Waters™

응용 자료

UPLC Analysis of Benzalkonium Chloride (BAC) in Consumer Products using ACQUITY UPLC CSH C_{18}

Christopher J. Hudalla, Kenneth J. Fountain

Waters Corporation

Abstract

Analysis of BAC homologues with the ACQUITY UPLC CSH C_{18} stationary phase offers a rapid, reproducible alternative to existing methods. The UPLC method reduces solvent consumption by 95% and analysis time by 80% relative to currently accepted methods, yielding significant cost savings while enabling high-throughput analyses. Additionally, scaling methods to utilize XSelect CSH C_{18} *XP* 2.5 µm Columns results in lower system operating pressures compatible with HPLC, maximizing the number of LC instruments that can be used for these analyses and facilitating the transfer of methods between facilities with combinations of UHPLC and HPLC instrumentation.

Benefits

- Accurate determination of benzalkonium chloride (BAC) content in consumer products
- Improved peak shape and stability relative to current USP method
- 95% reduction in solvents used relative to currently accepted methods
- 80% reduction in analysis time enables high throughput analysis

Introduction

Benzalkonium Chloride (BAC) refers to a series of quaternary ammonium chloride homologues with the structure shown in Figure 1. The pervasive use of BAC in consumer products results from its antiseptic and antifungal properties with widespread applications ranging from cleaning products and disinfectants to sanitizing wipes and ophthalmic solutions. Because of its extensive use, BAC has been the subject of numerous studies, including the evaluation of the reactivity of BAC with ocular tissue[1,2] and the study of worldwide municipal wastewater, which found BAC to be the most prevalent quaternary ammonium compound in wastewater, with concentrations ranging between 200 and 300 mg/L.[3,4]

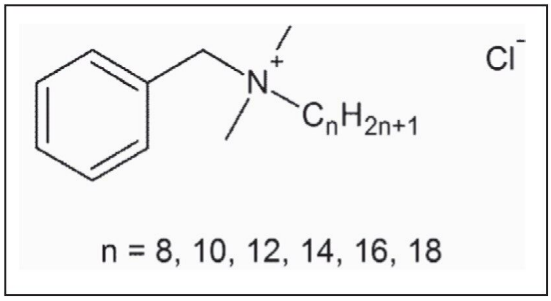

Figure 1. Structure of benzalkonium chloride (BAC). The C_{12}, C_{14}, and C_{16} homologues are the most common homologues found in consumer products.

The USP method for the quantitation of BAC utilizes a 10 µm particle size cyano column (L10) for the separation of the BAC homologues.[5] The isocratic method uses acetonitrile and 0.1 M sodium acetate (pH 5.0) as mobile phases, resulting in a separation requiring between 15 and 30 minutes. In addition to long analysis times, these separations suffer from reproducibility issues due to the traditionally poor chemical and mechanical stability of the cyano stationary phases.[6] Here we present an alternative method employing a Charged Surface Hybrid (CSH) C_{18} stationary phase under UPLC conditions, resulting in improved peak shapes with significant reductions in both analysis time and solvent consumption. Additionally, we include an example of this method that is transferred to conditions using the XSelect CSH C_{18} *XP* 2.5 µm stationary phase (UPLC and HPLC), demonstrating the ability to transfer methods across different instrument platforms.

Experimental

Sample Preparation

Standard Solution: Prepared from a USP Reference Standard containing 10% (w/v) of BAC. Diluent was 50:50 acetonitrile/water. Standard solutions were prepared at concentrations of 800, 500, 200, 100, 75, and 50 ppm (µg/mL).

Samples

Consumer products were prepared to a final concentration of approximately 80 ppm (0.008% w/v), based on

label claim, with 50:50 acetonitrile/water as diluent. Products in liquid form were diluted and analyzed without additional sample preparation or filtering.

