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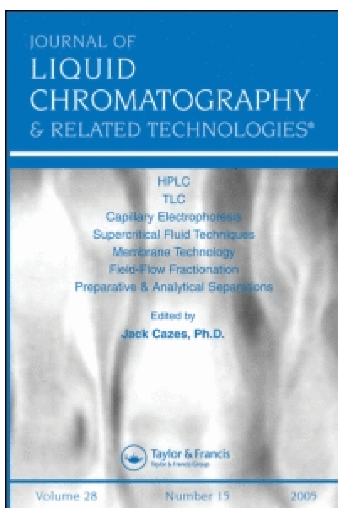

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Quantitative Analysis of a Biocide in Silicone Emulsions Using High Performance Liquid Chromatography

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QUANTITATIVE ANALYSIS OF A BIOCIDE IN SILICONE EMULSIONS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Traditional high performance liquid chromatographic (HPLC) methods are not typically used for characterization of silicones, due to a lack of absorption in the ultraviolet (UV) region of the spectrum. However, many additives in water-based, silicone formulations absorb in the UV and can be quantified using an HPLC method. Direct injection of a silicone emulsion can lead to irreversible deposition of siloxane polymer on an HPLC column and should be avoided.

This paper describes a sample preparation procedure in which the silicone emulsion is "broken" and the polymer precipitated prior to chromatographic analysis of the aqueous phase. A reverse-phase, HPLC method, originally developed by Rohm and Haas, was modified and used to quantify the two active components of Kathon® CG Biocide in Dow Corning® 1784 Emulsion using this sample preparation procedure. This

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procedure was also shown to be effective for quantitation of Kathon® LX in Dow Corning® Antifoam B and may be applicable to analysis of Kathon® in a wide variety of silicone emulsions.

INTRODUCTION

High performance liquid chromatography (HPLC) with ultraviolet (UV) detection is not commonly used to characterize silicones, due to lack of absorption for most siloxane polymers in the UV spectrum. However, many additives in water-based, silicone emulsions absorb in the UV and can be quantified using an HPLC method.

The method described in this paper was specifically developed to quantify the active components of Kathon® CG Biocide in Dow Corning® 1784 Emulsion. The method had to be compatible with an HPLC system already in place in a Quality Assurance (QA) Laboratory, which imposed some limitations on development and optimization of the method. Since the QA HPLC system did not have gradient capability, the method for Kathon® analysis had to use an isocratic solvent system. The method also had to leave no residual siloxane polymer or other contamination in the system, have no detrimental effect on the column, and otherwise cause no conflicts with the other analyses being run on the system.

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Most analyses of water soluble components in silicone emulsions start with separation of the siloxane polymer and aqueous phases in order to avoid any potential interference caused by the polymer.[1] However, in an effort to simplify the HPLC method, initial analysis of Kathon® in silicone emulsions was attempted using direct injection of the emulsion. Upon injection of the neat emulsion, the aqueous phase containing the Kathon® components passed through the chromatographic system, while the siloxane portion of the emulsion adsorbed onto the column.

The siloxane polymer was theoretically removed at the end of each run by flushing the system with tetrahydrofuran (THF). This early work only involved a few samples and the THF flush appeared to be removing the polymer from the column in between samples. However, once actual method development was initiated, involving a large number of samples, steadily increasing column back pressure indicated that the THF flush was not removing the siloxane from the column. A sample preparation procedure was developed in which the siloxane portion of the emulsion is precipitated by addition of methanol/acetic acid to the

sample followed by centrifugation, leaving the water soluble components in the aqueous phase. The aqueous phase can then be injected and analyzed without depositing polymer on the column.

MATERIALS

Dow Corning® silicone emulsions are widely used as additives in a variety of markets, including personal care products and food packaging. Water-based silicone emulsions include many application-specific formulations which contain a range of weight percent siloxane polymer as well as a variety of water soluble additives. These emulsions are complex mixtures and are challenging to characterize qualitatively or quantitatively.

