Application Note

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Modernization of USP Salicylic Acid HPLC Analysis Using Agilent InfinityLab Poroshell 120 Columns

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Abstract

This application note develops a method for salicylic acid drug substance and drug products analysis using HPLC equipment and superficially porous columns. Superficially porous HPLC columns such as 2.7 µm Agilent InfinityLab Poroshell 120 show comparable efficiency to sub 2 µm particles with about half the backpressure. Columns packed with these materials can be used in older HPLC instruments or in new, higher-pressure instruments for high resolution in longer column format. Additionally, since they use larger 2 µm frits commonly found in 5 µm columns, extra sample preparation is not necessary.[1]

Several stationary phases are evaluated with simple mobile phase additives on short 50 mm columns. Using five or six isocratic experiments, a retention model is built using Optichrom LC.[2] Multivariate optimization is performed within the model (resolution: 2, minimum time, pressure under 400 bar). Isocratic and gradient methods are considered.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. Superficially porous particle columns generate high efficiency at lower pressure, relative to their totally porous particle column counterparts. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column.[1] The current trend with superficially porous particles is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

As part of an effort to modernize USP monographs, modern HPLC and UHPLC methods are sought for analysis of active ingredients and impurities to replace older nonchromatographic methods such as titration. Salicylic acid is an over-the-counter (OTC) active occurring in hundreds of products in dozens of delivery forms. Given the market complexity, public standards need to contain chromatograph options to accommodate the wide variety of products and not create compliance issues.

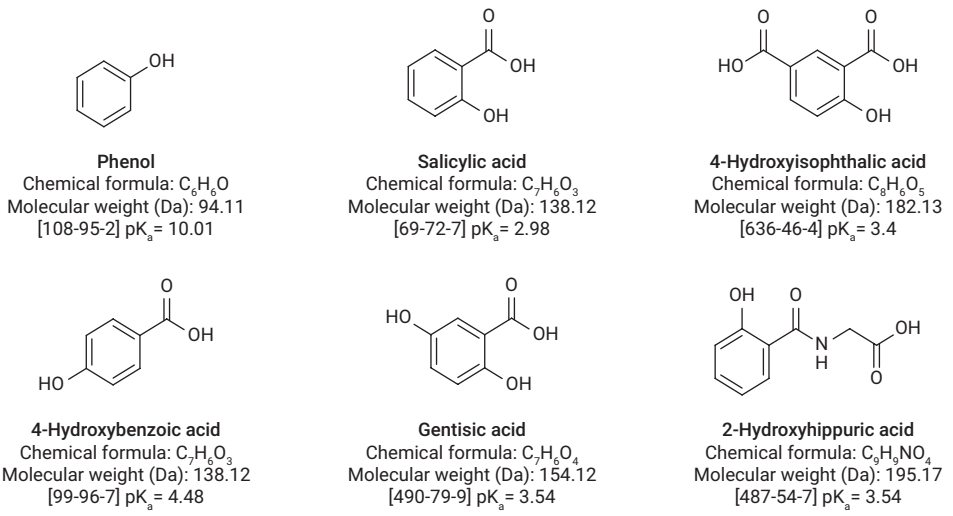
Several stationary phases are evaluated with simple mobile phase additives on short 50 mm columns. Using five isocratic experiments, a retention model is built using Optichrom LC.[2] Multivariate optimization is performed, within the model (resolution: 2, minimum time, pressure under 400 bar). Isocratic and gradient methods are considered.

Experimental

An Agilent 1260 Infinity LC was used in this work. The system was used in standard configuration, and is listed in Table 1. Salicylic acid and gentisic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA); 4-hydroxyiopthallic acid (4-HIPA, salicylic acid impurity B) and 2-hydroxyhippuric acid were purchased from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Phenol was purchased from EM Science. These compounds were prepared at 0.5 mg/mL in water except for salicylic acid, which was prepared at 1 mg/mL. Figure 1 presents structures of these compounds. Agilent InfinityLab Poroshell 120 Phenyl-Hexyl (p/n 699975-312) and Agilent Poroshell 120 SB-Aq (p/n 699975-314) 2.7 µm, 3 × 50 mm columns, as well as other columns, were evaluated. Trifluoracetic acid (TFA), acetic acid, and formic acid were purchased from Sigma-Aldrich. Methanol and tetrahydrofuran (GC/GPC grade) were purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA). A Milli-Q system (Millipore, Burlington, MA, USA) provided 18 MΩ·cm water that was passed through 0.2 µm filter. Samples of commercially available corn removal pads were examined.

