Separation of Salicylic Acid Impurities with Different Acid Mobile-Phase Modfiers

Application

Pharmaceuticals

Authors

William J. Long and John W. Henderson Jr.
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Optimization of chromatographic separations can best be accomplished using the selectivity tools of bonded-phase and mobile-phase choice. In this application, mobile-phase additives such as trifluoroacetic acid (TFA) and acetic acid change the selectivity of the separation of salicylic acid and several of its documented side products. The rapid sample throughput allowed by short 1.8-µm columns, and a rugged low-pH stationary phase, enabled quick evaluation of this important method-development parameter.

Introduction

Benefits of Low-pH HPLC

When analyzing organic acids, the ZORBAX StableBond SB-Aq becomes a frequent choice for bonded phase. It is durable at low pH conditions, and can be used with little or no organic solvent. Its patented bonded-phase chemistry includes diisopropyl side chain groups that sterically protect the key siloxane bond to the silica surface from hydrolytic attack at low pH. This ensures long column lifetime and reproducibility at low pH. Recent StableBond SB-Aq examples include organic acids found in fruit juices [1], water-soluble vitamins [2], and isomers of the pharmaceutical compound ceftibuten [3].

In method optimization, two key parameters that can be changed are the organic solvent and the mobile-phase pH. Examples of selectivity derived from changing from acetonitrile to methanol can be found in Reference 4.

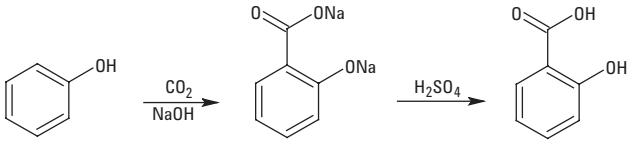
An ionizable compound will exist as a charged species in certain pH environments. For the ionizable compound benzoic acid, its retention and therefore its chromatographic interaction is very different at pH 3 and pH 7. At pH 3, benzoic acid is well retained (protonated), and at pH 7, it elutes near the void volume (ionized). Polar compounds, like benzyl alcohol and nitrobenzene, which are not ionizable but are polar, show much fewer shifts in retention with changes in pH.

Salicylic Acid Analysis

Salicylic acid, also known as 2-hydroxybenzoic acid is one of several beta hydroxy acids. It is the key additive in many skin-care products for the treatment of acne, psoriasis, calluses, corns, and warts. It treats acne by causing skin cells to slough off more readily, preventing pores from becoming clogged. This effect on skin cells makes salicylic acid an active ingredient in several shampoos to treat dandruff. Salicylic acid is also an active ingredient in gels that remove warts. The medicinal properties of salicylate (mainly for fever relief) have been known since ancient times. This colorless crystalline organic acid is also widely used in organic synthesis and functions as a plant hormone. The substance occurs in the bark of willow trees; the name *salicylic acid* is derived from *salix*, the Latin name for the willow tree [5].

Sodium salicylate is commercially prepared from sodium phenoxide and carbon dioxide at high

pressure and temperature in the Kolbe-Schmitt reaction. It is acidified to give the desired salicylic acid. The main reaction is shown in Figure 1; however, several additional materials are also formed in this reaction, including 2,5-dihydroxybenzoic acid, 5-hydroxy-1,3-benzenedicarboxylic acid, 4-hydroxybenzoic acid, phenol, and salicylglycine. These compounds are listed with their common names, structures, and pKa values in Figure 2.



Introduction to Organic Chemistry, Streitweiser and Heathcock, MacMillan, NY (1981). (Reference 6)

Figure 1. Synthesis of salicylic acid using the Kolbe-Schmitt reaction.

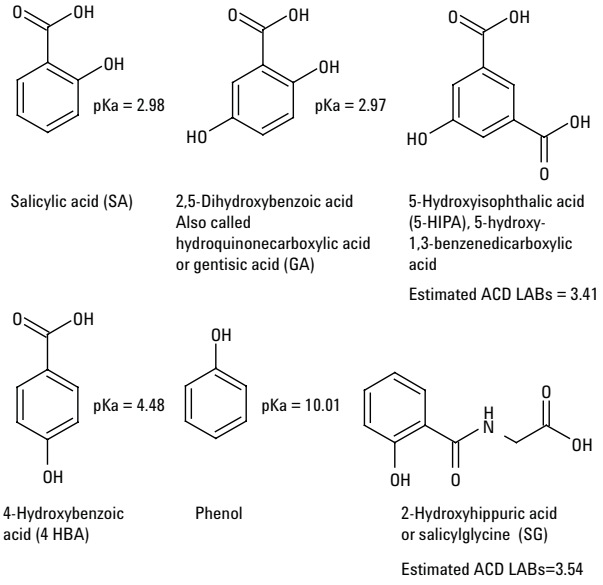


Figure 2. Structures of major salicylic acid impurities.

