Fast Analysis of Sulfa Drugs using the Agilent 1100 Series LC with Agilent Poroshell 120 EC-C18 columns

Application

Food

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Abstract

A method separating 10 sulfa drugs on a 4.6 × 250 mm, 5-µm column is transferred to an Agilent Poroshell 120 EC-C18 column using an Agilent 1100 Series LC. Simple guidelines for transferring a method are given. The new separation reduces the analysis from 30 min to 8 min, and should not require any changes to sample preparation since the columns both use 2-µm frits. While pressure is increased from the original method, it is still below 400 bar and should be easily transferred to almost any HPLC system. Lifetime of over 1000 injections is demonstrated using an unfiltered, protein-crashed milk sample, spiked with sulfadimethoxine.

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Introduction

Sulfonamides were the first chemical substances systematically used to treat and prevent bacterial infections in humans. Sulfonamides are bacteriostatic drugs; they work by inhibiting the growth and multiplication of bacteria without killing them. Currently, their most common use in humans is treating urinary tract infections. Other newer drugs are used to fight infections, such as beta-lactam antibiotics. Today, sulfa drugs are commonly used as antibiotics in veterinary applications; primarily, they are fed to animals to prevent infection. In addition, they are also used to fight disease in honey bees. Residues of these antibiotics are quite often found in honey samples and are of concern to consumers around the world due to toxic or allergic reactions. [1,2]

The Agilent Poroshell 120 EC-C18, 2.7-µm columns have similar performance to sub-2-µm completely porous materials, but since they use 2-µm column frits similar to those found on 5-µm columns, they require no additional sample preparation. This allows a more seamless method transfer.

In this work, a gradient method is transferred and optimized from a 4.6 × 250 mm, 5-µm column to a 4.6 × 100 mm Agilent Poroshell 120 EC-C18 column. Gradient time was decreased from 30 min to 8 min. Time can be further reduced to less than 4 min using a 4.6 × 50 mm column, with some loss of resolution.

Experimental

An Agilent 1100 Series HPLC modified for Rapid Resolution LC components was used for this work. This system consisted of a G1312A Binary Pump, capable of delivering up to 400 bar pressure; a G1316A Thermostatted Column Compartment (TCC), a G1376A High Performance Autosampler, a G1315B Diode Array Detector equipped with a semimicro flow cell with a 3-mm path length. When using the Poroshell 120 column all tubing was changed to 0.12 mm id tubing of the shortest lengths; the needle seat was changed to a 0.12 mm id part; and the detector data collection rate was increased to the fastest setting (40 Hz) in accordance with recommendations for optimization shown in previous documents and presentations. [3, 4] Data was collected using Agilent ChemStation version A.10.02. Columns used in this work include Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5-µm (p/n 959990-902) and Agilent Poroshell 120 EC-C18 4.6 × 100 mm columns (695975-902).

The following sulfa drugs were purchased from Sigma Aldrich: sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethazole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine. These compounds were prepared in 50/50 v/v acetonitrile:water at 1 mg/mL and then mixed in equal amounts to produce a solution of 0.1 mg/mL each.

The milk sample used was Similac. Similac is a commercial infant formula developed to be similar to breast milk. The composition of this material is strictly controlled by the manufacturer, so its protein composition is uniform, which is unlike that of milk (Abbott Laboratories Columbus Ohio). The most commonly used infant formulas contain purified cow's milk whey and casein as a protein source, a blend of vegetable oils as a fat source, lactose as a carbohydrate source, a vitamin-mineral mix, and other ingredients.

A 2-mL aliquot of Similac spiked with sulfadimethoxine was added to 18 mL of 200:800:1 solution of water:acetonitrile:formic acid and shaken vigorously for 1 min. The solution was then allowed to stand for 3 min and an aliquot was transferred to a centrifuge tube. The tubes were centrifuged at 5000 rpm for 5 min. The solution was transferred to an auto sampler vial without further filtration.

