「Application Note」

Analysis of Benzalkonium chloride in Hand Sanitizing Wipes by ChroZen HPLC

- HPLC Application

Abstract

Benzalkonium chloride (BKC or BAC) is one of the biocides which are widely used in industrial applications for preservatives, disinfectants, pesticides and antiseptics, etc.

Benzalkonium chloride, as one of the humidifier disinfectants ingredients, is a cationic surfactant with the structure of quaternary ammonium chloride homologs, and chronic exposure in contact with skin or if inhaled of this chemical can cause harmful effects to humans.

In Korea, there were severe social issues regarding the use of humidifier disinfectants that caused unexplainable lung disease in pregnant women, infants, and the weak. So, many national institutes strictly regulate its safety management in the use of household chemicals and biocides.

In this study, the determination of benzalkonium chloride in hand sanitizing wipes was conducted by ChroZen HPLC (High Performance Liquid Chromatograph) according to the test methods described in Korea Pharmacopoeia and the Ministry of Environment while ensuring analysis efficiency and data reliability.

General Test Method & Preservative Test by Korea Pharmacopoeia

Instruments and Software

Item	Description	Part No.
Pump	ChroZen HPLC Quaternary Gradient Pump with Vacuum degasser	9421011020
Autosampler	ChroZen HPLC Autosampler	5421011020
Column Oven	ChroZen HPLC Column Oven	3421011020
Detector	ChroZen HPLC UV/Vis Detector with dual wavelength	7411011020
Install. Option	HPLC Performance Kit	1601011890
CDS	YL-Clarity software for single instrument of YCM HPLC	5301011000
	Autosampler control of YL-Clarity	5301011040
Column	CN (4.6 mm x 150 mm, 5 μm)	-

Reagents and Standards

- Acetonitrile (CH_3OH), HPLC Grade
- Benzalkonium chloride, ≥ 50%
- Phosphoric acid, 85% purity
- Sodium phosphate dibasic, 99% purity
- Ultrapure water, 18.2 MΩ-cm resistivity

Preparation of Standard Solution

① Add 1g of Benzalkonium to 100 mL volumetric flask, then dilute to the 100 mL mark with the mobile phase.

② Take 0.1 mL of ① into the other 100 mL volumetric flask and dilute to the 100 mL mark with the mobile phase to make its concentration to 10 μg/mL.

Preparation of Sample Solution

① Take the sample depending on the % ingredients and dilute to 10 μg/mL with the mobile phase.

② Filtrate ① to use as sample solution.

Instrument Conditions & Chromatogram

ChroZen HPLC system	
Mobile phase	ACN : 1% Na_2HPO_4 = 50 : 50 (pH 5.2, phosphoric acid)
Column	CN (4.6 mm x 150 mm x 5 µm)
Flow rate	1.0 mL/min
Temperature	40°C
Injection volume	20 µL
Detection	UV/Vis detector 254 nm