UPLC Conditions

System:	ACQUITY UPLC H-Class with PDA Detector
Column:	ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 x 50 mm, part number 186005296
Mobile phase A:	100 mM ammonium acetate in water at pH 5.6 (adjusted with glacial acetic acid)
Mobile phase B:	100% methanol
Mobile phase C:	200 mM tetrabutyl ammonium hydrogen sulfate [TBAHS] in water for paired-ion chromatography (PIC)
Column temp.:	35 °C
Isocratic:	17% A/78% B/5% C (78% MeOH, 17 mM AmOAc, 10 mM TBAHS)
Flow rate:	0.6 mL/min
UV detection:	262 nm (40 pts/sec)
Injection volume:	8 µL
Needle wash:	50:50 acetonitrile/water
Purge:	50:50 acetonitrile/water
Seal wash:	50:50 acetonitrile/water

Note: After analysis, the chromatographic system and column were flushed with 50:50 acetonitrile/water followed by an additional flush with 100% acetonitrile to prevent any precipitation of the buffer or PIC reagent in the system or column.

Chromatography Data System

Empower chromatography software was used to control the chromatographic instrument, acquire the data, and generate the results. Empower provides the ability to create the necessary calculations such as %BAC which will be described later in this application note.

Results and Discussion

Methods for the analysis of BAC, based on the USP method, rely on cyano ligand bondings on silica base particles. Employing such methods using modern stationary phases based on high purity silica can result in a significant degradation of peak shape, with increased peak tailing and propensity for overloading. This is attributed to the lack of charged impurities in the highly pure silica base particles, resulting in an increase in the undesirable interactions between the charged analyte and the stationary phase. Paired Ion Chromatography [PIC] is a technique for separating charged analytes on reversed-phase columns by exploiting electrostatic interactions between the analyte and the charged PIC reagent. Paired-ion reagents added to the mobile phase are adsorbed onto the stationary phase, where they alter the interaction between the analyte and the stationary phase surface. For oppositely charged analytes, increased stationary phase interactions can result in analyte retention, whereas similarly charged analytes can exhibit decreased interactions and result in faster elution.[7] In this application, tetrabutyl ammonium hydrogen sulfate [TBAHS], a low UV absorbing PIC reagent, is used to reduce the unwanted interaction of the charged quaternary ammonium salt with the stationary phase, producing sharper peaks with reduced tailing. Figure 2 demonstrates the separation, based on the USP method, of BAC on a traditional Spherisorb Cyano column and on the high purity silica HSS Cyano Column, with and without the PIC reagent. Increased interactions between the BAC homologues and the high purity silica result in severe peak tailing. The addition of a similarly charged PIC reagent to the mobile phase reduces this interaction resulting in a decrease in retention and a significant improvement in peak shape.

