Evaluation of a Benchtop Preparative HPLC System to Determine Optimal Purification of Compounds via Reverse Phase Chromatography, Normal Phase Chromatography, and Flash Chromatography

Application Note FB0111

Keywords

Preservatives, Paraben, HPLC, Normal Phase Chromatography, PLC 2020 Personal Chromatography System, Preparative Chromatography, Purification, Resolution, trans-Stilbene Oxide, Flash, Normal Phase, Reverse Phase

Introduction

This study was presented at Pittcon 2011 held in Atlanta Georgia in March 2011.

Reverse phase (RP), normal phase (NP), and flash chromatography are selected for a variety of applications using compound purification based on a number of criteria. Not all compounds will separate adequately for the purposes of purification using just one mode of chromatography. This application demonstrates the purification of four common preservatives found in food and beverage, cosmetic, and pharmaceutical products using a benchtop system capable of performing RP, NP, and flash chromatography. Flexibility to select between RP, NP, and flash on a benchtop system assists a chemist with determining the optimal chromatography for a variety of purification applications.

Four common preservatives were injected at various mg loads using the PLC 2020 for performing RP, NP, and flash chromatography purification injections. Chromatography methods were optimized for time, separation, and fraction collection slope conditions of each preservative. Common column packing and mobile phase solvents were specifically chosen for each mode of chromatography.

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Resolution, total run time, fraction volume, fraction subdivisions per peak, peak width, and mobile phase consumption were calculated from multiple injections (n=5) using a 1 mL injection sample containing four common preservatives. Sample concentration was kept the same for all injections onto RP, NP, and flash chromatography columns. A resulting matrix was developed to assist a chemist with determining the optimal chromatography for compound purification.

Figure 1. Gilson PLC 2020 Personal Purification. System (Part No. 21130000)

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Materials & Methods

Samples and Solvents

Methyl 4-hydroxybenzoate (Sigma-Aldrich, P/N H5501-500G)
Propyl 4-hydroxybenzoate (Sigma-Aldrich, P/N P53357-500G)
4-aminobenzoic Acid (Sigma-Aldrich, P/N 100536-250G)
4-hydroxybenzoic Acid (Sigma-Aldrich, P/N H20059-500G)
Ethanol, (EMD Chemicals, P/N EX0278-6)
Methanol, (B&J, P/N 230-4)
Hexane, (B&J, P/N AH212-4)
Isopropyl Alcohol, (B&J, P/N AH323-4)
Milli-Q Water

Apparatus

Gilson PLC 2020 Personal Purification System

- 50SC pump heads
- 5 mL injection loop
- Preparative, 0.2 mm pathlength, 0.7 µL volume, quartz detector flow cell

Columns

- RP Column
  - Phenomenex, Luna 5 micron C18 (2), 50 mm x 21.2 mm, P/N 00B-4252-P0-AX
- NP Column
  - Phenomenex, Luna 5 micron Silica (2), 150 mm x 21.2 mm, P/N 00F-4274-P0
- Flash Columns
  - Macherey-Nagel, CHROMABOND® Flash RS 40SiOH 40g, P/N 732 803

Table 1. Preservative Solutions and Column Load Comparison.

	Column Load (mg)	Diluent
Methyl 4-hydroxybenzoate	0.5	RP: 60:40 Water:Methanol
Propyl 4-hydroxybenzoate	2	
4-aminobenzoic Acid	1	NP and Flash: Ethanol
4-hydroxybenzoic Acid	1.5	

Protocols

Table 2. Comparison of Reverse Phase, Normal Phase, and Flash PLC 2020 Methods.

	Reverse Phase	Normal Phase	Flash
Mobile Phase Solvents	A = Methanol B = Water	A = Hexane B = Isopropyl Alcohol	A = Hexane B = Isopropyl Alcohol
Mobile Phase Gradient %A	0–1 minutes = 38 1.4–3.6 minutes = 60 3.9 minutes = 66 4.5–5.5 minutes = 38	0 minutes = 99 7.5 minutes = 90 12 minutes = 75 12.1–12.5 minutes = 99	0–1 minutes = 100 8–13 minutes = 0 13.1–13.5 minutes = 100
Fraction Collection Conditions	Front Slope = 70 Back Slope = 60 Maximum Collection Volume per Tube = 20 mL	Front Slope = 70 Back Slope = 25 Maximum Collection Volume per Tube = 20 mL	Front Slope = 70 Back Slope = 25 Maximum Collection Volume per Tube = 20 mL
Run Time (minutes)	5.5	12.5	13.5
Flow Rate (mL/min)	25	30	30
UV Detection (nm)	254 and 280		

Figure 3. Reverse Phase Method for PLC 2020.

Figure 4. Normal Phase Method for PLC 2020.

Figure 5. Flash Method for PLC 2020.

Results

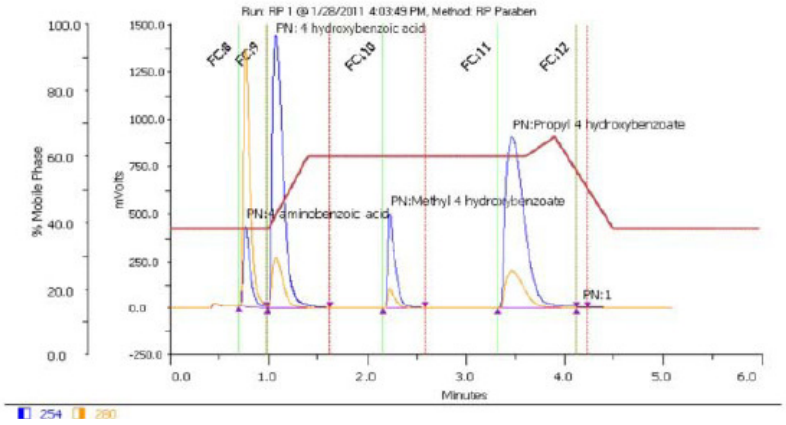

Figure 6. Reverse Phase Chomatogram.

