The analysis of impurities is an important part of the development process in chemical industries. Due to the fact that impurities can be structurally similar to the main compound, it can often be difficult to separate them chromatographically. A solution to this challenge can be the use of the Agilent 1290 Infinity 2D-LC. In a heart cutting experiment, the peak of interest is sampled from the first column and eluted into a loop capillary. This is then transferred by a switching valve to a second dimension column. In this way co-eluting peaks can be resolved. In this work the importance of the capillary. This is then transferred by a switching valve to a second dimension column.

Silica HPLC columns have a useful pH range between 2 and 9. The lifetime of these columns can be compromised by exposure to phosphate and bicarbonate buffers at elevated temperature or higher pH. In this regard, there are very limited options in super critically porous particles compared to totally porous particles. Unlike conventional supercritically porous particles, Poroshell HPH columns are designed to be stable over a wide pH range. Using these columns, chromatographers can enjoy the ultrahigh efficiency of these supercritically porous particles using phosphate or bicarbonate mobile phases making more selectivity options to facilitate method development.

In this work, we present a column packed with superficially porous particles that are efficient of these superficially porous particles using phosphate or bicarbonate mobile phases.

The Agilent 1290 Infinity 2D-LC consisted of the following modules:
- Agilent G1329A 1200 Auto-sampler
- Agilent G1311A 1200 series Quaternary Pump (600 bar)
- Agilent G1315C 1200 Diode Array Detector (1st dimension detector)
- Agilent G4220A 1290 Infinity Pump
- Agilent G1316C 1290 Infinity Thermostatted Column Compartment (TCC) with a 2-position/4-port – duo valve (G4226A) for 2D-LC.
- Agilent G4212A Infinity Diode Array Detector (2nd dimension detector).
- Open Lab version C.1.04 with 2D control software.

The first three components were recycled from an older system and were integrated with the 1290 Infinity Pump, 1290 Infinity DAD, 1290 Infinity TCC and valve by upgrading firmware.

**Figure 1 Agilent 2DLC Solution**

1. Dimension
- Agilent G1315C 1200 Quaternary Pump
- Agilent 1200 Infinity DAD
- Agilent 1200 Infinity Valve (optional)
- Agilent 1200 Infinity Diode Array Detector

2. Dimension
- Agilent G1329A 1200 Auto-sampler
- Open Lab version C.1.04
- G1316C 1290 Infinity Thermostatted Column Compartment (TCC)
- G4212A Infinity Diode Array Detector
- Open Lab version C.1.05

The benefit of high pH orthogonality is shown in a real example. Amphetamines are basic compounds. These compounds are better retained at high pH and have better peak shape using Ammonium Bicarbonate Buffer. Increased retention can be an advantage when injecting large volumes. Better peak shape is an advantage in minimizing overlapped peaks (selectivity) and increased sensitivity (sharper peaks are taller)

**Figure 4 Separation amphetamine mix at low pH 0.1% Phosphoric Acid and pH 10 ammonium bicarbonate.**

**Conclusions**
- Poroshell HPH is stable at pH 10 Bicarbonate Buffer
- Selectivity using pH can help generate highly non correlated separations
- Acidic compounds retain less at high pH than at low pH. Basic compounds are retained more at high pH than at low pH
- In 2D-LC separations it is possible to use pH to generate orthogonal gradients using the same column phase

Thank you to Professor Dwight Stoll and David C. Harmes at Gustavus Augustus College for the Amphetamine Data.

**Results and Discussion**

The stability of Poroshell HPH is discussed further in Poster P.M-0204. In that work it is demonstrated that the column is stable at pH 10 for 40,000 column volumes. In Figure 2, the retention time of individual compounds with the pH 3 gradient are plotted against pH 4.8 on a Poroshell 120 EC-C18 or pH 10 on a Poroshell HPH C18 gradient using Methanol as an organic modifier. In the top figure plotting pH 3 and pH 4.8 on the Poroshell 120 EC-C18 yields highly correlated data, most of the data falls on the trend line with an R² value of 0.94 indicating very little difference between the two conditions. In the lower figure the data is more scattered, the R² value of 0.46 indicates the data is not correlated. Figure 3 shows a similar plot with Acetonitrile as the organic modifier. In the top figure plotting pH 3 and pH 4.8 we observe a highly correlated data set, not as correlated as the methanol set with an R² value of 0.79. In the lower plot (pH 3 vs. pH 10) the data is more scattered with an R² value of 0.35. This data indicates that pH can be used to decrease the correlation of a separation. In this work the decreasing R² values indicate that the separations become more orthogonal as the pH differences are greater and will yield very different separations.

**Experimental**

**Two Dimensional HPLC using a High pH Stable Superficially Porous Column**

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Agilent Technologies (P-W-1923), HPLC 2014, New Orleans, USA

Gradient Conditions 1st Dimension A: High pH, B: Organic (MeOH or ACN) Gradient: 5% B at t0, ramp to 95% B in 6 min., hold at 95% B for 1 min. Flow Rate 0.42 mL/min 25 C. The data was transferred to Excel.

**Gradient Conditions 2nd Dimension A: Low pH, B: Acetonitrile**

Time | % B | Flow rate (mL/min)
--- | --- | ---
0 | 10 | 0.5
1 | 90 | 0.5
5 | 90 | 0.5
6 | 90 | 1

**Poroshell HPH C18, Methanol Gradient**

R² = 0.4627

**Poroshell HPH C18, Acetonitrile Gradient**

R² = 0.3465

**Poroshell 120 EC-C18, Methanol Gradient**

R² = 0.9261

**Poroshell 120 EC-C18, Acetonitrile Gradient**

R² = 0.4627

**Poroshell HPH C18, MeCN gradient**

R² = 0.3465

**Poroshell 120 EC-C18, MeOH gradient**

R² = 0.9261

**Poroshell HPH 2.7 x 150 mm D1 (high pH)**

Grad. B 50B/5 min

**Poroshell HPH 2.7 x 100 mm D1 (low pH)**

Grad. A 10 ml/min 20 C

**Poroshell HPH D1(high pH) Poroshell HPH (low pH) D2**

**30% B-50B/5 min**

**Data from Stoll Lab**

**Poroshell HPH C18 2.1 x 100 mm D1(high pH)**

**Diclofenac & Pioglitazone**

75 Acidic, basic and neutral compounds were injected individually or as a mixture on a 2.1 x 50 mm Poroshell HPH C18 or Poroshell 120 EC-C18 column on an Agilent 1260. The Agilent 1260 consisted of the following modules:

- Agilent G13370D Hi-ALS +
- Agilent G1312B Binary Pump (600 bar)
- G1315C Infinity Thermostatted Column Compartment (TCC)
- Agilent G4212A Infinity Diode Array Detector
- Open Lab version C 1.05

Gradient: A: 10 mM Buffer (Ammonium Formate pH 3, Ammonium Acetate pH 4.8, Ammonium Bicarbonate pH 10) B. Organic (MeOH or ACN) Gradient: 5% B at t0, ramp to 95% B in 6 min., hold at 95% B for 1 min. Flow Rate 0.42 mL/min 25 C. The data was transferred to Excel.

**Comprehensive 2D-LC (LCxLC):**

The complete effluent of the first column will be injected to the second column and will be analyzed with very fast gradient. A peak of the first dimension should be sampled at least 3 to 4 times. The run time of the 2nd dimension method matches the collection time of the 1st dimension finally. Initially, the peaks will be re-constructor.