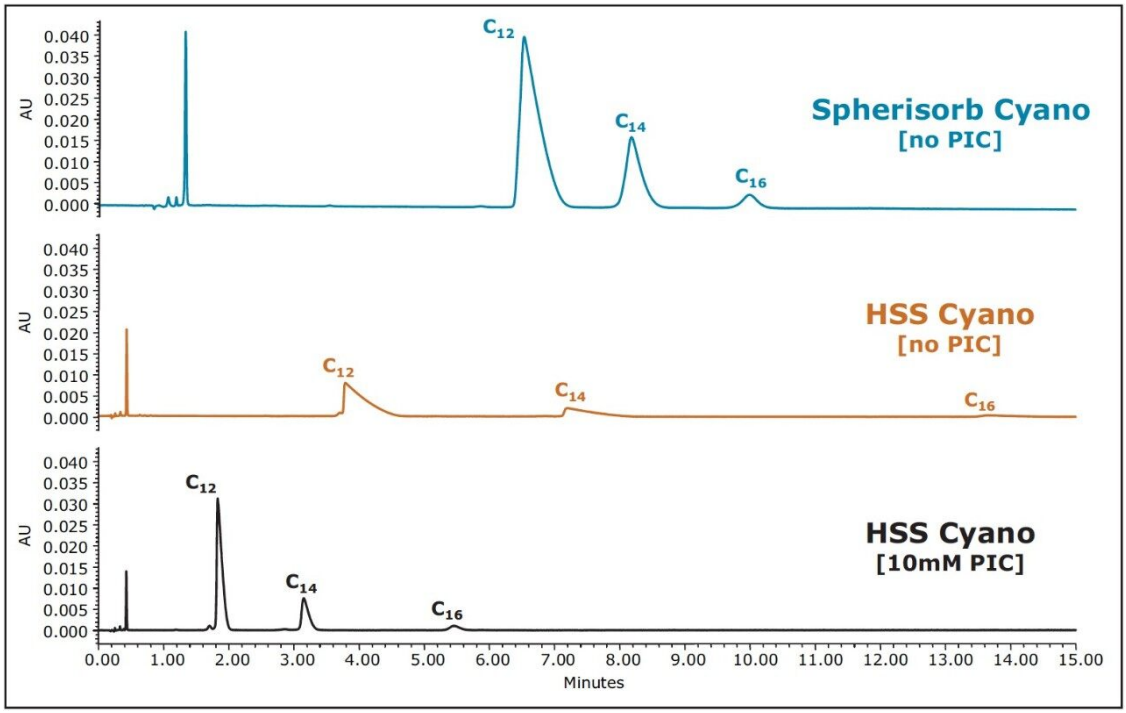

Figure 2. HPLC separations of the BAC reference standard on cyano columns: 5 µm, 4.6 x 150 mm Spherisorb Cyano (top), and on a 3.5 µm, 2.1 x 100 mm XSelect HSS Cyano, without PIC reagent (middle) and with the TBAHS PIC reagent (bottom). The isocratic separations, based on the USP method, used 45:55 acetonitrile/100 mM sodium acetate (pH=5.0) at flow rates of 1.8 mL/min for the 4.6 mm ID column and 1.2 mL/min for the 2.1 mm ID column.

Additional improvements in peak shape and analyte loadability are realized with the use of the Charged Surface Hybrid (CSH) C_{18} stationary phase, and the replacement of acetonitrile with methanol. Figure 3 demonstrates the improvement in peak shape and loading for the C_{12} BAC homologue on the CSH C_{18} Column as a function of analyte concentration and PIC reagent concentration.