Discussion

Faster analyses with small particle columns have been demonstrated for several years. However, unlike 5 or 3.5-µm columns, smaller frits are required to keep the packing material in the column. A 2-µm frit is used to hold in particles of 5 and 3.5 micron size. For particles that are 3 µm or smaller, frits of 1 or 0.5 µm are required. A totally porous particle will have a particle size distribution that is 25% greater than a superficially porous particle. A narrow particle size distribution material such as Poroshell 120 can be held in the column using a 2 µm frit. If the frit size on the new column is smaller than that on the older column, more care must be taken with sample preparation when transferring the method, to prevent clogging the frit with a dirty sample. [5] By keeping the column frit at a larger size no additional sample cleanup is required when transferring a method from a 5 or 3.5 µm column to a Poroshell 120 column.

The initial method shown in Figure 1 demonstrates a ten compound separation using a 4.6 × 250 mm, 5 µm column and a 30-min gradient. The separation yields baseline resolution on most compounds, and a minimum resolution of 1.7 on one peak pair. This separation may not have received further optimization due to the lengthy experiments required. The method is transferred to a 2.7 µm superficially porous column in several short steps:

1. Transfer of the gradient (gradient of the original method is proportionally shortened to the column length to maintain the original separation, preserving k'). Since the original method time was 30 min and the original column was 250 mm, using a 100 mm column at the same flow rate shortens the gradient time by 100/250 or 0.4. The injection volume is also decreased by the same amount. [4,6,7] This is shown in Figure 2.

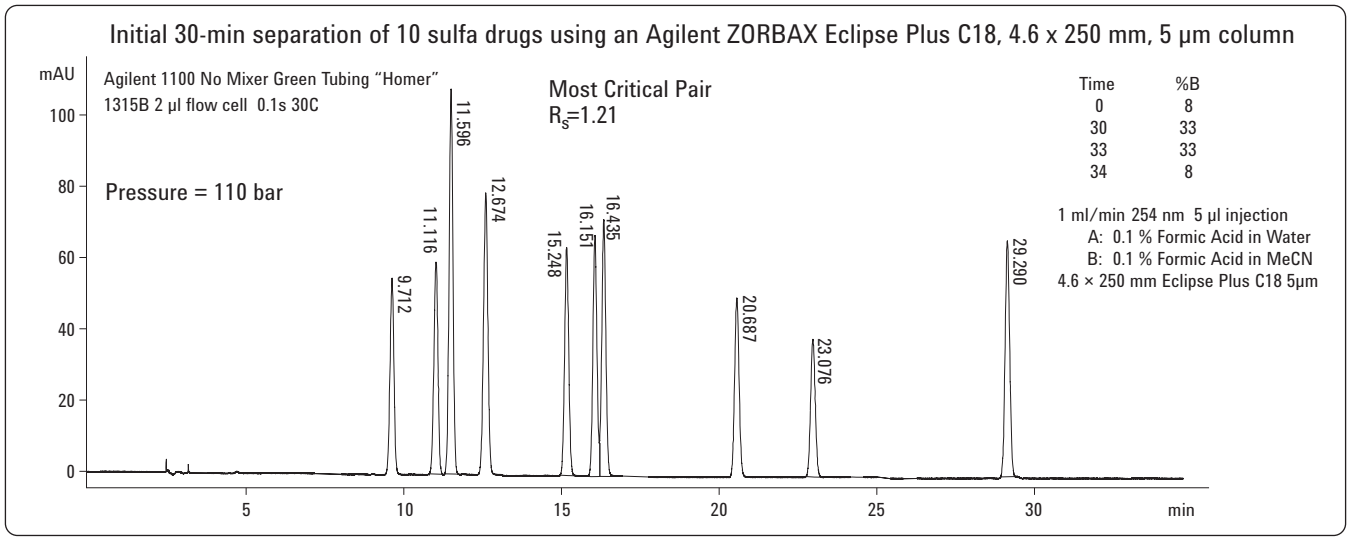

Figure 1. A separation of 10 sulfa drugs on an Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5 µm in 30 min using a formic acid/acetonitrile gradient.