Purity was tested typically using a 5-micron C-18 column with an acidic mobile phase of water, methanol, and glacial acetic acid (60:40:1). This, however, only addresses three of the major impurities (4-hydroxybenzoic acid, 4-hydroxyisophthalic acid, and phenol). The compendial method also states that changes to the composition of the mobile phase could result in changes of elution order [7]. A reason these peaks may change elution order is that those compounds are all ionizable. As the mobile-phase constitution changes, so does the pH of the mobile phase.

In this work, changes in chromatography are made by changing the mobile-phase additives used. Commonly used concentrations of TFA, phosphoric acid, acetic acid, and formic acid are used to help resolve salicylic acid and five impurities.

Experimental

LC System

An Agilent 1200 Rapid Resolution liquid chromatograph (RRLC) (degasser, binary pump, well-plate autosampler, and diode-array detector set at 220 nm) was used with a StableBond SB-Aq, 1.8 µm, 4.6 mm × 50 mm column (part number 827975-914). The binary gradient was 5% to 30% methanol (channel B) over 10 minutes. Methanol was from Burdick and Jackson (HPLC grade).

Analytes:

- Salicylic acid (SA)
- Phenol (Phe)
- 4-Hydroxybenzoinc acid (4-HBA)
- Gentisic acid (GA)
- 5-Hydroxyisophthalic (5-HIPA)
- 2-Hydroxyhippuric acid (SG)

All were obtained from Sigma Aldrich or ARCOS.

Modifiers added to channel A:

- Trifluoroacetic acid (TFA), 99 % purity, (pKa=0.23)
- Phosphoric acid ACS grade (85 % purity) (pKa= 2.12)
- Acetic acid, glacial 99% (pKa=3.8)
- Formic acid ACS grade 99 % (pKa= 4.8)

Phosphoric, formic, and acetic acids were obtained from EM Science. TFA was from Sigma Aldrich.

Results and Discussion

Phosphoric acid, trifluoroacetic acid, acetic acid, and formic acid are commonly used to control the pH of HPLC mobile phases. The addition of an acid to the aqueous component of a mobile phase is a simple and sometimes effective alternative to buffers. In addition, in rapid gradients using low-volume columns, the small volume of the rapid resolution high throughput (RRHT) column allows equilibration in only a few minutes. Figure 3 shows the gradient described previously, using those four different acid modifiers in commonly used concentrations. While the initial separation was developed using nonvolatile phosphoric acid, it is generally not considered compatible with MS detectors. TFA is also sometimes excluded from LCMS because TFA can cause ion suppression by ion pairing to the analyte. However, in some cases

TFA can be displaced from the ion-pair complex by exposing the effluent to a higher concentration of a different acid post-column and before the MS detector. One acid that works well for this approach is acetic acid. Another ion pair displacer is a solution of 75% propionic acid:25% isopropanol. TFA and phosphoric acids yield the best separations as shown in Figure 3, while commonly used formic acid and acetic acid concentrations resolve only at best five of six components. Furthermore, in the examples using formic acid and acetic acid, 4-HBA and GA switch elution order compared to the TFA and phosphoric acid examples. This may be due to changes in pH; however, additional work is needed to determine the actual mechanism. For the scope of this work, observing elution order change is sufficient.

Figure 4 shows the effect of higher acid concentration on the separation. In almost all cases there are sharper peaks, the SG peak showing the greatest improvement. In Figure 3, the mobile-phase pH was near the pKa values of all compounds except phenol, so the addition of more acid would be expected to lower the solution pH to a point where the compounds are more completely uncharged, causing better peak shape.