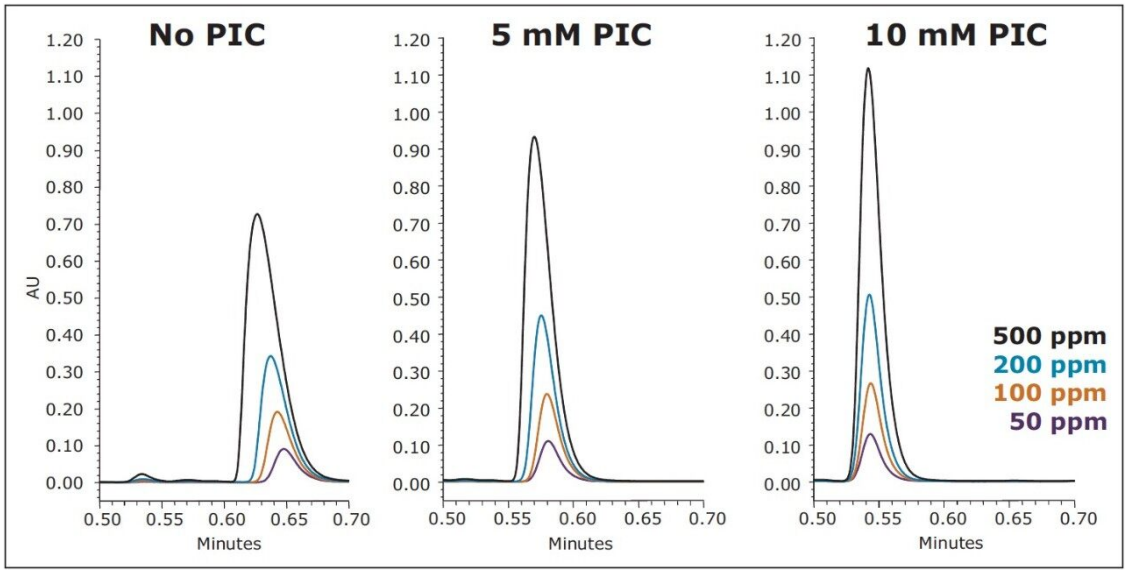

Figure 3. The UPLC separation of the C_{12} BAC homologue using a 1.7 µm, ACQUITY UPLC CSH C $_{18}$ Column (2.1 x 50 mm) under isocratic conditions with 80:20 methanol/100 mM ammonium acetate (pH 5.6). The BAC reference standard was prepared at four concentrations (500, 200, 100, and 50 ppm). Separations are shown using no PIC reagent (left), 5 mM PIC reagent (middle), and 10 mM PIC reagent (right).

Although there is an improvement in peak shape with the use of a PIC reagent, a decrease in analyte retention is also observed, as demonstrated in Figure 3. A simple adjustment in the organic concentration of the mobile phase, from 80% to 78% methanol, is all that is required to increase the retention factor, while still maintaining the improvement in peak shape. The resulting chromatography, shown in Figure 4 (bottom), under UPLC conditions, gives excellent peak shape and sensitivity for the USP reference standard for BAC, facilitating integration and quantitation. With the aid of the ACQUITY UPLC Columns Calculator, the method was also easily scaled to utilize the XSelect CSH C_{18} *XP*, 2.5 µm Column (3.0 x 75 mm) under UPLC (middle) and HPLC (top) conditions. The column dimension was chosen in order to maintain the same length to particle size ratio (L/d_p) as for the separation on the 1.7 µm particle size. When scaling methods between different column configurations, maintaining the L/d_p ratio, while scaling flow rates and injection volumes accordingly, results in similar chromatography, with different time scales.