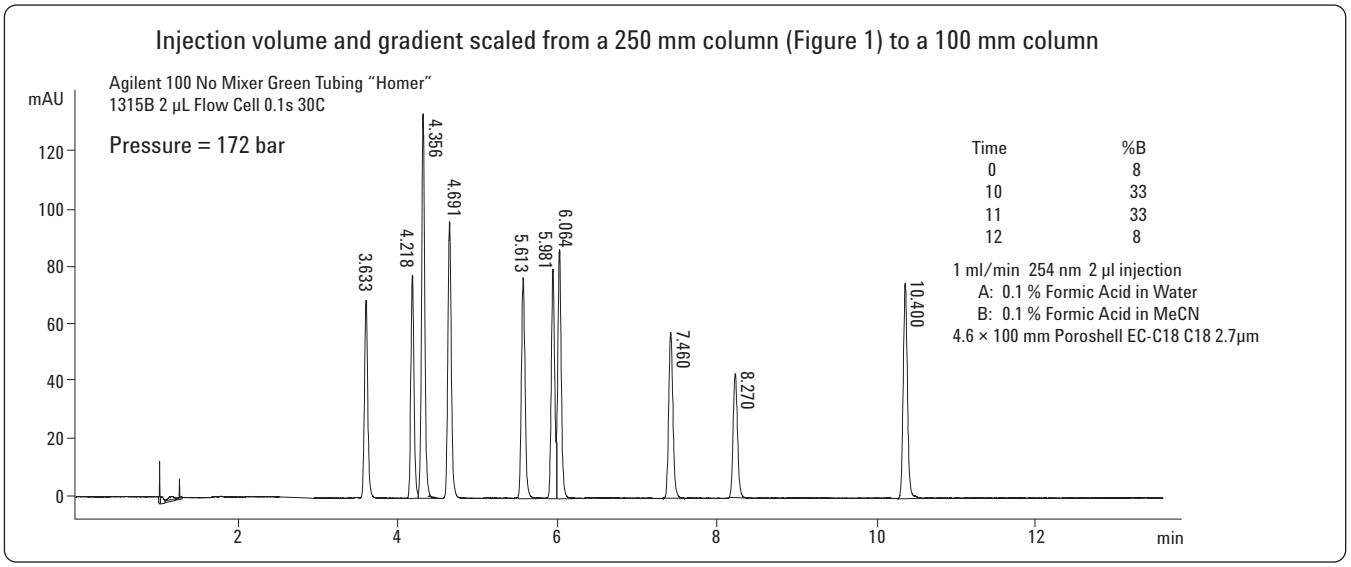

Figure 2. A separation of 10 sulfa drugs on an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column in 12 min using a formic acid/acetonitrile gradient.

2. Flow rate is increased, but the number of column volumes in the separation is maintained, as seen in Figure 3. k' is preserved in a separation by keeping gradient steepness (G_s) constant on similar columns. Using the formula, shown as Equation 1.

Equation 1. $G_s = (V_m/F)(\Delta\%B/t_G)$ [7],

and rearranging the formula to

Equation 2. $(F)(t_G) = ((\Delta\%B)(V_m) / G_s)$

where

F is flow rate,
t_G is gradient time,
V_m is column volume,
Δ%B is the change in solvent strength.

We find that we can shorten gradient time by increasing flow rate. So our initial separation of 8 to 33% B at 1 mL/min over 10 min is converted to 8 to 33 % at 1.5 mL/min over 6.7 min and finally 8 to 33 % B over 5 min at 2 ml/min. (1 mL/min × 10 min = 1.5 mL/min × 6.7 min = 2 mL/min × 5 min) following the formula below:

Equation 3. $$t_2 = t_1 \times \frac{F_1}{F_2}$$

where

t_2 is time of new gradient
t_1 is time of old gradient
F_2 is new flow rate
F_1 is old flow rate