Biocides are typically added to water-based silicone emulsions to inhibit bacterial growth with time. "Kathon" is a proprietary name for a family of microbiocides manufactured and marketed by the Rohm and Haas Company. Kathon® formulations all contain two active constituents: 2-methyl-4-isothiazolin-3-one (A) and 5-chloro-2-methyl-4-isothiazolin-3-one (B). The chemical structures of these compounds are shown in Figure 1. For the sake of brevity, they will be referred to as compounds or components A and B throughout the rest of this paper.

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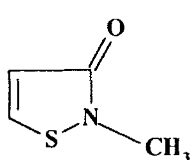
The formulation Kathon® CG (CG = cosmetic grade) is sold for use as a preservative in a very wide range of applications, including skin and body creams, shampoos, bubble bath, sun-screens, and mascara. Kathon® CG consists of 0.35% compound A, 1.15% compound B, 25% magnesium nitrate, and 73.5% water (percent by weight). According to the EEC (European Economic Community) Council Directive regarding cosmetic products, Kathon® CG is permitted for use as a preservative in cosmetic formulations with a maximum authorized concentration of 0.0015% (15 ppm) of a mixture of compound A and B in the ratio 1:3. An evaluation of a variety of analytical methods to quantify Kathon® CG in fully-formulated cosmetics was performed in 1989 at the request of the Commission of the European Communities.[2] The study concluded that a high performance liquid chromatographic method developed previously by Rohm and Haas[3] was the best method available at that time. This reverse-phase, HPLC method was modified and used with the sample preparation procedure described herein to quantify the active components of Kathon® CG in Dow Corning® 1784 Emulsion. This same method was also used to quantify Kathon® LX (food contact grade) actives in Dow Corning® Antifoam B. Both Kathon® CG and Kathon® LX contain the same concentrations of components A and B.



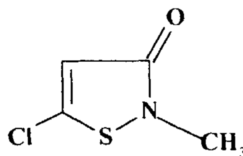


Figure 1. Chemical structure of active components in Kathon® CG and LX biocides.

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EXPERIMENTAL

HPLC Instrumentation and Conditions

A Perkin-Elmer HPLC Series 4 liquid chromatograph was used for all sample and standard analyses included in this report. The HPLC configuration used for analysis of 1784 Emulsion samples included the components/conditions as listed. Injections were performed manually.

Mobile Phase : 65/35 0.4% acetic acid in H_2O / methanol (v/v); (Millipore® H_2O, glacial acetic acid, HPLC-grade methanol).

Pump: Perkin-Elmer Series 4, single head, gradient HPLC pump; Solvent Flow Rate : 1 mL/min.

Injector : Rheodyne® Model 7126 Syringe-Loading Sample Injector (manual); Injection Volume (via injection loop) = 50 microliters.

Column Temp: Ambient.

Columns : Alltech Spherisorb® ODS-1 Classic 5 micron Analytical, 250mm x 4.6mm, catalog # 8364; Alltech PRP-1 Reversed-Phase Guard Column (Spherisorb® ODS-1, 5 micron), 25mm x 2.3mm with stainless steel holder, catalog # 79447; (total run time = 15 min.).

Detection : Waters® M-490 programmable, multi-wavelength UV Detector wavelength = 280 nm, range = 0.1 AUFS; Sampling Rate : 0.2 points per second; Temperature = ambient.

Note that the original HPLC method developed by Rohm and Haas[3] does not include acetic acid in the eluent mixture. However, of the different eluent systems evaluated by de Kruijf,[2] water with acetic acid, combined with methanol, gave the best results. The pH of Kathon® CG, as received, is low relative to Dow Corning® 1784 Emulsion (emulsion pH = 6.0 - 8.0). The lot of Kathon® used for spiking DC® 1784 Emulsion and making calibration standards had a pH of 2.6 (per Rohm and Haas). Maintaining a low pH by adding acetic acid to the HPLC eluent greatly improved the resulting chromatography. A 65/35 pure water/methanol eluent mixture was attempted during this study and the resulting peaks for Kathon® in the aqueous phase of a DC® 1784 Emulsion sample, prepared without acetic acid, were small and poorly shaped relative to an injection of the same sample using eluent and sample preparation with acetic acid. Acetic acid is a common modifier added to reverse-phase HPLC eluent systems and, in this case, may be preventing interaction of the active Kathon® components with free silanols present on the stationary phase.[4]