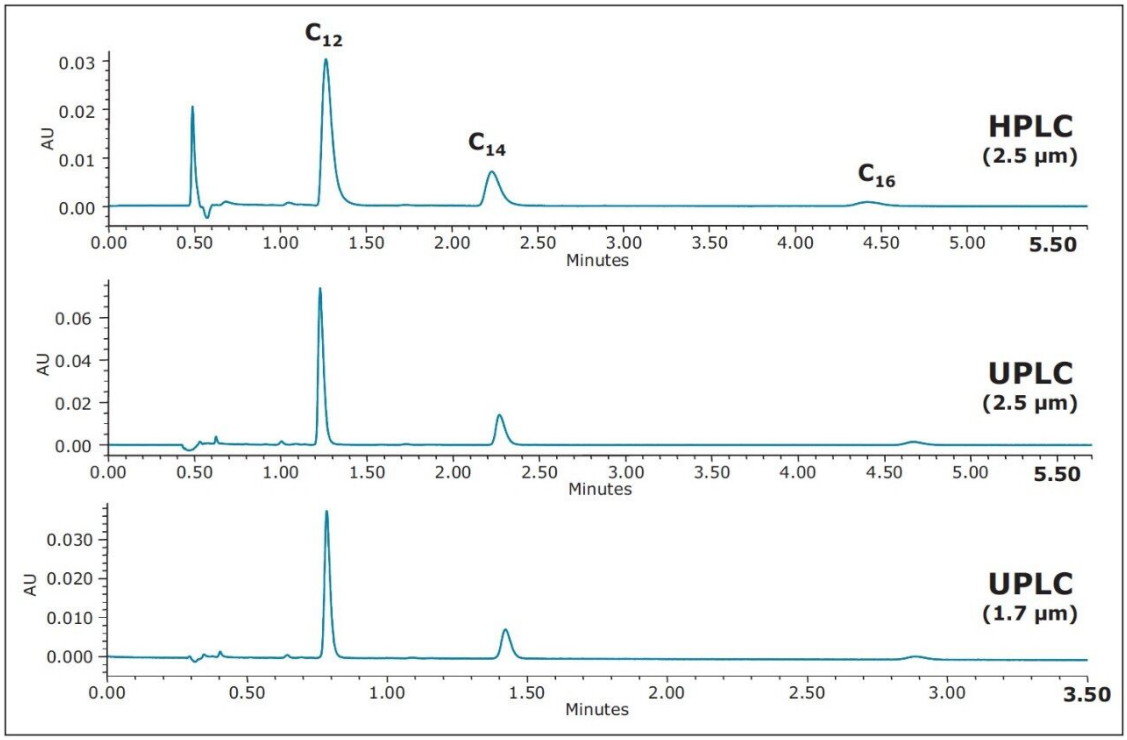

Figure 4. The UPLC separation of BAC homologues using a 1.7 µm, 2.1 x 50 mm ACQUITY CSH C_{18} Column (bottom). The UPLC isocratic separation was achieved using 78% methanol at a flow rate of 0.6 mL/min. The ACQUITY UPLC Columns Calculator was used to scale the method to utilize the 2.5 µm, 3.0 x 75 mm, XSelect CSH C_{18} XP Column under UPLC (middle) and HPLC (top) conditions. The USP reference standard for BAC was prepared at a concentration of 100 ppm.

Integration of UPLC chromatograms for the C_{12}, C_{14}, and C_{16} homologues in the BAC reference standard, prepared at various concentrations from 50 to 800 ppm (µg/mL), shows excellent linearity of detector response versus concentration with R^2 values greater than 0.999 (Figure 5).