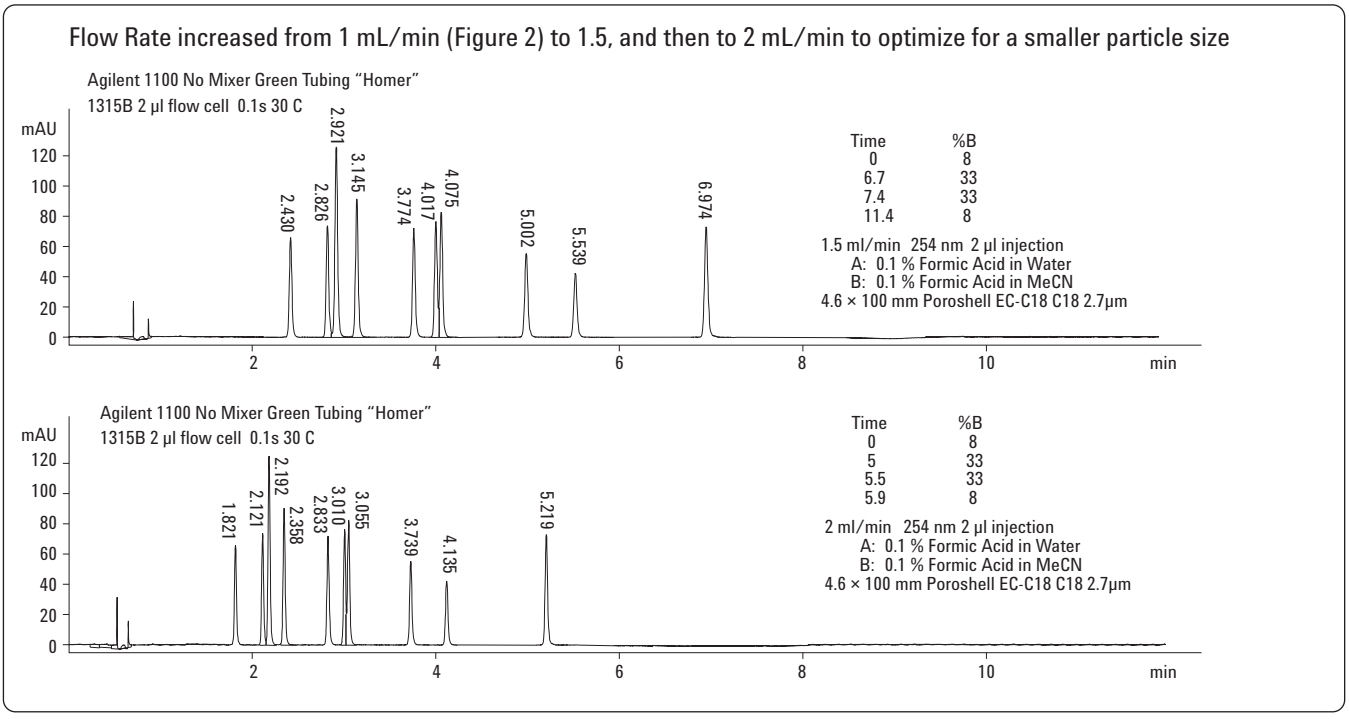

Figure 3. A separation of 10 sulfa drugs on an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column showing the flow rate and gradient scaled from 1 mL/min (Figure 2), to 1.5 mL/min, and finally to 2 mL/min, resulting in a 6 min separation using a formic acid/acetonitrile gradient.

3. Optimization of the gradient. Since the experiment can be run in a fraction of the time required in the original experiment, several quick gradient changes are made leading to an even faster separation or perhaps better resolution. This is accomplished in less than 1 hr. Some of this work could have been performed on the longer column, but due to the longer analysis times, analysts would need to spend 4 to 8 hr to accomplish this step. Figure 4 demonstrates the steps taken to optimize selectivity on the Poroshell 120 column.