Table 1. Instrument configuration details.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 SB-Aq, 3 × 50 mm, 2.7 µm, part number 699975-314 Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3 × 50 mm, 2.7 µm, part number 699975-312
Mobile Phase	Methanol: 0.1% TFA in water (varies)
Flow Rate	0.63 mL/min
Column Temperature	35 °C
Injection Volume	1 µL
Degasser	Agilent 1260 Infinity micro degasser (G1379B)
Pump	Agilent 1260 Infinity binary pump (G1312B)
Autosampler	Agilent 1260 high performance autosampler (G1367C)
Column Oven	Agilent 1290 Infinity thermostatted column compartment (G1316C)
Detector	Agilent 1260 Infinity II diode array detector WR (G7115A)



Phenol
Chemical formula: C_6H_6O
Molecular weight (Da): 94.11
[108-95-2] pK_a = 10.01

Salicylic acid
Chemical formula: $C_7H_6O_3$
Molecular weight (Da): 138.12
[69-72-7] pK_a = 2.98

4-Hydroxyisophthalic acid
Chemical formula: $C_8H_6O_5$
Molecular weight (Da): 182.13
[636-46-4] pK_a = 3.4

4-Hydroxybenzoic acid
Chemical formula: $C_7H_6O_3$
Molecular weight (Da): 138.12
[99-96-7] pK_a = 4.48

Gentisic acid
Chemical formula: $C_7H_6O_4$
Molecular weight (Da): 154.12
[490-79-9] pK_a = 3.54

2-Hydroxyhippuric acid
Chemical formula: $C_9H_9NO_4$
Molecular weight (Da): 195.17
[487-54-7] pK_a = 3.54

Figure 1. Structures of salicylic acid and related compounds.

Results and discussion

Several stationary phases were evaluated with simple mobile phase additives used to control pH. Data from experimental chromatographic runs were acquired and used to build an empirical retention model. Optichrom LC optimizes the remaining variables, as experimental data are required to build a retention model. These are collected after selecting a single column and after selecting and fixing the weak (A) and strong (B) mobile-phase components, pH, and temperature. The software is only programmed for binary mobile phase optimization; however, these can contain blends of solvents, modifiers, or buffers. The various experimental log k values for each solute are regressed against %B. As few as two values of %B may be used, but we generally use five different %B values and regress using a quadratic model. The regression coefficients are later used to predict k values of each peak as a function of %B.

Individual standards were run after enough equilibration at 5, 10, 15, 20, and 25% methanol. The data was entered in the Optichrom LC spreadsheet, and using the solver function of Excel, the best isocratic and gradient solutions for each column and condition were determined. Based on this study, isocratic conditions consisting of 15% methanol/85% water with 0.1% TFA was chosen. A gradient after the analysis was initially proposed to remove excess excipient material from the column but was not needed for this work. It may be re-added if column lifetime and pressure increase become an issue.

The best isocratic separations found used InfinityLab Poroshell 120 SB-Aq and Poroshell 120 Phenyl-Hexyl columns with 0.1% TFA in water with methanol. Separations using formic acid or acetic acid suffered from lower resolution, unresolved peaks, or poor peak shape. While these mobile phase additives are easier to use with MS, the availability of inexpensive standards and desire for low-cost analysis limit the need for MS.

A plot of selectivity versus %B was developed and appears in Figure 2B for the InfinityLab Poroshell 120 SB-Aq TFA/MeOH separation. The plot shows a flat region between 15 and 25% methanol. Use of the higher methanol concentration would speed up the separation, but may also require a faster data collection speed. To allow the use of a wider range of detectors, the lower organic mobile phase was used.