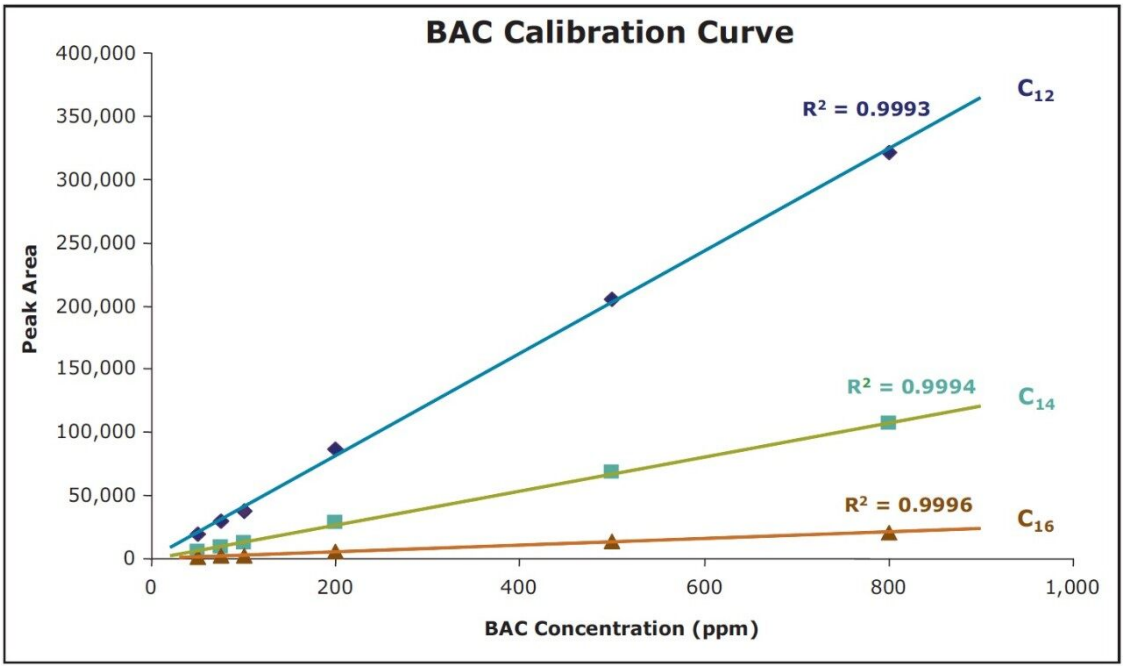

Figure 5. Calibration curve generated under UPLC conditions for the C_{12}, C_{14}, and C_{16} homologues in the USP BAC reference standard. Samples were prepared at concentrations of 800, 500, 200, 100, 75, and 50 ppm.

The UPLC method developed using the USP reference standard was applied to a variety of consumer products. Figure 6 shows a small sampling of products tested, each confirming the applicability of this method.

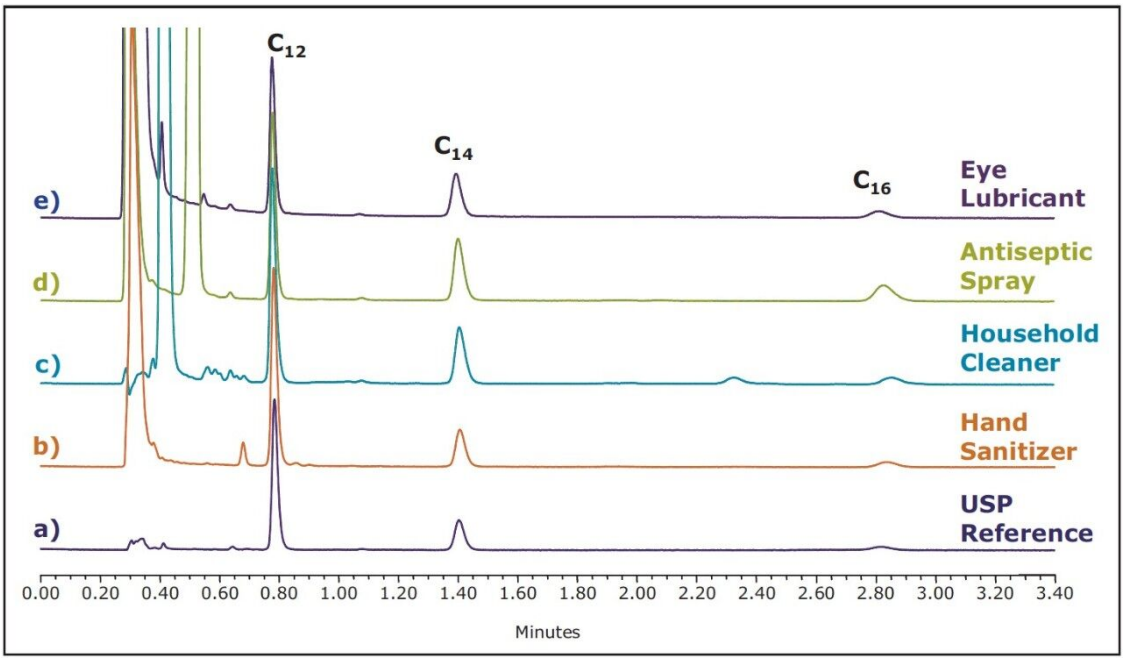

Figure 6. Application of the current method to various consumer products (from bottom to top), (a) USP reference standard-100 ppm, (b) hand sanitizer, (c) household cleaner, (d) antiseptic spray, and (e) eye lubricant. Chromatograms shown were collected on the 1.7 µm ACQUITY UPLC CSH C_{18} Column (2.1 x 50 mm) using 78% methanol at a flow rate of 0.6 mL/min.