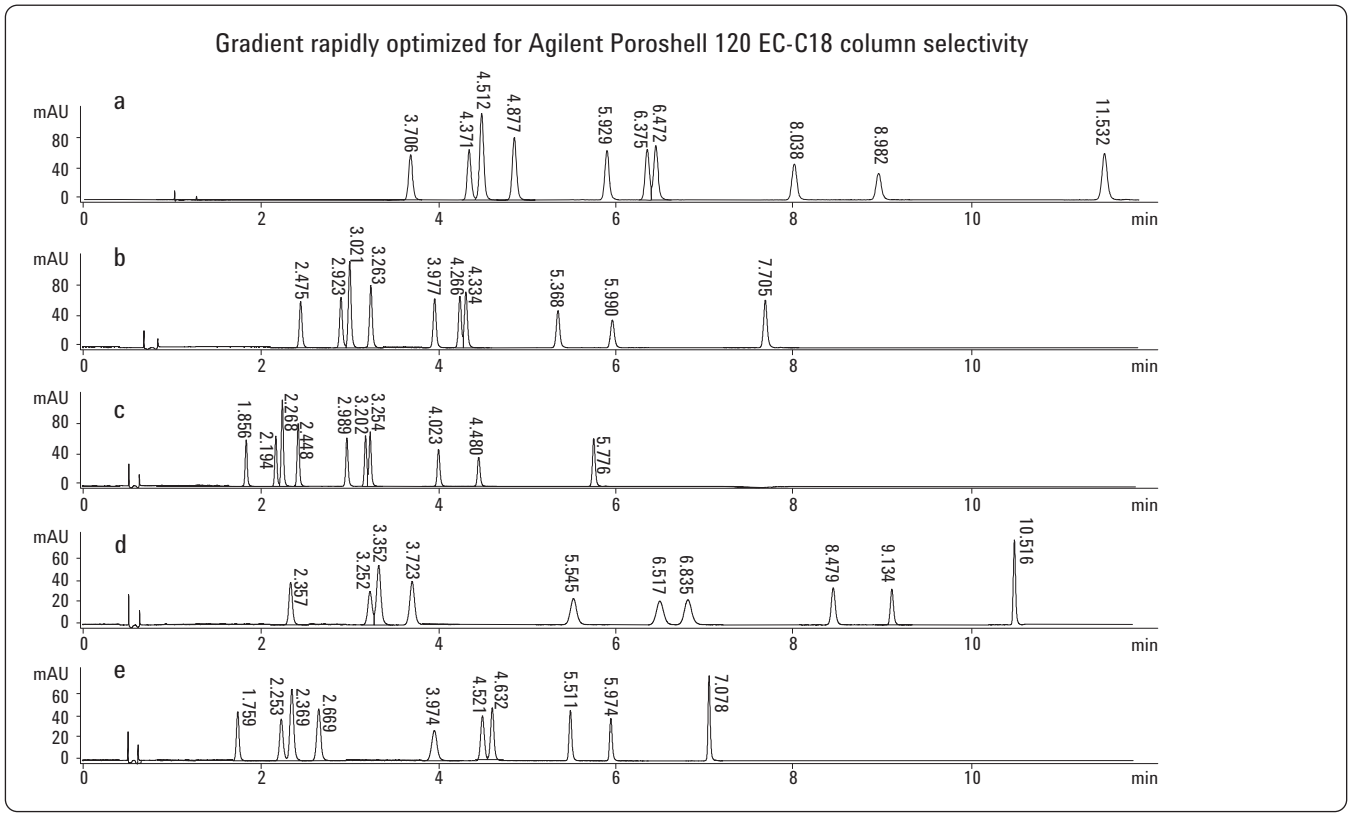

Figure 4. Optimizing a separation of 10 sulfa drugs on a 2.7 µm Agilent Poroshell 120 EC-C18 column, by adjusting the formic acid/acetonitrile gradient. (a) Original Scaled Chromatogram from 250 mm column, at 1 ml/min.(also shown in Figure 2) (b) Chromatogram scaled to faster flow rate of 1.5 ml/min shortening analysis time by 33 %. (also shown in Figure 3)(c) Chromatogram scaled to faster flow rate of 2 ml/min shortening analysis time by 50 %. (d) Adjusting gradient program to improve selectivity of peak pair 6,7. (e) Adjusting gradient program to improve selectivity of peak pair 2,3.

4. Complete optimization of the Agilent 1100 LC system. With small columns, additional performance gains can be made with attention to instrument configuration. The most important parameter is detector speed. In this case, the detector is set to maximum collection rate (40 Hz). Other important factors include: minimizing the tubing, changing the flow cell to smaller volume to reduce peak broadening, and changing the injector seat to a smaller volume. The initial 250-mm method is overlaid with the new 100-mm method in Figure 5. These chromatograms show the significant time savings, with the last peak on the new, faster method eluting before the first peak on the older 5-µm method.

