

Automated 2-D HPLC Using Trap Columns for the Fractionation, Isolation and Screening of Natural Products

Application Note 227

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Introduction

One aspect of 2-D chromatography that is being explored uses small reverse-phase columns to trap the eluent from the ion-exchange column at defined timed intervals. Components are then eluted from the “trapping” reverse-phase columns onto an analytical reverse-phase column with fraction collection for further analysis using techniques such as mass spectroscopy and bioactivity assays.

All types of 2-D chromatography have an attractive characteristic: they enhance the separation of a mixture that is not possible to separate using 1-D chromatography. While manual 2-D chromatography can be a difficult process, automation of the procedures alleviates the variables and increases repeatability and throughput.

A resurgence in natural product evaluation for biological activity is evident based on articles noted in scientific journals and the other media. One such natural product is Mugwort (*Artemisia vulgaris*), or black sage, whose components have been associated with digestive stimulant, diuretic and nerve tonic. The main chemical components of Mugwort extracts are alpha, beta-thujones, 1, 8-cineole, camphene and camphor, which have associative affects of neurotoxicity and abortifacient.

Materials & Methods

Instrumentation and Software

215 Liquid Handler: equipped with 819 Injection Module with 500- μ L injection loop, low-mount fraction collection valve

(3) VALVEMATE[®] Valve Actuator: equipped with 7610 Rheodyne (1), 10-port, 2-position; and 7060 Rheodyne (2), 6-position, selector valve

(2) UV/VIS Detector: high-pressure analytical flow cell (1), 5-mm; and analytical flow cell (1), 5-mm

(2) 32X HPLC Pumping System

307 Isocratic HPLC Pump: 5-mL pump head

UniPoint[™] System Software

Accessories and Reagents

Columns

- Ion-exchange: AX300 250 x 4.6 mm, 6 micron
- Trap reverse-phase: (5) Aquasil C-18 30 x 4.6 mm, 3 micron
- Reverse-phase: Beta Basic C-18 100 x 4.6 mm, 3 micron

Mobile Phase (ion-exchange)

- (A) 20 mM tris acetate, pH 7.8
- (B) 20 mM tris acetate, 100 mM sodium acetate
- Gradient: 0–50% B in 25 minutes, 50% B from 25–27 minutes

Mobile Phase (reverse-phase)

- (A) water, 0.1% TFA
- (B) ACN, 0.1% TFA
- Gradient: 0–100% B in 12 minutes, 100% B from 12–14 minutes

Natural Products Evaluated

- Mugwort (black sage): methanol/water extracts, 100 mg/mL of plant was extracted.

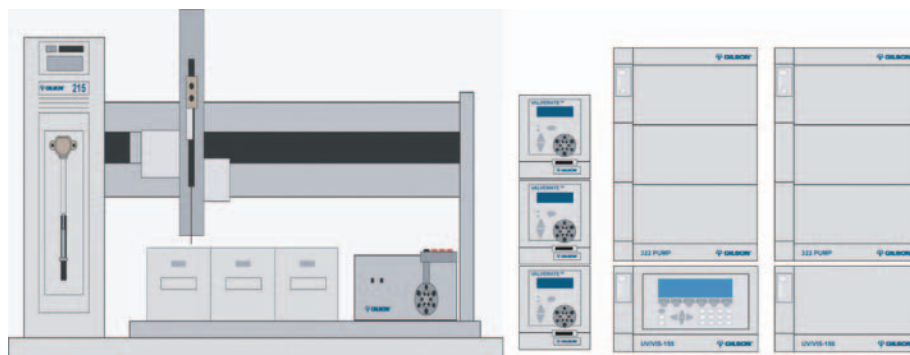


Figure 1. 2-D System illustration.

