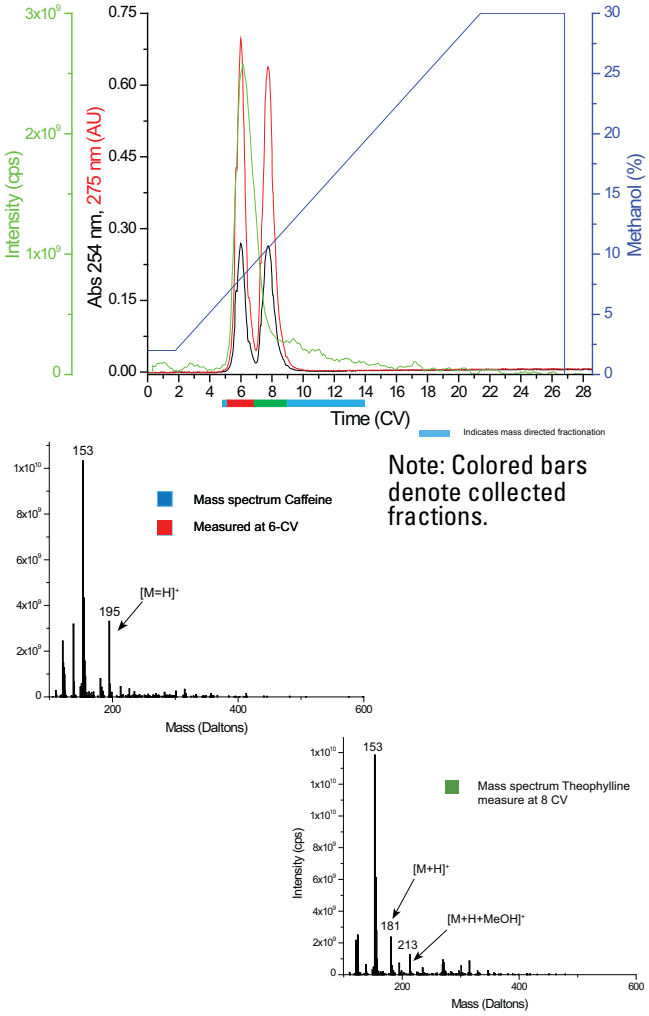

Figure 3: Purification of caffeine from theophylline. Although the MS was programmed for SIM, mass spectra are collected for all compounds.

Extracted Ion Current (XIC)

The Extracted Ion Current (XIC) was run in the same fashion as the single ion experiment (on a 12 g Redi*Sep* Rf column, PN 69-2203-312) except 4.5 g alkaloids on Celite was run (4% column load). The mass spectrometer was programmed to collect compounds within the range of 180-200 Daltons. Even with the heavy load, the mass spectrometer was able to detect both compounds (Figure 4).

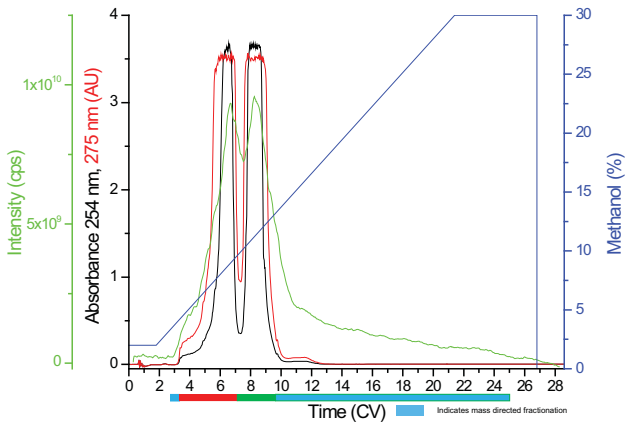

Figure 4: Purification of caffeine and theophylline by FCMS using XIC range 180-200 Daltons

Synthesis of Benzidine and Purification by XIC

Nitrobenzene (6.15 g, 50.0 mmol) was dissolved in 50 mL methanol containing an iodine crystal and placed in a 250 mL round bottom flask fitted with a reflux condenser. Magnesium powder (3.00 g, 123.4 mmol) was added. After the reaction proceeded for 10 minutes, an additional 25 ml methanol was added.