Finally, a sample of milk simulant, Similac, spiked with sulfadimethazine is extracted by precipitation of proteins with acidified acetonitrile. The sample is shaken and allowed to stand for 5 minutes before being decanted. As can be seen in the scatter plot, (Figure 6), no change in the pressure is noted over the period of testing. In addition, the efficiency remains nearly constant. The analysis of this unfiltered protein precipitated milk sample demonstrates the ruggedness of the Agilent Poroshell 120 EC-C18 column.

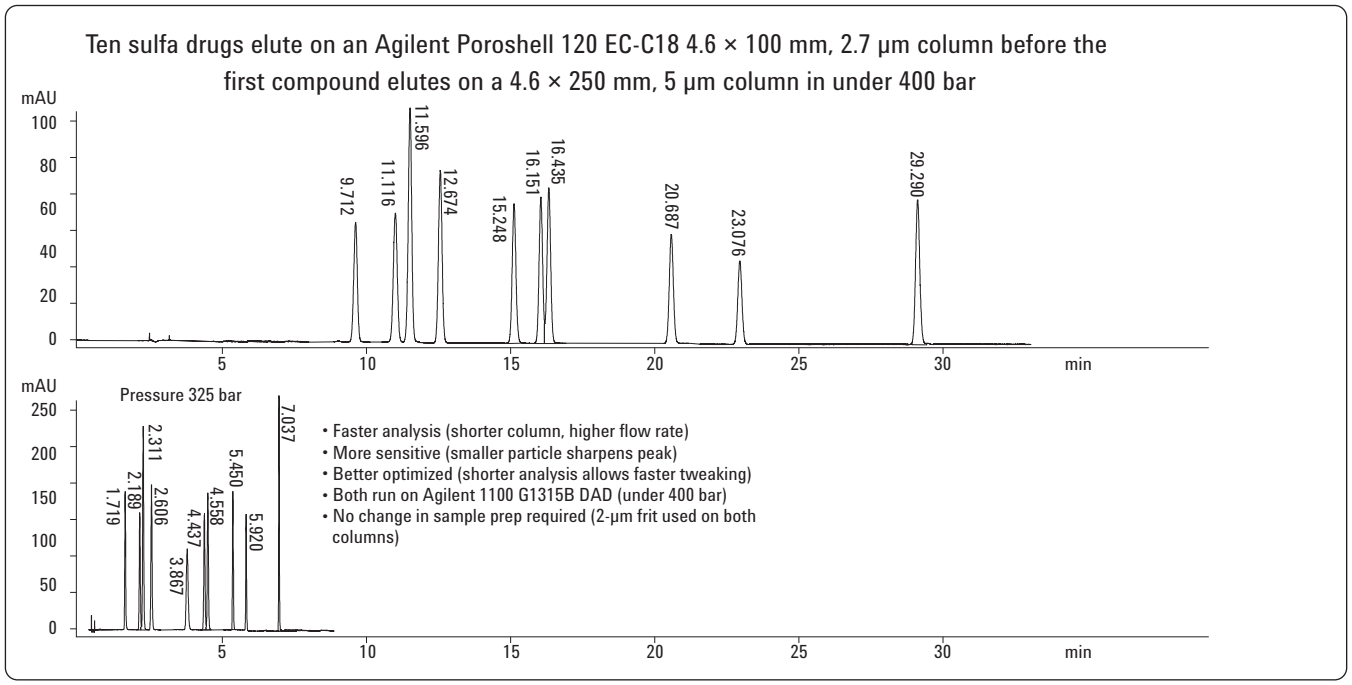

Figure 5. A separation of 10 sulfa drugs scaled from an Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5 µm column to an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column showing analysis time decreased from 30 min to 8 min using a formic acid/acetonitrile gradient.