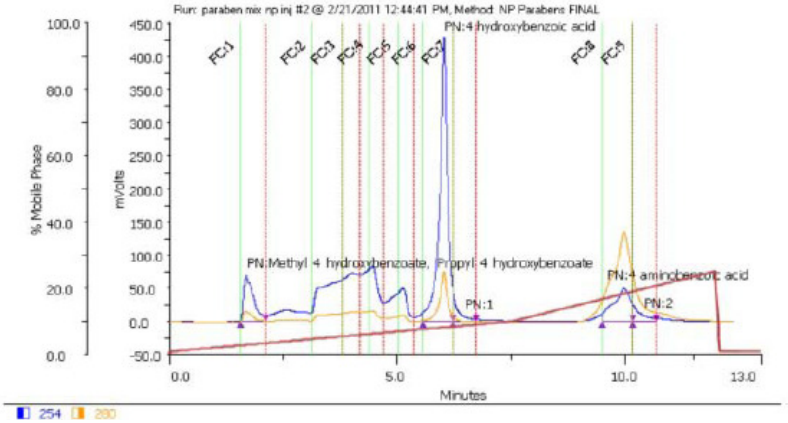

Figure 7. Normal Phase Chomatogram.

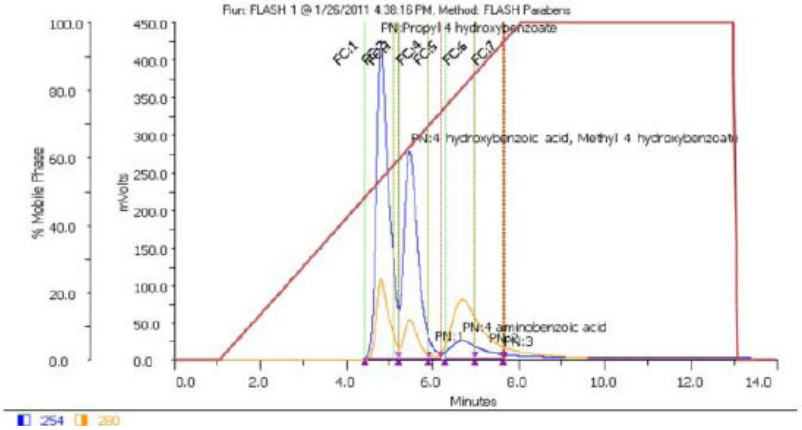

Figure 8. Flash Chomatogram.

Table 3. Resulting Matrix – Reverse Phase, Normal Phase, and Flash Purification of Four Preservative Compounds

		Reverse Phase	Normal Phase	Flash
Methyl 4-hydroxybenzoate (Average values n=5)	Resolution	2.252	NA	Note: Co-elution was confirmed with 4-hydroxybenzoic Acid
	Peak Width (min)	0.422	0.440	
	Number of Fractions Collected	1	1	
	Fraction Volume (mL)	10.6	13.8	
Propyl 4-hydroxybenzoate (Average values n=5)	Resolution	2.020	1.221*	NA
	Peak Width (min)	0.800	3.849*	0.626
	Number of Fractions Collected	1	5*	1
	Fraction Volume (mL)	20.0	57.6*	18.8
4-hydroxybenzoic Acid (Average values n=5)	Resolution	0.685	0.661	0.867
	Peak Width (min)	0.605	1.169	0.767
	Number of Fractions Collected	1	2	2
	Fraction Volume (mL)	15.4	24.9	25.4
4-aminobenzoic Acid (Average values n=5)	Resolution	NA	3.456	0.966
	Peak Width (min)	0.274	1.146	1.049
	Number of Fractions Collected	1	2	2
	Fraction Volume (mL)	7.0	34.3	33.2
Average Values per Injection (n=5)	Total Run Time|(minutes)	5.5	12.5	13.5
	Mobile Phase Consumption per Injection (mL)	137.5	375	405

* Co-elution was confirmed between Propyl 4-hydroxybenzoate and Methyl 4-hydroxybenzoate

Summary

This application demonstrated purification of four preservative compounds at different mg loads by reverse phase (RP), normal phase (NP), and flash chromatography (see Table 1). In general, purification is optimal when compound peak width is minimal. Smaller peak widths save dry-down time because fewer fraction collection peak subdivisions and lower overall fraction volumes are generated. The resulting matrix comparison of RP, NP, and flash purification of the four preservative compounds injected is provided in detail in Table 3.

Optimal peak width and resolution for the four preservative compounds was generated using RP chromatography. Both flash and NP chromatography saw some co-elution of compounds and generally larger peak widths and fraction volumes. NP chromatography and flash chromatography showed adequate resolution and peak width values for specific compounds; however, resolution was generally greater with NP chromatography.

Mobile phase consumption and total run time are also important considerations when selecting the optimal purification chromatography; helping to reduce laboratory expense for time and solvent waste. RP chromatography was the optimal method for these compounds, resulting in approximately half of the run time and solvent consumption of the NP and flash chromatography methods. NP and flash chromatography resulted in very similar solvent consumptions and total run times. A benchtop solution that allows for the flexibility to select between RP, NP, and flash chromatography for multiple applications assists with optimization of compound purification.