After an additional 20 minutes of reaction time, the mixture was heated to 85 °C, and an additional 2.00 g (82.3 mmol, 205.7 mmol total) of magnesium powder was added and the reaction run for an additional 30 minutes. The mixture was poured into a beaker and the flask was washed with 100 mL water which was added to the reaction mix. Glacial acetic acid (30 mL) was added with stirring and the mixture was rotary evaporated to remove the methanol. Concentrated hydrochloric acid (15 mL) was added and the mixture heated to 65 °C for 30 minutes. Concentrated ammonium hydroxide was added until the solution was basic and then extracted 3 times with ethyl acetate.

The combined extracts were dried over magnesium sulfate and evaporated to yield 4.87 g (4.60 g expected) of product. The mixture was adsorbed onto 18.74 g silica (PN 60-3874-091); 5.0 g of the silica/sample mixture (1.03 g reaction mixture) was run on a 40 g Redi*Sep* Rf silica column with a hexane/ethyl acetate gradient. The mass spectrometer detection range was set to 175-300 Daltons; the carrier solvent was methanol containing 0.1% formic acid. The recovery from the FCMS system was 0.76 g (Figure 5).

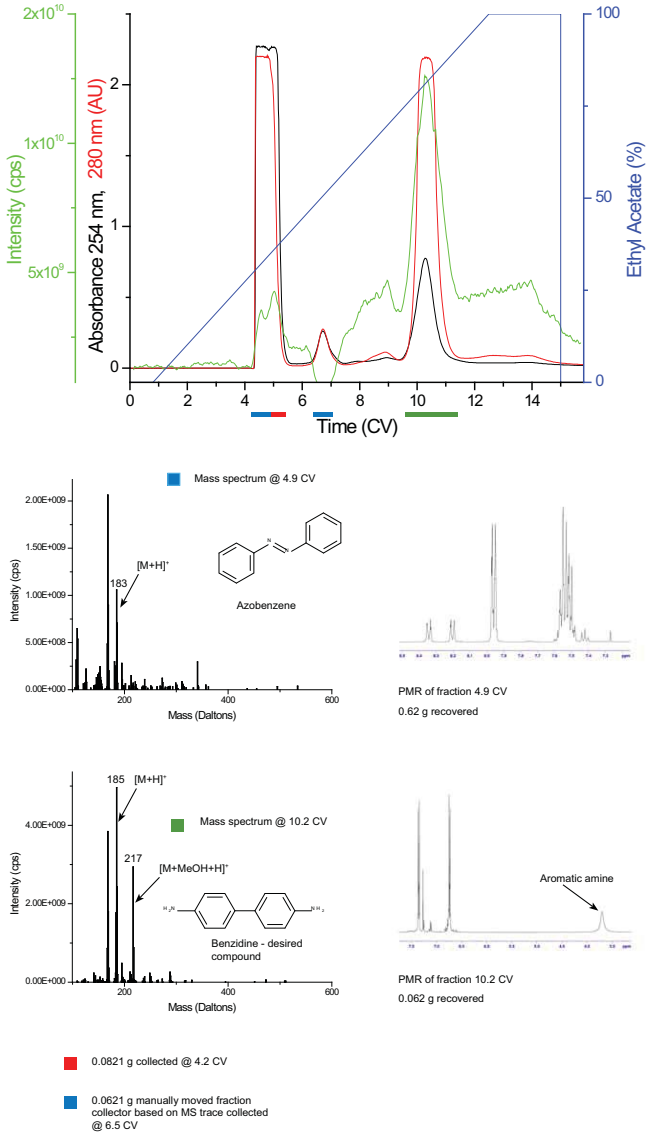

Figure 5: Purification of benzidine using XIC and real-time mass spectra, XIC 175-300 Daltons with structure assignments by MS, structure verification by Proton Magnetic Resonance (PMR). Color bars denote collected fractions.

Conclusion

FCMS is a useful tool for validating the results of a reaction as well as ensuring the desired reaction product is collected. In the benzidine synthesis, the major compound eluted is actually an oxidation product and could be ignored due to observation of the mass spectrum in real-time. The ion trace can be run as a "Single Ion Current," allowing a single component to be collected or as a mass range allowing several compounds of interest to be fractionated.

All purifications were performed with ACS grade solvents with standard flash columns. Nothing was found from these columns or solvents that interfered with the purification by mass spectroscopy. The Combi*Flash* Rf$^+$ PurIon system acquires a full range mass spectrum from 50-1200 Dalton even if SIC is run allowing evaluation of interesting peaks during the purification.

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