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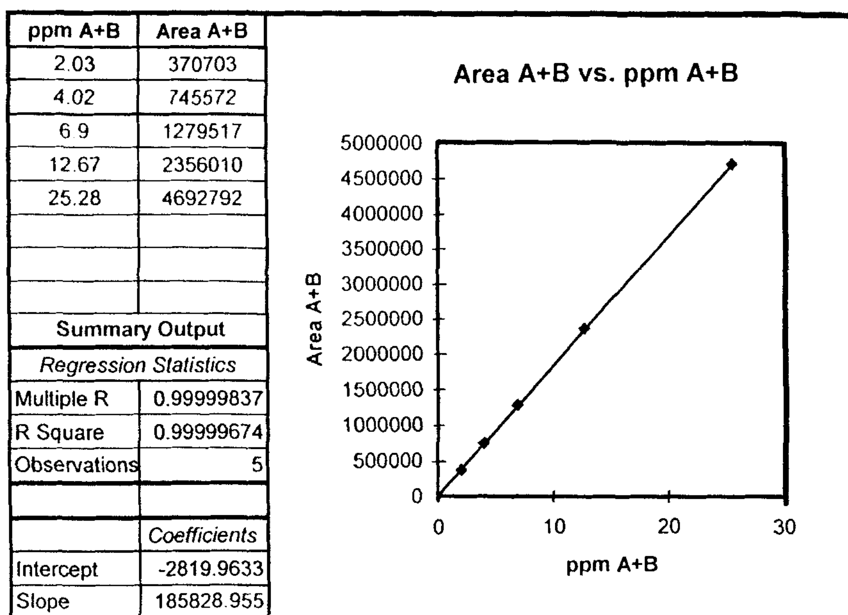
Sample Preparation

Approximately 5 gm of the emulsion sample was weighed accurately into a 1/2 oz glass bottle using an analytical balance. The weight was recorded to four decimal places. The aliquot of emulsion sample was then diluted to approximately 10 gm with methanol, containing 0.6% glacial acetic acid (v/v). The weight of diluted emulsion was also recorded to four decimal places. The dilution factor for the sample was calculated by dividing the total (diluted) sample weight by the original emulsion aliquot weight. The bottle was capped with a polyethylene-lined lid and placed on a wrist-action shaker for 30 minutes. The sample was then centrifuged for 30 minutes at 3000 rpm. The siloxane precipitated from the emulsion and was visible as a clear layer on the bottom of the bottle while the aqueous layer remained on top. An HPLC syringe was used to collect a portion of the aqueous phase for injection. Sometimes a "skin" was observed on the surface of the aqueous layer, which could be easily pushed aside before inserting the syringe needle.



ppm A+B	Area A+B
2.03	370703
4.02	745572
6.9	1279517
12.67	2356010
25.28	4692792
Summary Output	
Regression Statistics	
Multiple R	0.99999837
R Square	0.99999674
Observations	5
	Coefficients
Intercept	-2819.9633
Slope	185828.955

Figure 2. Calibration curve for Kathon® active components : area A+B *vs* ppm A+B.

Preparation of Standards and Calibration

A sample of Kathon® CG, containing 1.54%, by weight, active Kathon®, (A+B) was used to prepare a stock solution of 1526 ppm active, by dilution in the HPLC eluent. A total of 5 calibration standards were prepared by dilution with HPLC eluent, from this stock solution, at concentrations which bracketed the expected Kathon® concentration of the samples. The standards were analyzed using the conditions listed previously and a calibration curve was generated using the raw peak areas for each standard (A+B) *vs* the solution concentration for each standard (A+B).

