Chromatography of Nitrogen-Containing Compounds Without Triethylamine

Application

Pharmaceutical/Nutraceutical

Authors

William J. Long and John W. Henderson, Jr.
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE
USA

Abstract

Triethylamine (TEA) is widely used as a mobile phase additive to reduce peak tailing when analyzing bases at neutral pH. In this work, Eclipse Plus C18 demonstrated sharp symmetrical peaks for difficult compounds without TEA. Eclipse Plus C18 has a lower silanol activity with similar selectivity to other C18 columns, making it a preferred HPLC column for analyses of pharmaceutical and other amine-containing compounds.

Introduction

Peak tailing in reversed phase HPLC is of great concern to most chromatographers when separating basic compounds. Basic and nitrogen-containing compounds can form asymmetrical peaks that can compromise separation and quantitation. Since many bioactive compounds contain nitrogen, this can be particularly worrisome to those analyzing pharmaceutical or phytochemical compounds by HPLC.

A common cause of peak tailing in reversed phase HPLC is the ion-exchange interaction that takes place between a positively charged solute (for example, an amine) and a negatively charged silanol on the surface of silica stationary phase support particles. It is observed most often when using HPLC columns packed with stationary phases that have significant silanol activity. It usually is worse in a neutral pH mobile phase than in an acidic pH mobile phase.

There are several possible “fixes” for peak tailing caused by silanol interactions. The usual corrective action is to lower the pH of the mobile phase, typically below pH 3, where the silanols do not cause tailing. Operating at a pH below 3 protonates silanol groups on the silica stationary phase support (pKa of silanol is ~ 3.5) and thereby makes the silanols less available for interacting with solutes. This, however, can change the selectivity and resolution of the separation and therefore may not be an acceptable alternative.

A second corrective action is to increase the buffer strength of a mobile phase. By adding additional buffer capacity to the mobile phase, the interactions between the basic compound and the silica can be overcome. This, however, can cause problems when the increased buffer reagents precipitate in or about the instrument, especially the pump seals. One should be mindful of using an appropriate buffer, considering the controlling range of a given buffer. Table 1 lists several of the most common buffers.

Another potential fix is to add triethylamine (TEA) to the mobile phase. TEA acts as competing base, reducing the availability of stationary phase silanols and interaction of the analyte with the silanols.

Table 1. Commonly Used HPLC Buffers

Buffer	pKa	pH Range
Phosphate		
pK1	2.1	1.1–3.1
pK2	7.2	6.2–8.2
pK3	12.3	11.3–13.3
Formate	3.8	2.0–4.8
Acetate	4.8	3.8–5.8
Citrate		
pK1	3.1	2.1–4.1
pK2	4.7	3.7–5.7
pK3	5.4	4.6–6.4
Ammonia	9.2	8.2–10.2

Some chromatographers object to adding a competing amine to mobile phase because it adds an additional component to their mobile phase and alters the HPLC column in a way that is not easily reversed. Strong amines, such as TEA, are significantly difficult to wash off the column. An example of this is in positive ion mode of electrospray LC MS where an ion at 102 *m/z* that persists after TEA use in a system, contaminating not only the column but also the tubing[1]. In order to remove the TEA it is necessary to wash the system with 5% to 10% acetic acid as the flushing solvent. This means that a column thus modified by TEA is not suitable for applications that do not use TEA in the mobile phase. TEA acts as a competing base, essentially saturating available silanols, thus reducing the potential for tailing. About 10 mM TEA is sufficient for most applications, but in many cases more can be used. TEA, however, is very basic and if added to an unbuffered or lightly buffered mobile phase, it can cause the pH of the solvent to dramatically rise, perhaps to the detriment of the column, possibly affecting the separation and ultimately shortening column life. The best fix is to use a column that minimizes the need for TEA.