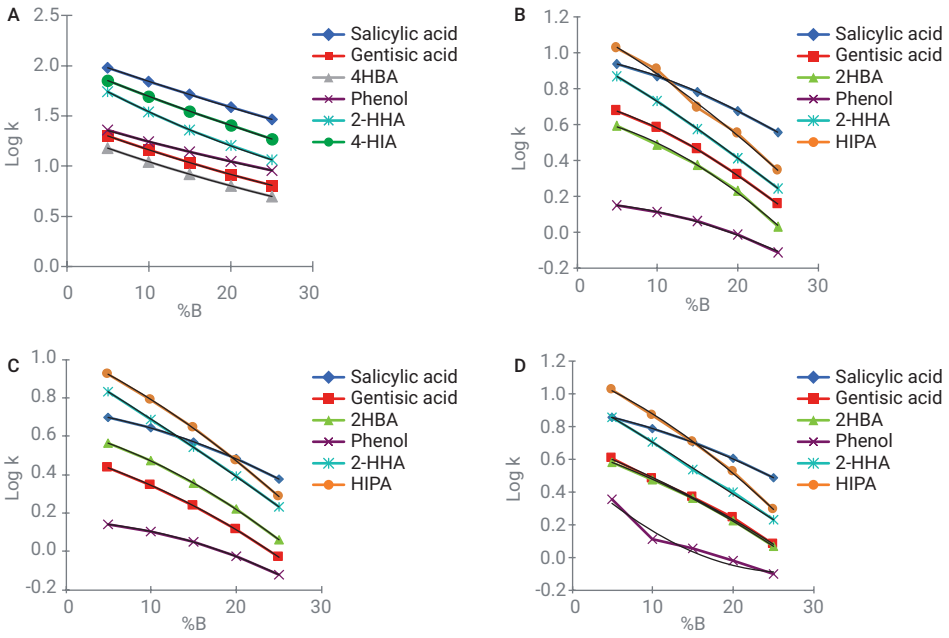


Figure 2. Agilent InfinityLab Poroshell 120 Phenyl-Hexyl and SB-Aq retention model data Log k versus %B (Optichrom LC fit data). (A) InfinityLab Poroshell 120 Phenyl-Hexyl: 0.1% TFA in water/methanol; (B) InfinityLab Poroshell 120 SB-Aq: 0.1% TFA in water/methanol; (C) InfinityLab Poroshell 120 SB-Aq: 0.1% acetic acid in water/methanol; (D) InfinityLab Poroshell 120 SB-Aq: 0.1% formic acid in water/methanol.

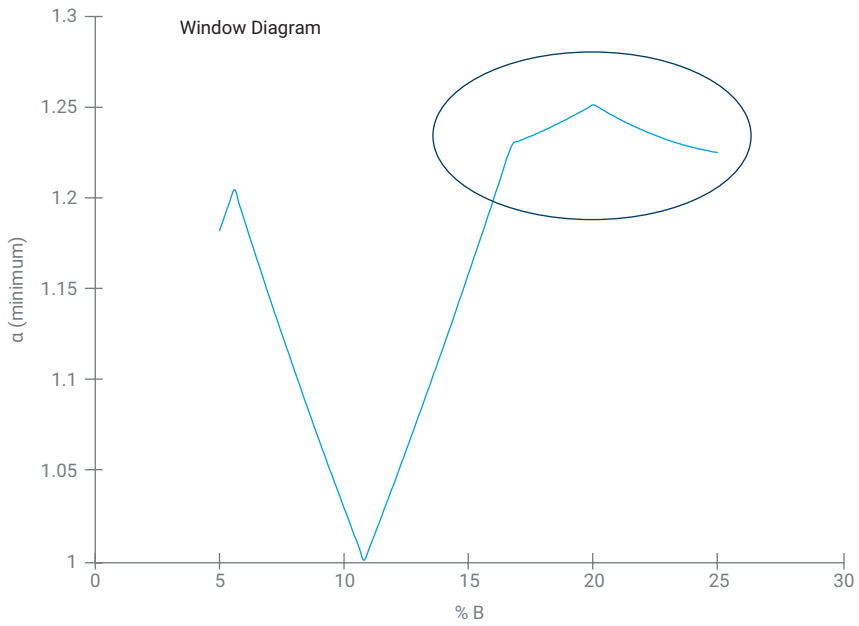


Figure 3. Resolution map (minimum α versus %B plot).

Figure 4 shows a comparison of the optimized method using the InfinityLab Poroshell SB-Aq and Phenyl-Hexyl columns. As the modeling predicted, the InfinityLab Poroshell 120 SB-Aq column delivered better Rs ≥ 2 resolution for all peak pairs (minimum Rs of 2.17 between 2HBA/GA, and Rs = 2.76 for HIPA/SA). The Phenyl-Hexyl separation at the same isocratic conditions shows a different elution order, although with salicylic acid eluting last in both cases. In addition, at these conditions, a minimum Rs of 0.89 for 2-HBA/HIPA and 1.70 for HIPA/GA was determined. The retention time for SA on the SB-Aq column is roughly half that on the Phenyl-Hexyl column, leading to shorter analysis times.