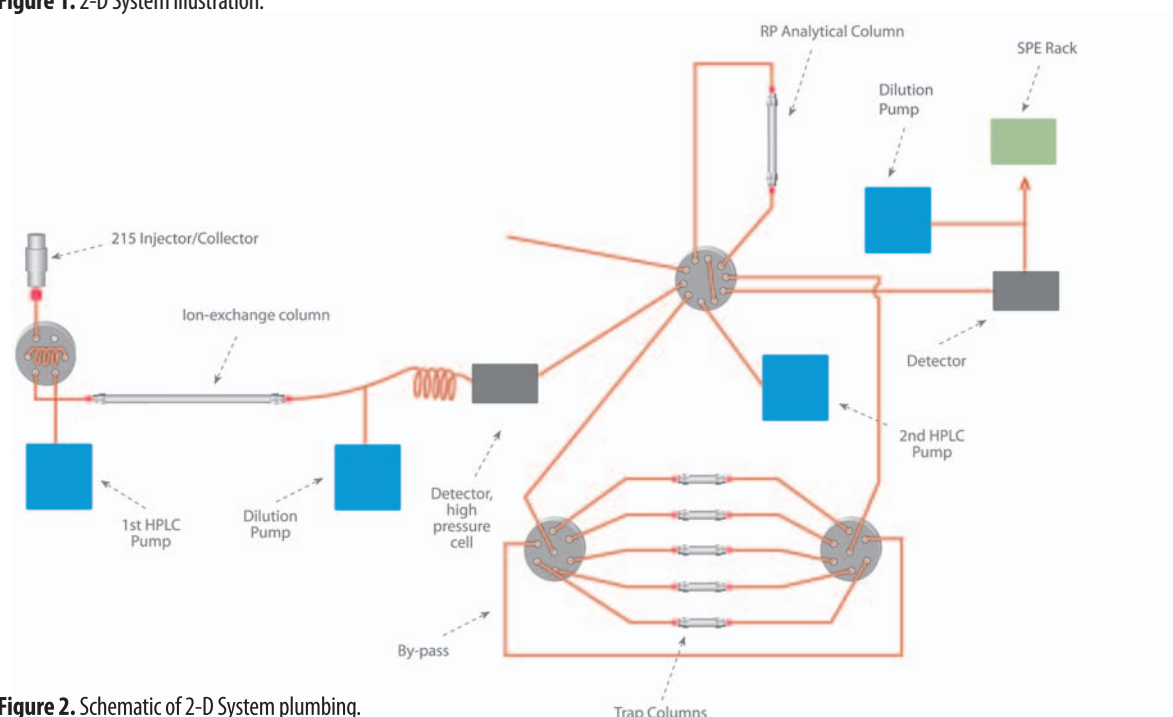


Figure 2. Schematic of 2-D System plumbing.

Methodology

- Mugwort (black sage) ground plant (flowers, leaves, stems) was extracted with water and methanol.
- The extracts were filtered with a 0.45- μm filter.
- The aqueous and organic extracts were injected onto the ion exchange column. The injection volume was 500 μL to overload the column.
- The sample was eluted through the ion exchange column.
- The column eluent prior to the detector was mixed with a constant volume (0.35 mL/min) of water from a dilution pump.
- The eluent was “trapped” onto small reverse-phase columns (5) post detector at timed intervals.
- The “trapped” RP columns were then switched on line to an analytical reverse-phase column, and the components were separated and fractions were collected. Fractions could then be analyzed via MS or other methods for biological activity.

Bidirectional System Control

- The two HPLC systems are controlled through “actions” written to the display of the 215 Liquid Handler (e.g., “ready”, “busy”).
- Two operations lists are run independently of each other to maintain sample and fraction tracking in unique data files.
- This allows each system to complete their application without delay and employ one 215 Liquid Handler for both systems to minimize required bench space.
- Collected fractions are then analyzed via selective methods (e.g., MS analysis).