A typical calibration curve is shown in Figure 2. Solution concentrations of the active Kathon® components in the aqueous phase of prepared emulsion samples were experimentally determined using raw peak areas and linear regression of the calibration curve. Multiplying the solution concentration of a sample by its dilution factor gave the concentration of active Kathon® present in the original emulsion sample.

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Data Collection and Chromatographic Method of Analysis

Chromatographic data acquisition and measurement of raw peak areas was performed using PE Nelson® Access*Chrom software, version 1.8. Most chromatography software packages allow use of external and internal standard methods as well as normalized area percent for quantitative analysis. An external standard method was chosen for the analysis of Kathon® CG in DC® 1784 Emulsion in order to confirm the linearity of response *vs* concentration for the two active Kathon® components. Because the two active components have different detector responses (see Discussion), response factors would have to be experimentally determined for each component in order to generate accurate concentration values using an internal standard or normalized area percent method.

A standard additions method could also be used, in which the analyte of interest is spiked into the sample, with the level of analyte originally contained in the sample being determined by extrapolation.[5] The method of standard additions is especially useful if matrix effects interfere with analyte detection. The use of a standard additions method in this case could potentially help minimize the impact of the chromatographic interferences observed with DC® 1784 Emulsion analysis (see Discussion). However, most chromatography software does not currently include standard additions as a method option and the required calculations must be done manually. Since this method was intended for implementation in a QA laboratory, it was desirable to have a method which could eventually be automated.

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RESULTS

Extraction Efficiency

The sample preparation procedure developed during this study, to enable HPLC analysis of silicone emulsions, includes both precipitation of the siloxane and extraction of the Kathon® components into the aqueous phase. Before quantitative analysis could be performed, it was necessary to determine to what extent the active Kathon® components were extracted into the aqueous phase. Extraction methods typically do not result in 100% recoveries, due to partitioning of the analyte between phases.[6] However, due to the hydrophobic, nonpolar nature of the bulk siloxane polymer, high recoveries of the active Kathon® components were expected.

Table 1

Percent Recoveries of Active Kathon® Components from Spiked Silicone Emulsion

Active Spiked (A+B)	Active Detected (A+B)	% Active Recovered (A+B)
7.67 ppm	7.36 ppm	96%
11.57 ppm	12.22 ppm	106%
15.49 ppm	14.68 ppm	95%

Three DC® 1784 Emulsion samples, spiked with a typical range of Kathon ® concentrations, were used to determine the efficiency of the extraction. The percent recovery was estimated using the difference between the amount spiked and the amount detected by the method after sample preparation (single injection). The percent Kathon® CG actives recovered from the three spiked DC® 1784 Emulsion samples are shown in Table 1. These analyses were performed on the same day that the samples were spiked.

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A recovery of 95% to 106% is a broader range than that reported previously for determining active Kathon® CG in cosmetic samples using a similar reverse-phase HPLC method, in which the cosmetics were emulsified in mobile phase and then filtered prior to injection.[2] This method reported 98% to 102% recoveries in a 0.1 to 40 ppm active Kathon® range, but it is unknown if these values were based on single injection results or averages of multiple injections. The repeatability of this previously reported method is, therefore, unknown and it is possible that the cosmetic samples analyzed did not experience the baseline fluctuation and peak interferences observed with the DC® 1784 Emulsion samples.

Repeatability and Accuracy

In order to determine the repeatability of the entire method, including sample preparation, data was generated using 11.57 ppm and 15.49 ppm active Kathon®-spiked samples. Five solutions each, of the two spiked samples, were prepared and analyzed separately on five different days. Data was generated using both combined and separate calibration curves for components A and B. The same calibration standards were used for all the analyses and were refrigerated when not in use. A new calibration curve was generated for each day of analysis.