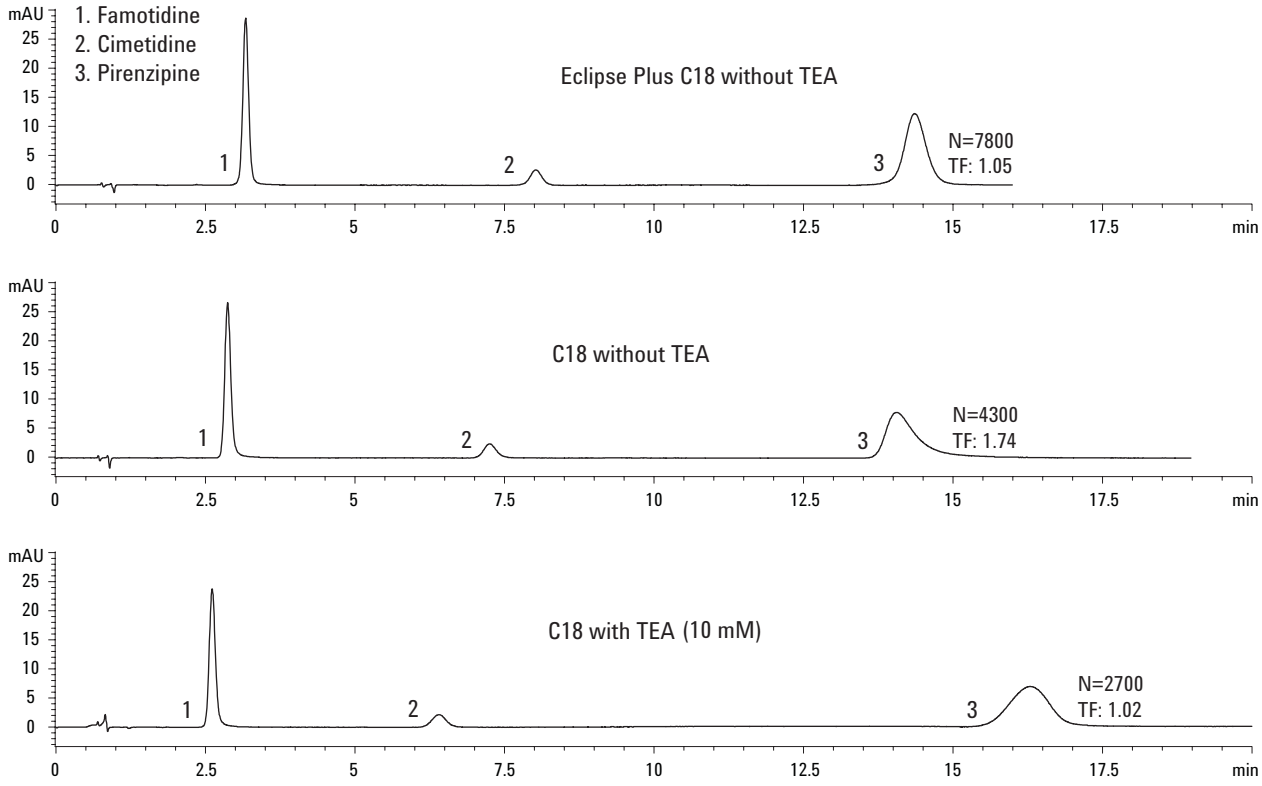
Eclipse Plus takes advantage of a proprietary silica treatment, improved bonding and endcapping procedures, leading to a column that naturally exhibits low peak tailing without the addition of TEA.

Experimental

All analyses were performed on an Agilent 1200, consisting of an autosampler, diode array detector, binary pump, degasser, and thermostatted column compartment column. Samples of famotidine, cimetidine, and pirenzipine were purchased from Sigma Aldrich (St. Louis, MO, USA). These materials were dissolved in 50/50 MeCN/water. The goldenseal sample was purchased locally, and extracted in 70/30/0.1% v/v/v water/MeCN/phosphoric acid, sonicated, centrifuged, and diluted 1/5 with extraction solvent according to the procedure in reference 2. Standards of palmatine, berberine, and hydrastine were purchased from Sigma Aldrich and prepared in mobile phase. These standards were used for identification of the goldenseal extract.

