



Analysis of Drugs of Abuse at Low and High pH Using an Agilent Poroshell HPH C18 by LC/MS

Application Note

Small Molecule Pharmaceuticals

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Introduction

In this application note, LC/MS of several compounds associated with drugs of abuse are compared at high and low pH using a generic gradient in positive ion mode electrospray (ESI+). Normally, it could be expected that the ionization state of analyte molecules would be dependent on the pH of the mobile phase, and the ionization efficiency in LC/MS with electrospray in positive ion mode will be dramatically lowered in high pH mobile phases since the compounds become neutral. However, many researchers investigating different types of samples (including proteins, peptides, and amino acids) have observed either insensitivity to an increase of mobile phase pH, or even increases in sensitivity [1-6]. High pH mobile phases do not suppress the ionization of basic compounds in ESI+. Positive ions are formed abundantly, and analyte responses are often better in high pH compared to low pH acidic mobile phases. This finding is significant as it extends the applicability of generic elution methods to the analysis of polar basic compounds that were previously difficult to retain.

However, many of the conditions that could be used at high pH have been shown to be harmful to conventional silica HPLC columns. Control of pH can be used to adjust selectivity and sensitivity without sacrificing column lifetime at elevated pH using new high pH stable columns such as the Agilent Poroshell HPH C18. Using these columns, chromatographers can now explore a wider range of pH in method development using superficially porous particle technology, which is being increasingly adopted due to its high efficiency and speed [7].



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Experimental

An Agilent 1290 Infinity LC system with an Agilent 6460 Triple Quadrupole Mass Spectrometer was used, consisting of:

- Agilent 1290 Infinity Binary Pump, capable of delivering up to 1,200 bar (G4220A), modified with a PEEK seal in the solvent bypass valve (Rotor Seal PEEK FL p/n 5068-0171)
- Agilent 1290 Infinity Thermostatted Column Compartment (TCC) (G1316C)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) with PEEK rotor seal (5068-0170 Rotor Seal PEEK FL)
- Agilent Poroshell HPH C18, 3 × 100 mm, 4 μm (p/n 695970-502)

PEEK FL seals should be used with ammonium bicarbonate buffer. The standard rotor seals in the Agilent 1290 Series Instruments are made out of Vespel, and support a limited pH range (1-10). The PEEK FL rotor seals are made out of a special PEEK blend material that covers a broader pH range (1.0-12.5). The UHPLC was mounted on an A-line Rack. The system was plumbed with 0.075-μm tubing to minimize dispersion. This creates more backpressure than conventional 0.12 or 0.17 mm id tubing, but this is not a problem as a 4 μm Poroshell HPH C18 generates little pressure. Table 1 lists the MRM transitions and monitored ions.

Ammonium formate and formic acid were prepared at 5 mM, and used to prepare a low pH buffer at pH 3. Ammonium formate was purchased from Sigma-Aldrich, Corp. Double distilled formic acid was brought from GFS. Ammonium bicarbonate and ammonium hydroxide were used to prepare a pH 10.5 buffer, and were supplied by Sigma-Aldrich.

Table 1. MRM Transitions for compounds used in this work.

Compound	Precursor ion	Fragmentor voltage	Product ion 1	Collision energy 1	Product ion 2	Collision energy 2
Strychnine	335.2	195	184	41	156	53
Alprazolam	309.1	179	281	25	205	49
MDMA	194.1	97	163	9	105	25
Amphetamine	136.1	66	119.1	5	91	17
Trazadone	372.2	159	176	25	148	37
Meperidine	248.2	128	220.1	21	174.1	17
Verapamil	455.3	158	165	37	150	45
Methadone	310.2	112	265.1	9	105	29
Proadifen	354.2	153	167	29	91.1	45
Diazepam	285.1	169	193	45	154	25
THC	315.2	150	193.2	20	123.3	30

Results and Discussion

In this application note, comparing elution of these compounds in basic mobile phase to acidic mobile phase, we see the bases are retained longer in basic mobile phase. Basic compounds are fully protonated, and are retained better than charged compounds in reversed-phase HPLC. Since the compounds are retained better, more solvent adjustments can be made, which allows better peak spacing, as shown in Figure 1. Gradient and mobile phase conditions are described in Figure 1. Finally, some of the analyte peaks are larger in basic solvent than in acidic mobile phase. Peaks associated with trazodone, verapamil, methadone, and proadifen are substantially larger in the basic mobile phase. This may be a pH effect, but it may also be due to the compound being retained longer and eluting in a higher organic mobile phase.

Conclusions

It was previously shown that an Agilent Poroshell HPH-C18 can be used for extended periods (over 2,000 injections) with high pH mobile phases such as pH 10 ammonium bicarbonate buffer. Therefore, chromatographers can now explore a wider range of pH in method development using superficially porous particle technology, which is being increasingly adopted due to its high efficiency and speed. The use of high pH mobile phase with superficially porous particles is both beneficial and practical [8].

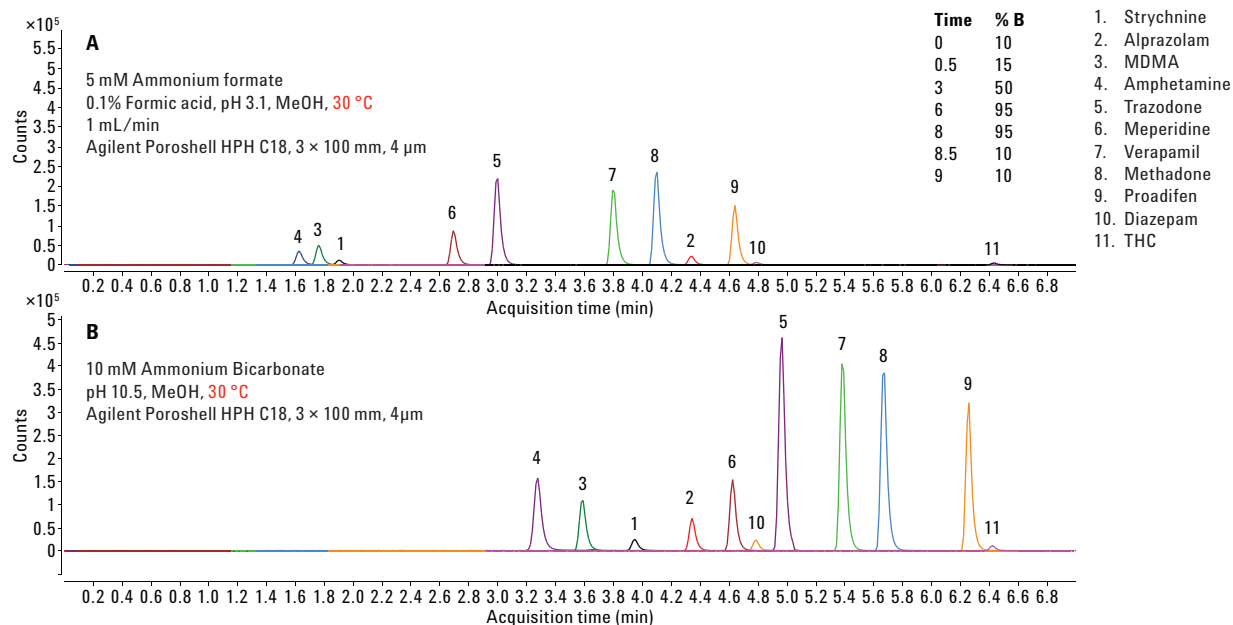


Figure 1. Separation at Low and High pH using an Agilent Poroshell HPH C18, 4 μm column.

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