Table 2

Repeatability of HPLC Results for Spiked Samples using Combined Calibration Curve (Conc. A+B *vs* Area Response A+B)

Kathon® Spike Conc'n. =	11.57 ppm active (A+B)	15.49 ppm active (A+B)
Sample Number		
1	12.22 ppm active (A+B)	14.68 ppm active (A+B)
2	11.67 " " "	14.72 " " "
3	11.96 " " "	15.22 " " "
4	12.34 " " "	14.70 " " "
5	12.24 " " "	14.72 " " "
	Mean = 12.09	Mean = 14.81
	S.D. = 0.2716	S.D. = 0.2309
	R.S.D = 2.2%	R.S.D. = 1.6%

The repeatability results for the method are listed in Tables 2 and 3. The relative standard deviation values listed represent ± one standard deviation. The linearity of all the calibration curves generated during this work was excellent, with correlation coefficients of 0.9999+.

Note the excellent agreement between the spiked concentrations and the experimentally determined levels of active Kathon® listed in Tables 2 and 3. In all cases the results are within the percent recovery range, i.e., 95% to 106% active. Since the average percent recovery is approximately 100% for the levels of spiked Kathon® evaluated, this method of sample preparation and quantitative analysis of DC® 1784 Emulsion using HPLC is estimated to be accurate to within 6% relative, in the range of 7 ppm to 15 ppm active Kathon®.

Detection Limit

The limit of detection is the analyte concentration which gives an instrument signal significantly different from the blank or background signal.[5] There are several ways of calculating detection limit but, for comparative purposes, the definition stated by de Kruijf,[2] i.e., 2 x baseline noise, will be used here.

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Table 3

Repeatability of HPLC Results for Spiked Samples Using Separate Calibration Curves for Components A and B

1. 11.57 ppm Active Kathon® Spike: (actual conc'n. = 2.927 ppm A, 8.640 ppm B)

Sample No.	ppm Component A	ppm Component B	ppm Component A+B
1	3.117	9.092	12.21
2	2.999	8.648	11.65
3	2.907	9.110	12.02
4	3.205	9.095	12.30
5	3.090	9.146	12.24
	Mean = 3.064	Mean = 9.018	Mean = 12.08
	S.D. = 0.1143	S.D. = 0.2081	S.D. = 0.2643
	R.S.D. = 3.7%	R.S.D. = 2.3%	R.S.D. = 2.2%

2. 15.49 ppm Active Kathon® Spike: (actual conc'n. = 3.919 ppm A, 11.57 ppm B)

Sample No.	ppm Component A	ppm Component B	ppm Component A+B
1	3.962	10.59	14.55
2	4.004	10.57	14.57
3	4.265	10.75	15.02
4	4.061	10.46	14.52
5	3.891	10.74	14.64
	Mean = 4.037	Mean = 10.62	Mean = 14.66
	S.D. = 0.1419	S.D. = 0.1228	S.D. = 0.2060
	R.S.D. = 3.5%	R.S.D. = 1.2%	R.S.D. = 1.4%

The detection limit for the active Kathon® components, using this HPLC method, is very good in the absence of any baseline disturbances or peak interference. Using peak heights, the detection limits for components A and B were estimated to be 0.013 ppm and 0.045 ppm, respectively, for a 50 microliter injection of a standard solution containing 0.59 ppm A and 1.75 ppm B. However, due to the presence of interfering peaks and baseline drift in chromatograms of the aqueous phase of DC® 1784 Emulsion samples, the detection limit in the actual sample matrix is not as low. Again, using peak heights, the detection limit for a 50 microliter injection of aqueous phase from

the 7.67 ppm spiked sample (1.94 ppm A + 5.73 ppm B) was estimated to be 0.50 ppm for component A and 1.4 ppm component B. A detection limit of 2 ppm total active Kathon® CG in DC® 1784 Emulsion, using this method, compares well with the 1 ppm detection limit reported by Rohm and Haas for analysis of Kathon® CG actives, using their recommended HPLC method for water-dilutable samples.[3]

DISCUSSION

Detector Response

The two active components in Kathon® CG do not respond equally with UV detection at 280 nm. In Table 4 are listed the peak area values for components A and B from chromatograms of five standard solutions. The area values have been normalized by dividing the raw peak area by the component concentration so that the values listed represent area response per ppm component. The decimal point was added to the area values to aid in visual comparison of the data.