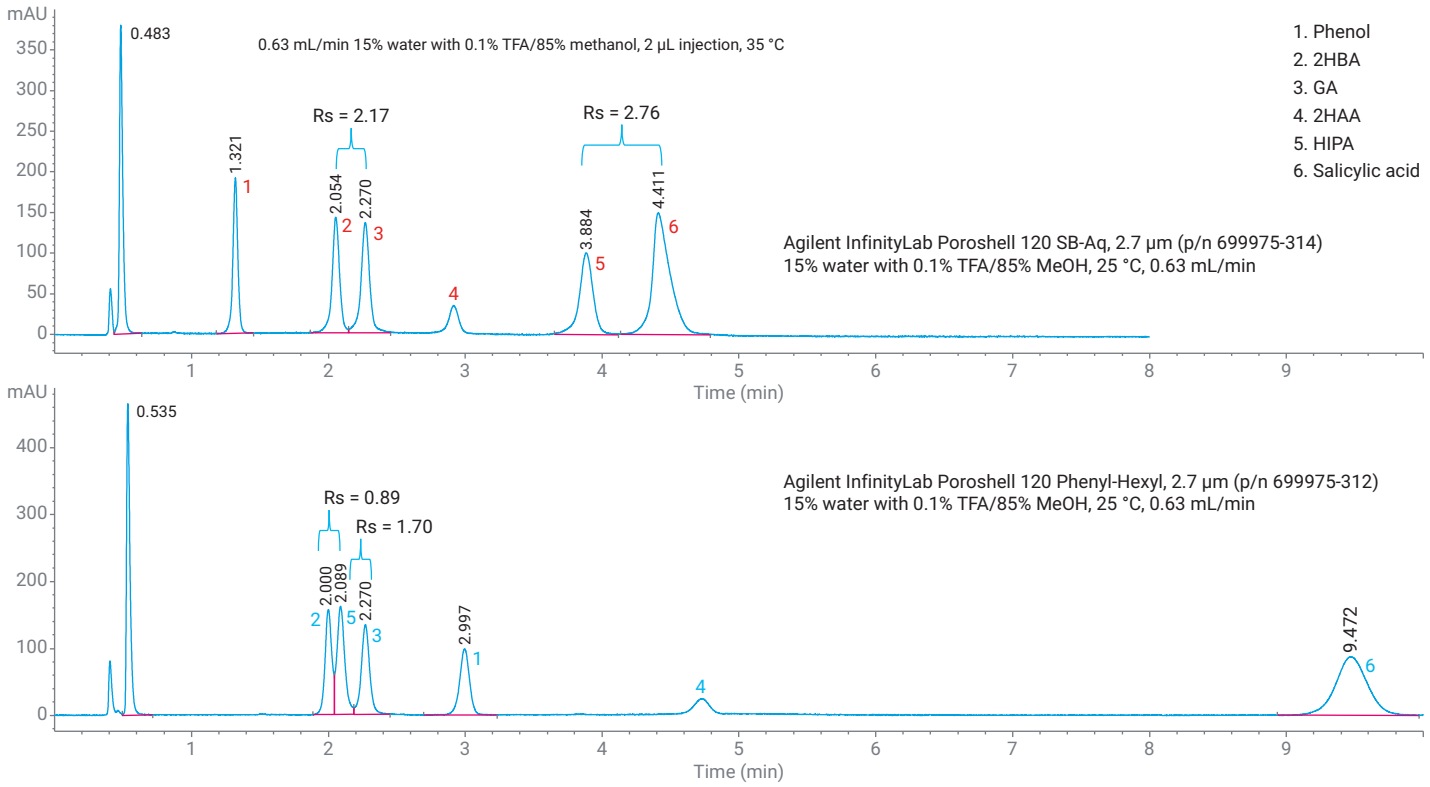


Figure 4. A comparison of the optimized isocratic method using the Agilent InfinityLab Poroshell SB-Aq and Phenyl-Hexyl columns (0.63 mL/min 15% water with 0.1% TFA/85% methanol, 2 µL injection, 35 °C).