Results

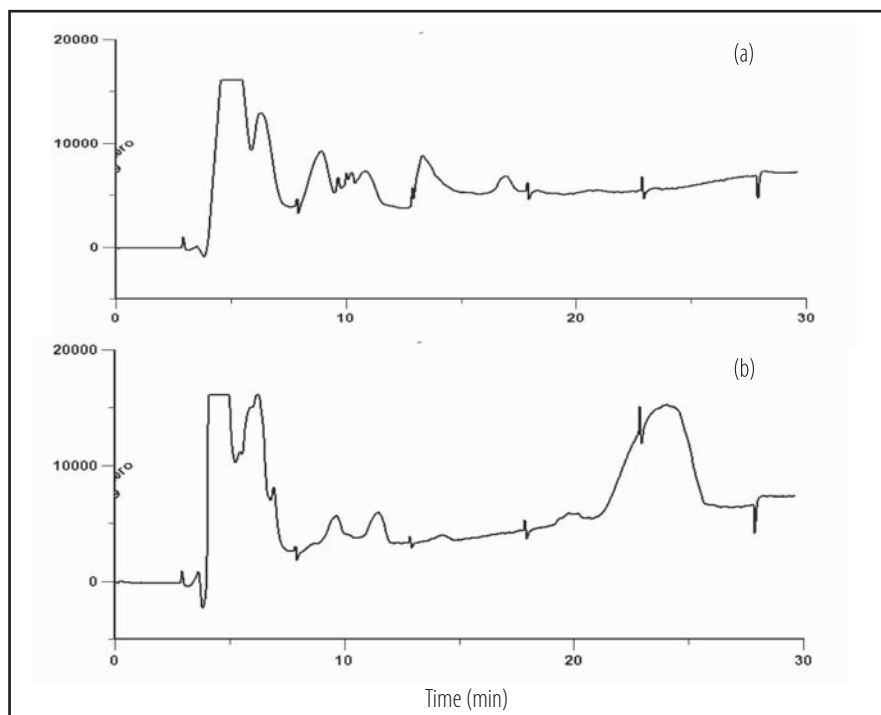


Figure 3. Ion exchange chromatograms from mugwort extractions using (a) water and (b) methanol. Spikes represent switching between the trapping columns. Column was exposed to an excess of sample for collection and post-run evaluation.

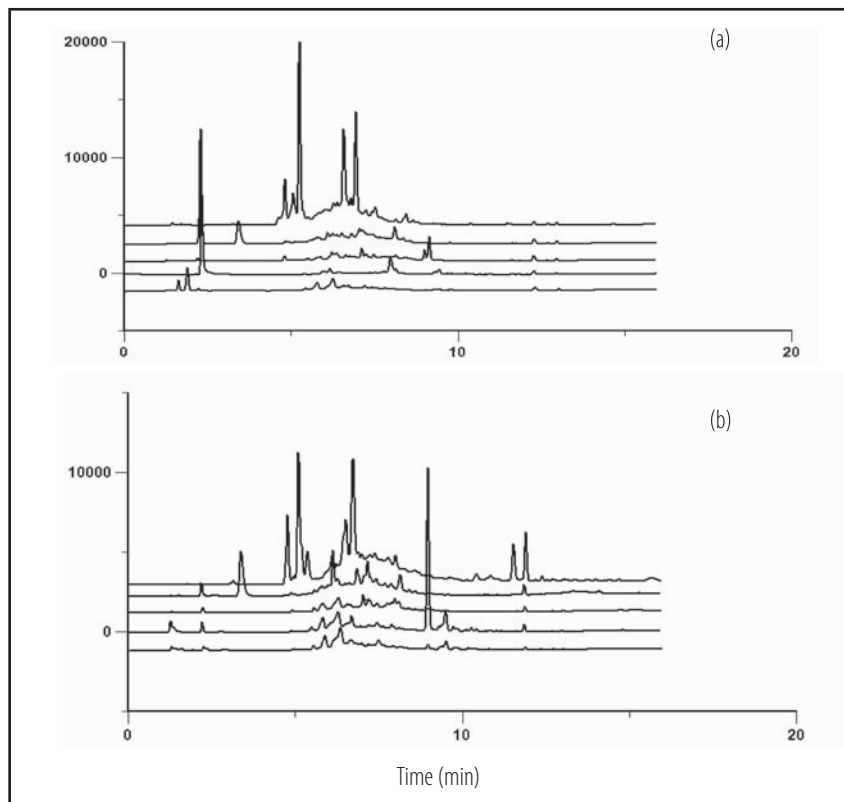


Figure 4. Overlays of the reverse-phase chromatograms from the “trapped” columns; trap 1–5, top to bottom. (a) Water extract traps and (b) methanol extract traps. Each column was switched in line with the reverse-phase analytical column and run under basic RP conditions. Fractions were collected via time for each run into microplates. Specific fractions were then analyzed via MS.