The normalized data in Table 4 clearly show that component A responds more strongly at 280 nm than does component B. However, as long as the ratio of component A to B remains relatively constant at 1:3 in the sample, the total peak area response (A+B) can be used to calculate the ppm active Kathon®. If the ratio of the two components does not remain constant, for example, if one component is consumed or otherwise degraded with time relative to the other component, then each component must be quantified separately. This can be accomplished by using a separate calibration curve for each component or by using a calculated response factor for each component to correct for their difference in response. Response factors are instrument-specific and should be determined experimentally on the same system to be used for the actual analysis.

At no time during this work was a significant change in the ratio of the normalized area percent for the two Kathon® components in emulsion samples observed. Therefore, unless specified otherwise, all the sample concentrations of active Kathon® listed in this paper, determined using HPLC, were calculated using calibration curves of concentration A+B *vs* total area response. For a comparison of the repeatability of results using a calibration curve for A+B *vs* separate calibration curves for the two components, see Tables 2 and 3.

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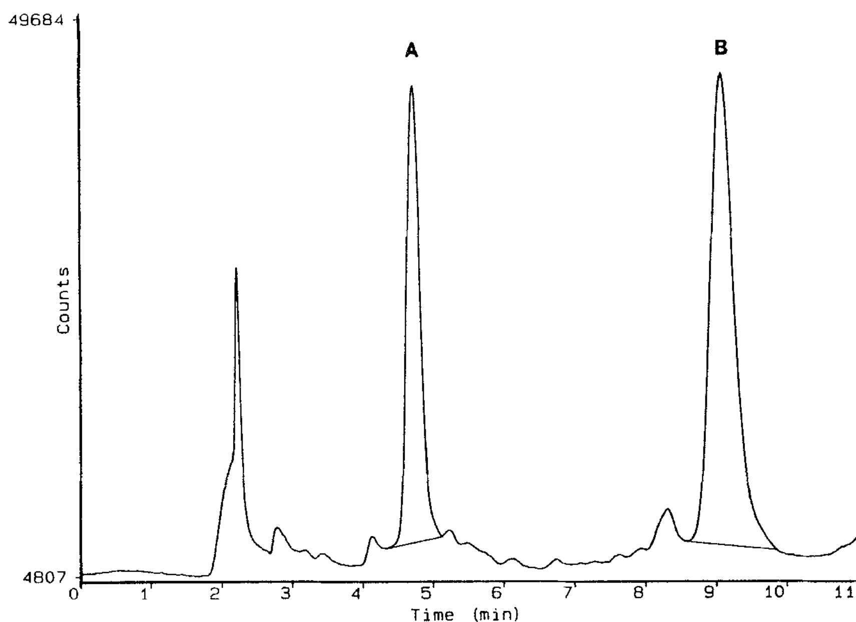

Figure 3. Active Kathon® CG components in Dow Corning® 1784 Emulsion after precipitation of siloxane.

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Table 4

Normalized Area Response Values for Components A, B and A+B

	ppm A	Area% A / ppm A	ppm B	Area % B / ppm B	ppm A+B	Area % A+B / ppm A+B
Std 1	0.51	250.076	1.52	163.291	2.03	185.094
Std 2	1.02	247.392	3.00	167.358	4.02	187.665
Std 3	1.75	251.256	5.15	165.754	6.90	187.440
Std 4	3.21	247.677	9.46	166.116	12.67	186.780
Std 5	6.40	249.064	18.88	166.367	25.28	187.303