The BAC concentrations in each sample can be calculated by integration of the individual peak areas for the C_{12}, C_{14}, and C_{16} homologues, and comparing those values with the peak areas from the BAC reference standard using the following equations (results shown in Table 1).

$$Average\ Relative\ Molecular\ Mass\ (ARMM) = \sum_{i=12,14,16} W_i \text{ x } \frac{\text{A}_\text{i}}{\text{A}_\text{T}}$$

$$Percentage\ of\ each\ homologue,\ \%C_i = \frac{W_i \text{ x } \text{A}_\text{i}}{\sum_{i=12,14,16} W_i \text{ x } \text{A}_\text{i}} \text{ x } 100$$

$$Assay\ of\ BAC\ (\%w/v) = \left(\frac{\sum_{i_{12,14,16}} W_i \text{ x } \text{A}_\text{i}}{\sum_{k_{12,14,16}} W_k \text{ x } \text{A}_\text{k}} \text{ x } Conc_{Std} \text{ x } DF \right) \div 10000$$

Where:

$W_{i,k}$ = Relative molecular mass for the given homologue: 340, 368, and 396 for the C_{12}, C_{14}, and C_{16} homologues, respectively.

A_i = Area of the peak due to the given homologue in the sample preparation.

A_k = Area of the peak due to the given homologue in the reference standard preparation.

A_T = Sum of the areas of the peaks due to all homologues in the sample preparation.

$Conc_{Std}$ = Concentration of BAC reference standard (100 ppm)

DF = Dilution Factor for sample preparation.

10000 = conversion factor from ppm to %w/v.

BAC Analysis	USP Tailing (C_{12})	USP Efficiency (C_{12})	ARMM	% C_{12}	% C_{14}	% C_{16}	Assay of BAC (% w/v)
Eye Lubricant	1.28	8,576	353.2	59.3%	30.9%	9.8%	0.011
Antiseptic Spray	1.30	7,135	357.2	50.9%	32.7%	16.4%	0.213
Household Cleaner	1.45	7,250	351.9	62.6%	29.4%	8.1%	0.178
Hand Sanitizer	1.39	7,485	349.4	70.3%	23.0%	6.7%	0.132
BAC Ref Std	1.34	7,521	349.4	69.5%	24.9%	5.5%	10.0

Table 1. Summary of BAC concentrations in consumer products.

Figure 7 shows an Empower report for a sample which was tested.