Results and Discussion

Figure 1 shows a comparison of a separation carried out on Eclipse Plus C18 and a C18 column with and without TEA for three ulcer medications: famotidine, cimetidine, and pirenzipine. The structures of these three amine-containing drugs are shown in Figure 2. Cimetidine and famotidine are histamine H2-receptor antagonists and act against ulcers by turning off the pathway that produces gastrointestinal fluids. Pirenzipine is an example of an M1-selective muscarinic antagonist, which inhibits production of acid in the stomach and gut. Ulcer medications are among the most commonly used medications. As can be seen in Figure 1, the peak shape of the three analytes on the C18 column has been improved with the addition of 10 mM TEA. The tailing factor of the pirenzipine peak changed from 1.74 without TEA to 1.02 with TEA. Simply by substituting Eclipse Plus C18, however, we realize an improvement in both efficiency and peak shape of the peaks corresponding to cimetidine and pirenzipine. In fact, the tailing factor corresponding to these peaks is very close to that of the C18 using TEA and the efficiency is better. For this Eclipse Plus C18 analysis, no mobile phase modifiers are required for great peak shape and efficiency.



Columns: 4.6 x 75 mm, 3.5 µm
Mobile phase: 20% MeOH, 80% 20 mM phosphate pH 7.0
Flow rate: 1 mL/min. UV 254 nm, semi micro flow cell

Figure 1. Effect of ionized silanols on peak shape using Eclipse Plus C18 and another C18.