Chromatographic Interference

All of the data listed in this report for DC® 1784 Emulsion was generated by injection of the aqueous phase of the sample after precipitation of the siloxane polymer. Unfortunately, other water soluble components in DC® 1784 Emulsion remain in the aqueous phase and cause minor interference with elution of the Kathon® components. Figure 3 is a chromatogram of the aqueous phase of a production lot of DC® 1784 Emulsion (15.5 ppm active Kathon®) after precipitation of the siloxane. The chromatographic peaks representing these other components tended to shift in position, relative to the Kathon® peaks, from injection to injection. This made manual selection of the endpoints of the active component peaks necessary. The methanol/acetic acid solution added to the emulsion was adjusted to try to match the pH of the aqueous phase of the sample to the HPLC solvent in order to minimize baseline disturbances and fluctuating retention times. The use of an autosampler, so that the time between sample injections is constant, and/or a gradient elution system could potentially make the retention times of all the eluting peaks more repeatable and enable the chromatographic software system to automatically analyze the samples. However, at the time this method was developed, the QA HPLC system did not include an autosampler or gradient elution capability, so these two options were not pursued. Another option would be to remove the interfering components from the aqueous phase before injection. This could be very difficult and would most likely result in further dilution of the active Kathon® components, which would, in turn, reduce the sensitivity of the method. In the interest of keeping sample preparation as simple as possible with minimal dilution and handling, cleanup of the aqueous phase of the sample was not attempted.

The sample preparation procedure developed for Dow Corning® 1784 Emulsion was also found to work very well for HPLC analysis of Kathon® LX in Dow Corning® Antifoam B. DC® Antifoam B is a more complex formulation than DC® 1784 Emulsion, containing a greater number and concentration of water soluble components in addition to silicone. Some of these other components are visible in the chromatogram, but the baseline variability and retention time fluctuations observed in the DC® 1784 Emulsion analyses were not seen with the DC® Antifoam B samples. A chromatogram of the aqueous phase from a DC® Antifoam B sample (19 ppm active Kathon® LX) is shown in Figure 4. The fact that this sample preparation procedure allows HPLC analysis of the aqueous phases of these two, very different emulsions demonstrates that this method may be applicable to a wide variety of silicone emulsions containing Kathon® as a preservative.

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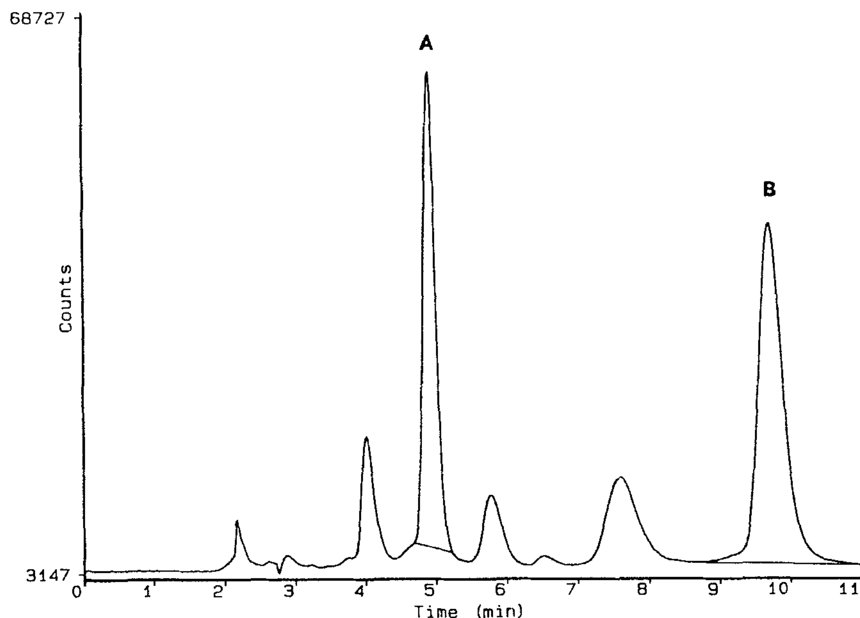

Figure 4. Active Kathon® LX components in Dow Corning® Antifoam B after precipitation of siloxane.

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