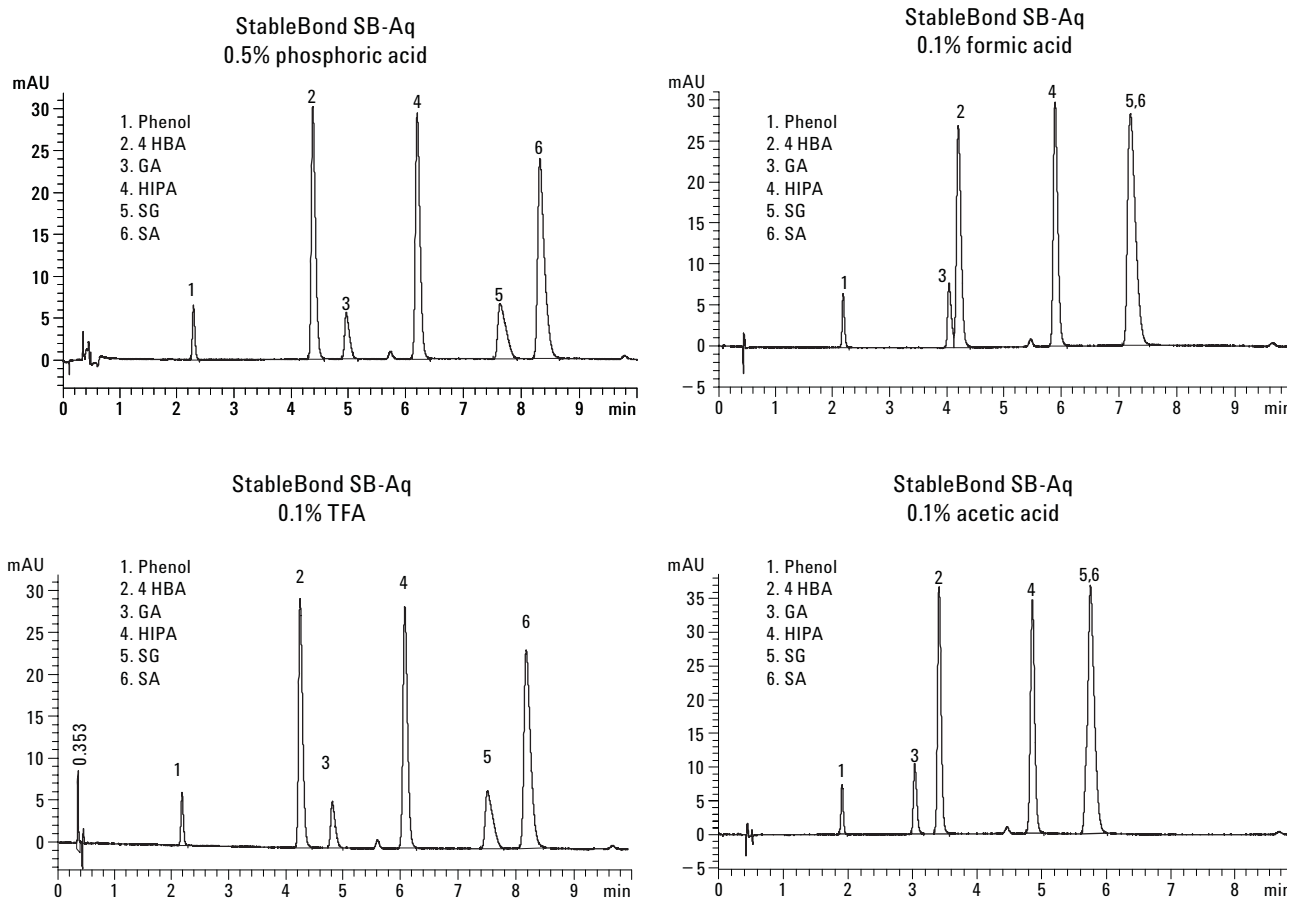


Figure 3. Effect of different acid modifiers on the separation of salicylic acid and its impurities.

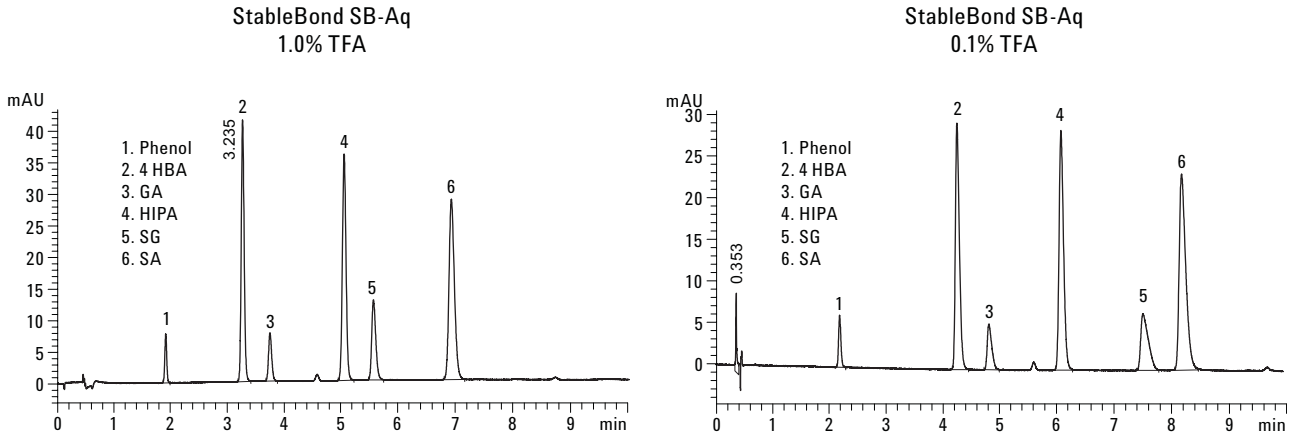


Figure 4. Effect of acid modifier concentration on separation and peak shape.

Conclusions

Evaluating several aqueous mobile-phase modifiers in method development has become more practical due to the short equilibration and analysis times afforded by RRHT 50-mm columns. Better peak shape and resolution may be achieved by simply substituting one acid modifier for another. Additionally, lowering pH (increasing acid strength) away from the pKa of the analytes can improve peak shape. This extra method development takes significantly little time when short, low-volume columns such as RRHT SB-Aq columns are used. In these cases, simple acids were used to control pH, but this concept can be extended to the evaluation of different mobile-phase buffers and optimization of buffer concentration.

StableBond columns are ideal for low-pH applications. Their unique column chemistry and variety of bonded phases, such as SB-Aq, provide broad selectivity, long lifetime, and reproducibility at low pH.

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