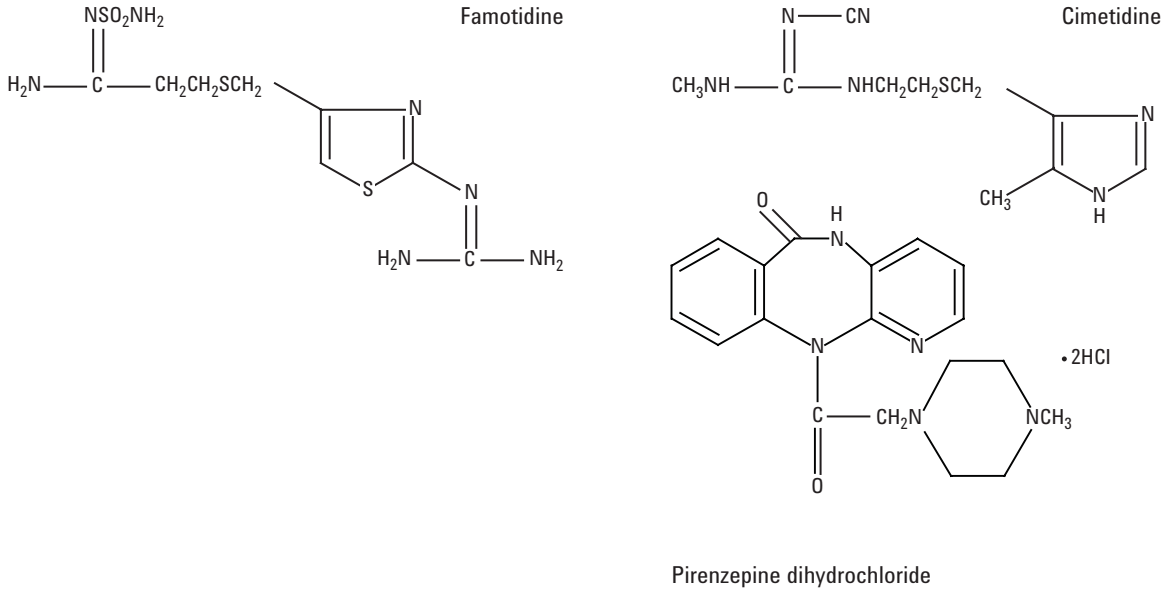
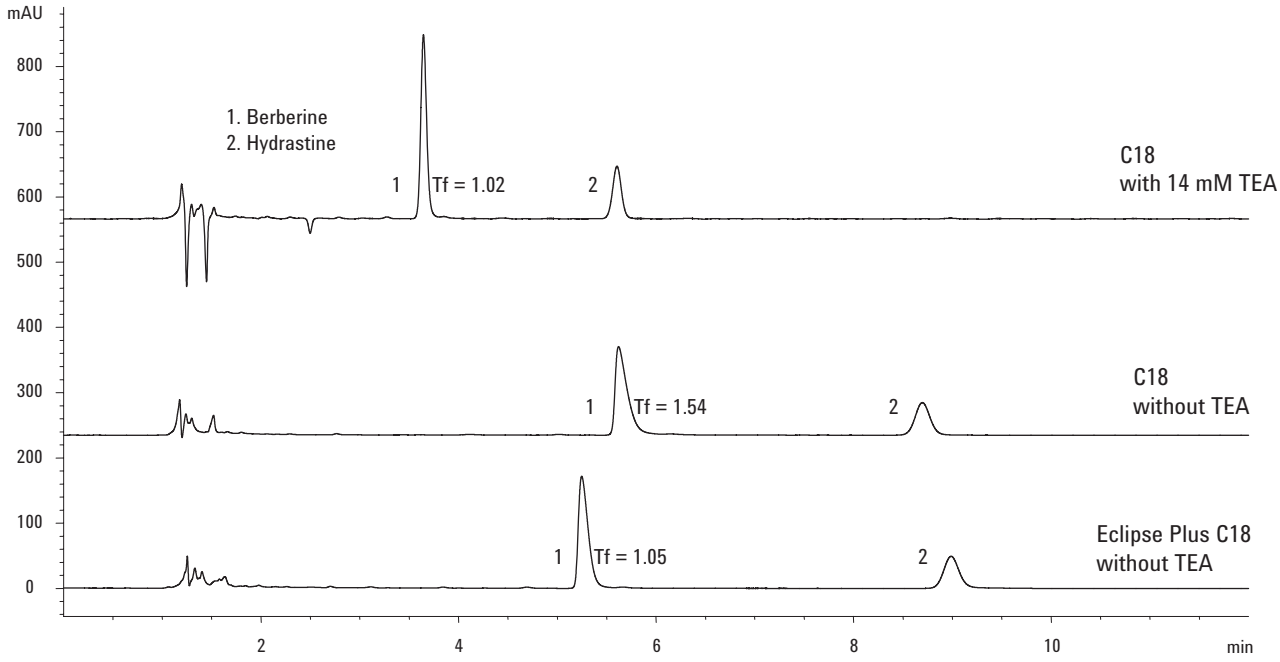


Figure 2. Structures of amine-containing ulcer medications.

Figure 3 demonstrates the use of the Eclipse Plus C18 in the analysis of the nutraceutical goldenseal. Goldenseal has been used as an antibiotic, an anti-inflammatory, a decongestant, as a treatment for a variety of intestinal disorders, and is one of the most widely used nutraceutical materials. Using a widely cited method [2], the Eclipse Plus C18 is evaluated against a C18 column with and without TEA.

In this case, we examined an extract of 500-mg sample of goldenseal root from a commercial source. The two principal components of goldenseal are berberine and hydrastine. Palmatine is cited in the method but is not found in the species of plant examined, *Hydrastis Canadensis L.* The structures of these amines are shown in Figure 4.