Using the Poroshell 120 SB-Aq method conditions, a calibration curve was developed. It was found to be linear across the range of interest.

Analysis of real samples was attempted. Corn removal pads were purchased from a local pharmacy; these pads contained 40% salicylic acid in a synthetic rubber matrix. The rubber pads were carefully peeled from the bandage before extraction. Before extraction, the pad should be examined for inert bandage material. Extracting the pads in 100% water yielded inferior results, with only low levels found. Because a synthetic rubber is used in the pad, 2 mL THF was added as a first step in the extraction with dramatic improvement in extraction.

The analyses are very consistent. In the case of the water extraction, sample 3 was found floating on the top of the extraction vessel, with samples 1 and 2 completely submerged. In every case, the water extraction results yielded very low recovery. However, with the addition of THF, better extraction was found, but a calculated recovery was nearly 100%. It is important to carefully scrape off any adhesive or backing so as not to dilute the sample with inert material. No additional optimization of extraction time was carried out (due to lack of samples), but the 45-minute extraction after the initial THF soak could possibly be reduced.

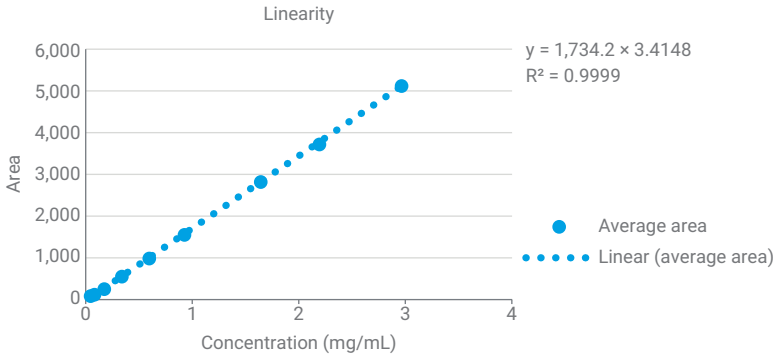


Figure 5. Salicylic acid calibration curve.

Table 2. Summary of extraction study. Two pads: extracted with 25 mL water; sonicated for 60 minutes. Recovery based on two pads weighing 50 mg consisting of 40 wt % SA.

Injection	Area	Concentration (mg/mL)	% Recovery
Sample 1, Injection1	57.59	0.9957	4.98
Sample 1, Injection 2	52.43	0.9215	4.60
Sample 2, Injection1	51.94	0.9145	4.58
Sample 2, Injection 2	51.07	0.9020	4.50
Sample 3, Injection1	38.21	0.7170	3.58
Sample 3, Injection 2	38.07	0.7150	3.56

Table 3. Summary of extraction study. Two pads: extracted with 2 mL THF; soaked for 15 minutes; 23 mL water added; sonicated for 45 minutes. Agilent InfinityLab Poroshell 120 SB-Aq, 2.7 µm (p/n 699975-314) 15% MeOH/85% water with 0.1% TFA, 25 °C, 0.63 mL/min.

Injection	Area	Concentration (mg/mL)	% Recovery
Sample 1, Injection1	1372.36	19.90	99.5
Sample 1, Injection 2	1366.53	19.82	99.1
Sample 2, Injection1	1369.58	19.86	99.32
Sample 2, Injection 2	1339.30	19.42	97.14
Sample 3, Injection1	1352.50	19.62	98.1
Sample 3, Injection 2	1358.63	19.71	98.52

Conclusion

A fast and low-cost isocratic method for the analysis of an OTC medicine is presented. The method is demonstrated on an Agilent 1260 Infinity LC and run at approximately 170 bar (2,470 psi) at 35 °C. Chromatographic run time is approximately five minutes. The extraction procedure as demonstrated takes approximately 60 minutes, but multiple samples could be extracted simultaneously. An optimized extraction procedure could be faster.

Thank you to The Proctor & Gamble Company for allowing use of Optichrom LC.

References

1. Gratzfield-Hugsen, A.; Naegele, E., Maximizing Efficiency Using Agilent Poroshell 120 Columns. Agilent Technologies application note, publication number 5990-5602EN, **2016**.
2. Chester, T. L. Business-Objective-Directed, Constraint-Based Multivariate Optimization of High-Performance Liquid Chromatography Operational Parameters. *J. Chromatogr. A* **2003**, 1016(2), *181–193*.

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