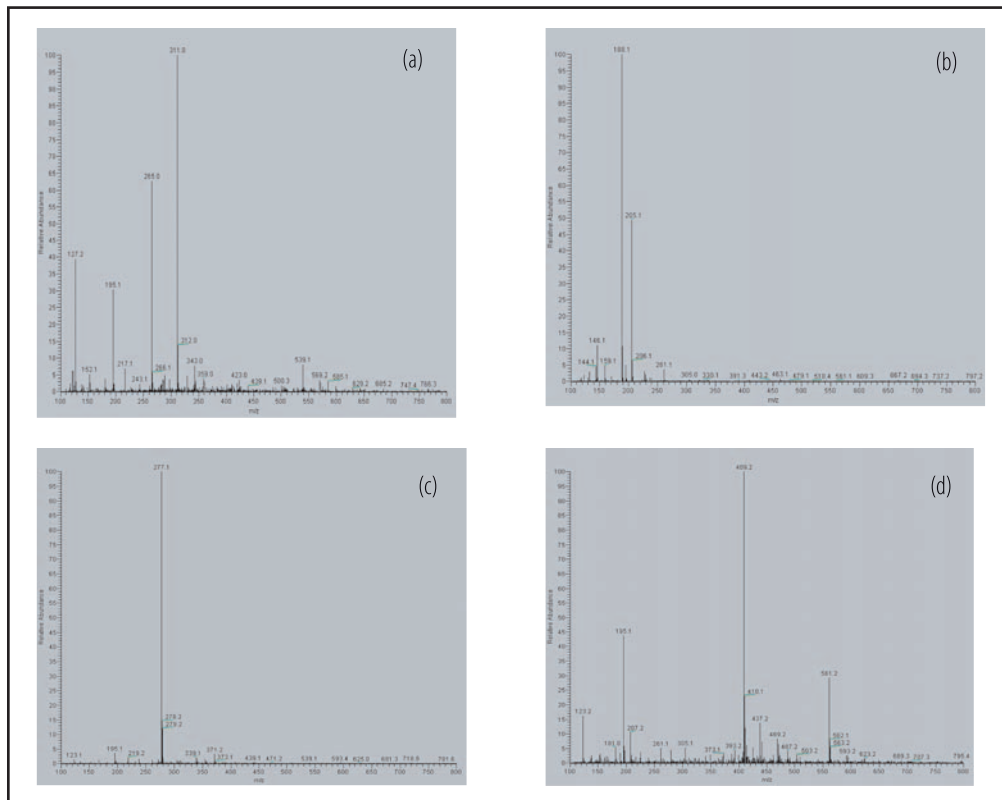


Figure 5. Mass spectral data taken on specific fractions from the methanol extract. Evaluation of the data was based on a published database for mugwort: ARS Phytochemical and Ethnobotanical (permission for use granted by Dr. Duke). Although additional evaluations and studies would need to be done to confirm all structural determinations, possibilities are offered as follows: (a) carotene-type compound, (b) coumarin/cineole, (c) coumarin (d) plant steroid-type compound.

Conclusion

2-D HPLC as presented in this application offers separation advantages for complex components such as natural products (e.g., Mugwort). Complex constituents found in natural products require tandem separation techniques which are manually challenging. The use of 2-D automated HPLC in this perspective allows for a significant amount of the natural product to be loaded onto the ion exchange column in order to circumvent the requirement for a significant initial separation—which will require adjustments for each natural product evaluated.

Employing small RP columns to trap the eluent, in conjunction with the dilution pump, reduces the salt concentration, eliminating the concern that the sample will precipitate (“salt out”) under RP conditions. Retaining the compounds from the initial ion exchange onto the small “trap” columns simplifies the 2-D reverse-phase chromatography. Extremely reproducible fractionation of the secondary RP separation deliver the components in an acceptable state for mass spectral evaluation.

Having the entire process automated on one bed layout eliminates confusion with sample transfer and sample and fraction tracking. The entire process is controlled through the software, and sample and fraction tracking data are identified and can be placed in discrete folders for future evaluation. Automation of 2-D HPLC offers a degree of consistency and throughput that enhances the process and evaluation of the components to be determined.

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