Columns: 3.5 µm 4.6 x 150 mm. C18 or Eclipse Plus-C18
Mobile phase: 68% 30 mM ammonium acetate, 14 mM TEA, 32% acetonitrile adjust mobile phase to pH 4.85
230 nm 1 mL/min 30 °C

Figure 3. Effect of ionized silanols on peak shape of goldenseal root extract.

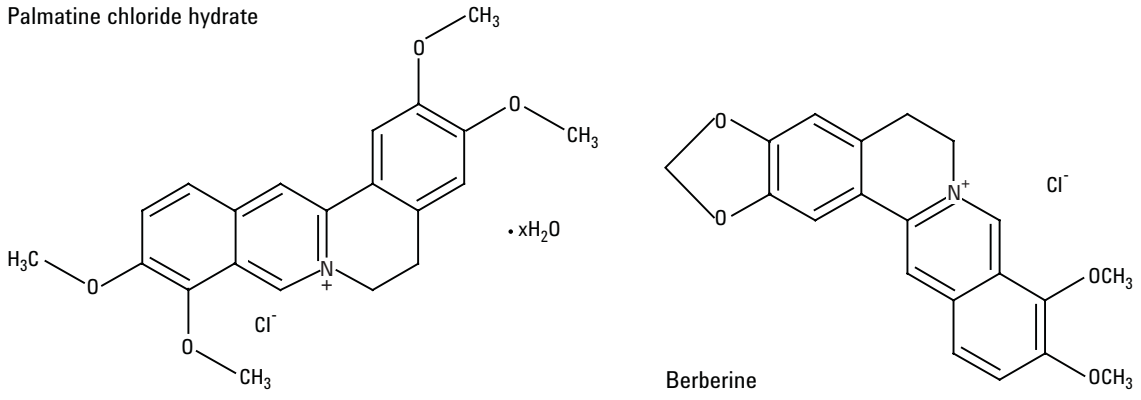


Figure 4. Amine-containing active components of goldenseal.

Two main points are visible upon examination of the chromatograms. First, the peak shape of berberine on the Eclipse Plus C18 is improved, compared to a C18 column. Tailing factor is reduced and efficiency is improved. Second, the selectivity of the C18 column changes significantly with the addition of TEA in this separation. A final point of note is the abundance of vacancy peaks or negative peaks at the beginning of the chromatogram containing TEA. Since an additional component is added to the mobile phase (14 mM TEA) as well as additional acid to readjust the pH, the mobile phase simply becomes more complicated as well as potentially dirtier. Best lab practices, including high-quality fresh reagents, can help avoid this pitfall, but the potential would still exist. A better alternative is to avoid the use of TEA.

Conclusions

Peak tailing problems due to ionized amines and silanols are usually mitigated by lowering the pH of the mobile phase, increasing buffer strength, or adding a competing base, such as TEA. These remedies reduce tailing but may bring about other method development challenges, such as poor selectivity or resolution or reduced column lifetime. A new solution is to use Eclipse Plus C18 stationary phase. It is designed to have low silanol activity and provide excellent peak shape and efficiency of bases.

References

1. H. Rütters, Th. Möhring, J. Rullkötter, J. Griep-Raming, and J.O. Metzger, "The persistent memory effect of triethylamine in the analysis of phospholipids by liquid chromatography/mass spectrometry," *Rapid Commun. Mass Spectrom.* 2000, 14, 122-123.
2. H. A. Weber, M. K. Zart, A. E. Hodges, H. M. Malloy, B. M. O'Brien, L. A. Moody, A. P. Clark, R. K. Harris, J. D. Overstreet, and C. S. Smith, "Chemical comparison of goldenseal (Hydrastis canadensis L.) root powder from three commercial suppliers," *J Agric Food Chem.* 2003 Dec 3; 51(25):7352-8.

Columns used in this work.

Eclipse Plus C18 4.6 x 150 mm, 3.5 µm (PN 959963-902)

Eclipse Plus C18 4.6 x 75 mm, 3.5 µm (PN 959933-902)

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