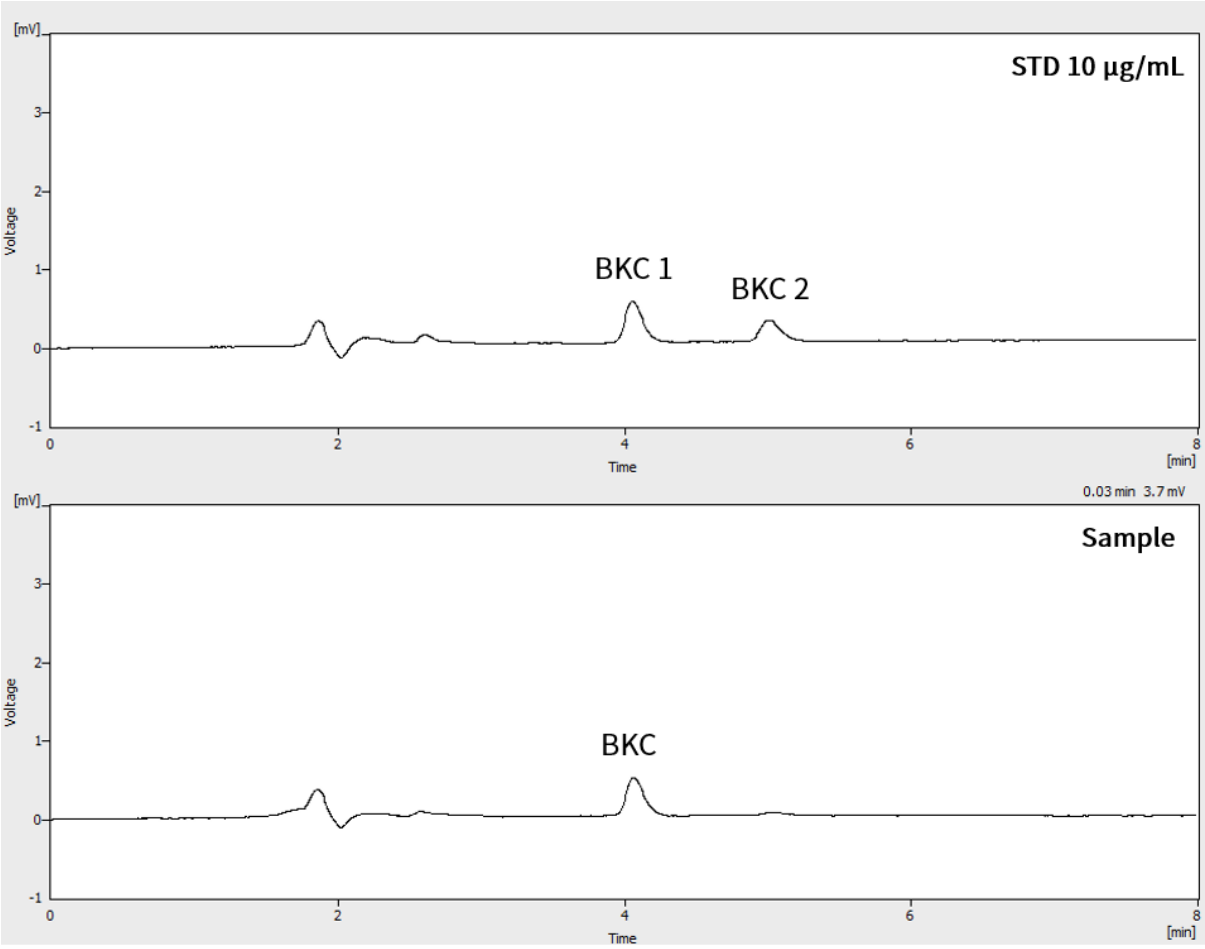


Figure 1. Chromatogram of Benzalkonium Chloride Standard & Sample

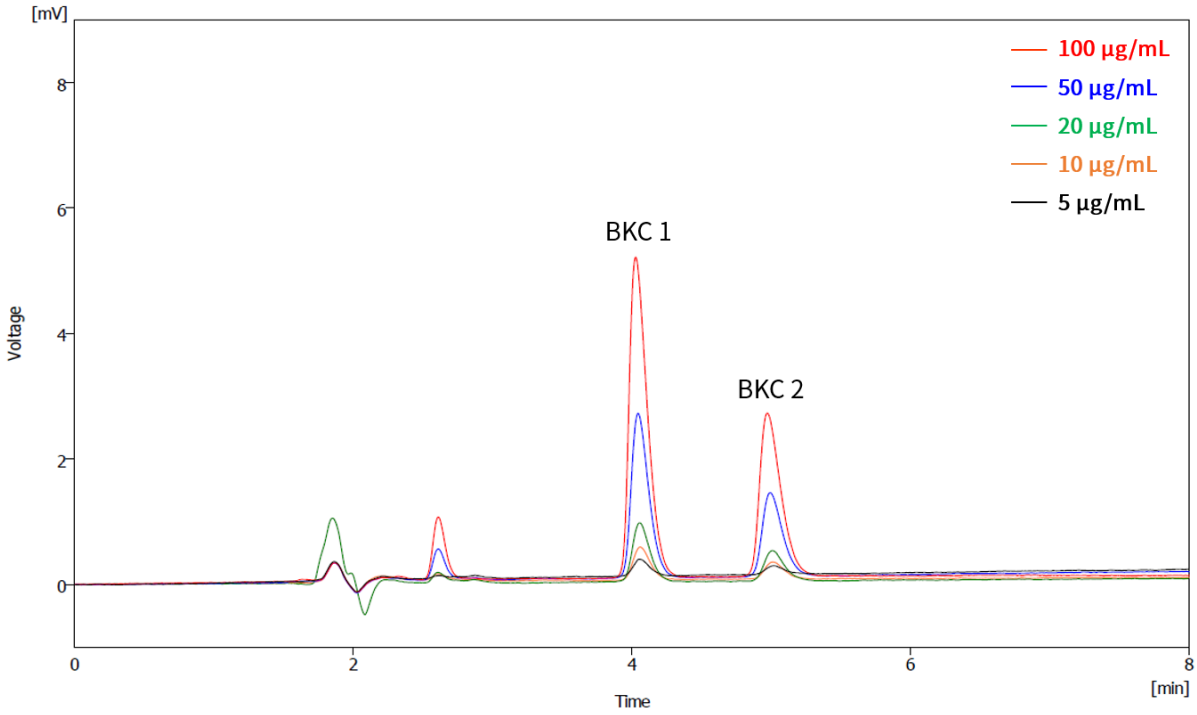
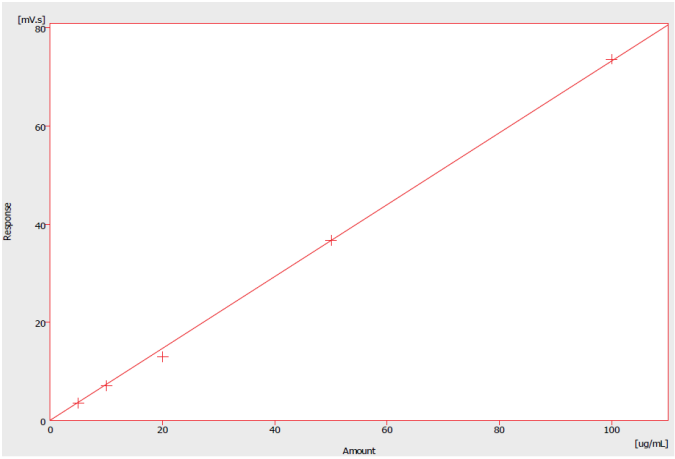