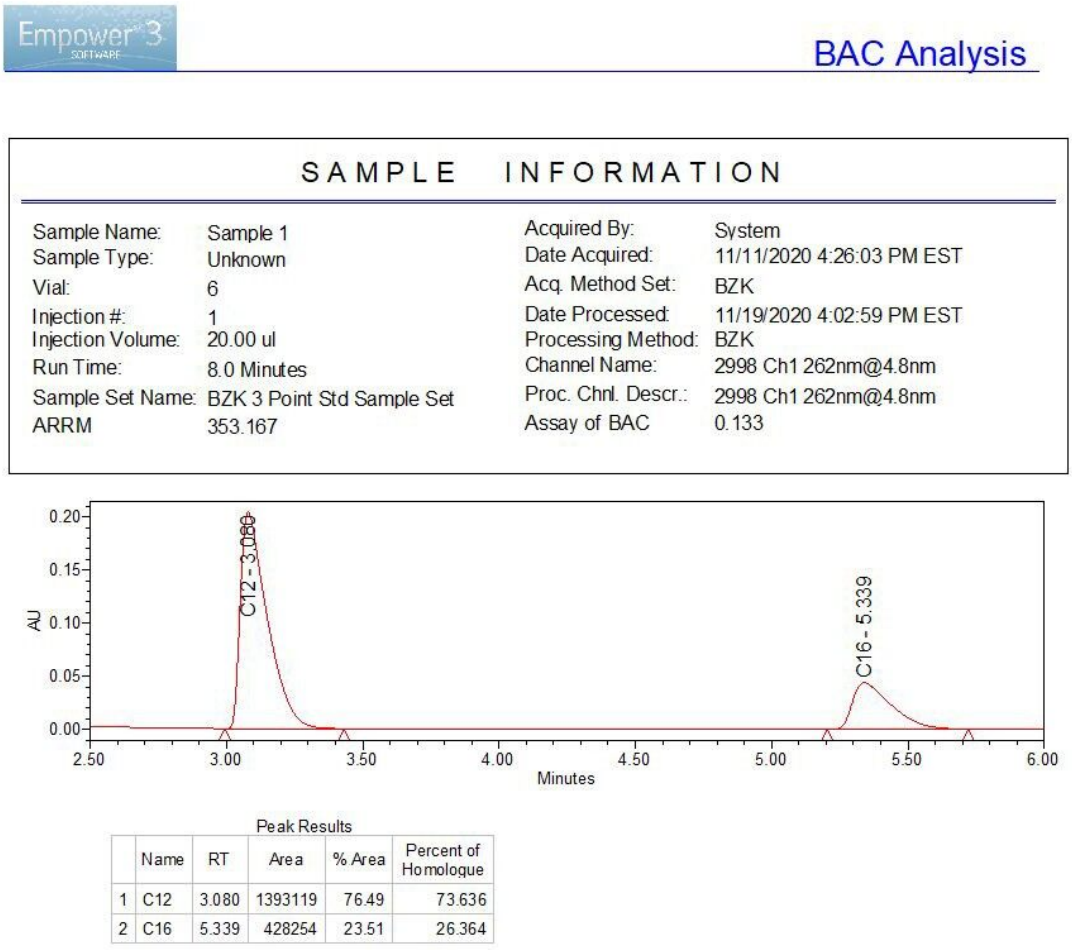

Figure 7. An Empower report showing ARRM, Assay of BAC, and percent of each homologue.

Conclusion

Analysis of BAC homologues with the ACQUITY UPLC CSH C_{18} stationary phase offers a rapid, reproducible alternative to existing methods. The use of a PIC reagent reduces the undesirable interactions between the charged quaternary ammonium analyte and the stationary phase, resulting in significant improvements in peak shape. The improvement in peak shape, in combination with the excellent linearity of response, facilitates quantitation. The UPLC method reduces solvent consumption by 95% and analysis time by 80% relative to currently accepted methods, yielding significant cost savings while enabling high-throughput analyses. Additionally, scaling methods to utilize XSelect CSH C_{18} *XP* 2.5 µm Columns results in lower system operating pressures compatible with HPLC, maximizing the number of LC instruments that can be used for these analyses and facilitating the transfer of methods between facilities with combinations of UHPLC and HPLC instrumentation.

References

1. B.J. Tripathi and R.C. Tripathi, *Lens Eye Toxic Res.*, 6(3), 395–403 (1989).
2. K.C. Swan, Reactivity of the Ocular Tissues to Wetting Agents, *Am. J. Ophthalmol.*, 27, 118 (1944).
3. C. Zhang, U. Tezel, K. Li, D. Liu, R. Ren, J. Du, and S.P. Pavlostathis, *Water Res.*, 45, 1238–1246 (2011).
4. E. Martinez-Carballo, A. Sitka, C. Gonzalez-Barreiro, N. Kreuzinger, M. Furhacker, S. Scharf, and O. Gans, *Environ. Pollut.*, 145, 489–496 (2007).
5. USP Monograph. Benzalkonium Chloride, USP35–NF30 [1708]. The United States Pharmacopeial Convention, official from August 1, 2012.
6. J.E. O'Gara, B.A. Alden, C.A. Gendreau, P.C. Iraneta, and T.H. Walter, *J. Chrom. A*, 893, 245–251 (2000).
7. B.A. Bidlingmeyer, S.N. Deming, W.P. Price, B. Sachok, and M. Petrusek, *J. Chrom. A*, 186, 419–434 (1979).

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

720004647, Revised December 2020