Conclusions

This work has shown that an existing method with a 4.6 × 250 mm, 5 µm C18 column, can be easily adapted to a faster, but similar, method using an Agilent Poroshell 120 EC-C18 4.6 × 100 mm column. This method can still be used with a traditional HPLC system, as the maximum pressure is well below 400 bar. Additionally, sample preparation can stay the same, since both columns use 2-µm frits. The time and solvent savings gleaned from this method transfer may allow analysts to further optimize their method parameters, an option that may not have been reasonable with a significantly longer analysis time.

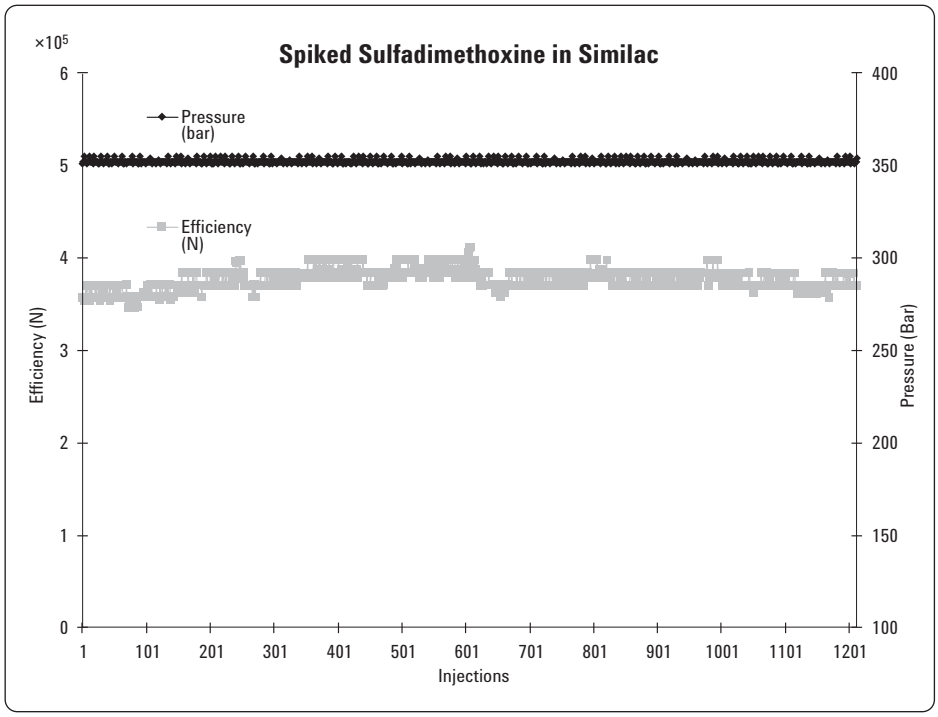

Figure 6. Scatter plot showing the Agilent Poroshell 120 100-mm column (Sulfadimethoxine enduring over 1000 injections of precipitated Similac, efficiency of sulfa drug plotted, no increase in pressure detected.)

References

1. William J. Long and John. W. Henderson Jr., “Analysis of Sulfa Drugs on Eclipse Plus C18”, September 11, 2006, 5989-5436EN
2. Carol A. Gonzalez and Karyn M. Usher, Anne E. Brooks and Ronald E. Majors, “Determination of Sulfonamides in Milk Using Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry”, March 11, 2009, 5990-3713EN
3. Fast, Faster Ultrafast: Optimization of Cycle Times in Liquid Chromatography for Optimal Speed and Resolution. Agilent Technologies February 1, 2005, 5989-2108EN
4. Michael Woodman, “Rapid Screening and Analysis of Components in Nonalcoholic Drinks”, August 3, 2006, 5989-5178EN
5. Dorothy J. Phillips, Mark Capparella, Uwe Neue, Zouibar El Fallah, “A small, new particle packing for faster analysis with high resolution”
6. L.R. Snyder and J.W. Dolan, “High Performance Gradient Elution”, Wiley-Interscience, New York, 2007
7. L.R. Snyder, J.J. Kirkland, J.L. Glajch, “Practical HPLC Method Development”, 2nd ED., Wiley-Interscience, New York, 1997.

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