Figure 2. Chromatogram Overlay of Benzalkonium Chloride Standards



STD	Standard Concentration (µg/mL)	Area (mV.s)
1	5	3.5105
2	10	7.0135
3	20	12.8988
4	50	36.6985
5	100	73.6270
Linearity		0.9997043

Figure 3. Verification of calibration curve

Calculation

$$Amount\ of\ Benzalkonium\ Chloride(\text{mg}) = \text{Concentration of Standard (mg/mL)} \times \text{Dilution Ratio of Sample (mL)} \times \frac{A_T}{A_S}$$

A_T = Peak Area of BKC in Sample (Sum of BKC 1 & BKC 2)

A_S = Peak Area of BKC in Standard (Sum of BKC 1 & BKC 2)

$$\text{Ex.}\quad 0.01\ \text{mg/mL} \times 100 \times \frac{3.708}{7.060} = 0.525\ \text{mg}$$

Test Methods in Product of Risk Concern by the Ministry of Environment

Instruments and Software

Item	Description	Part No.
Pump	ChroZen HPLC Quaternary Gradient Pump with Vacuum degasser	9421011020
Autosampler	ChroZen HPLC Autosampler	5421011020
Column Oven	ChroZen HPLC Column Oven	3421011020
Detector	ChroZen HPLC UV/Vis Detector with dual wavelength	7411011020
Install. Option	HPLC Performance Kit	1601011890
CDS	YL-Clarity sofrware for single instrument of YCM HPLC	5301011000
	Autosampler control of YL-Clarity	5301011040
Column	C18 (4.6 mm x 250 mm, 5 μm)	-

Reagents and Standards

· Benzalkonium chloride, ≥ 50%

· Methanol, HPLC grade

· Sodium perchlorate, ≥ 98.0%

· Ultrapure water, 18.2 MΩ-cm resistivity

Preparation of Standard Solution

① Add 0.1g of Benzalkonium to 100 mL volumetric flask, then dilute to the 100 mL mark with the pure water to make its concentration to 1,000 mg/L

② Take 0 ~ 5 mL of ① with 1 mL increment to 50 mL volumetric flask and dilutie to the 50 mL mark with the pure water.

Preparation of Sample Solution

① Take 1~4 g of sample to 50 mL volumetric flask, add 10~20 mL of methanol, and then ultrasonicate for efficient extraction of sample.

② Filtrate ① to use as sample solution.

Instrument Conditions & Chromatogram

YL9100Plus HPLC system			
Mobile phase	A : 1% $NaClO_4$ B : MeOH		
Column	C18 (4.6 mm x 250 mm x 5 µm)		
Flow rate	0.7 mL/min		
Temperature	30°C		
Injection volume	20 µL		
Detection	UV/Vis detector 220 nm		
Gradient programs	Time (min)	% A (1% $NaClO_4$)	% B (MeOH)
	Initial	35	65
	20	5	95
	25	5	95
	30	35	65
	40	35	65

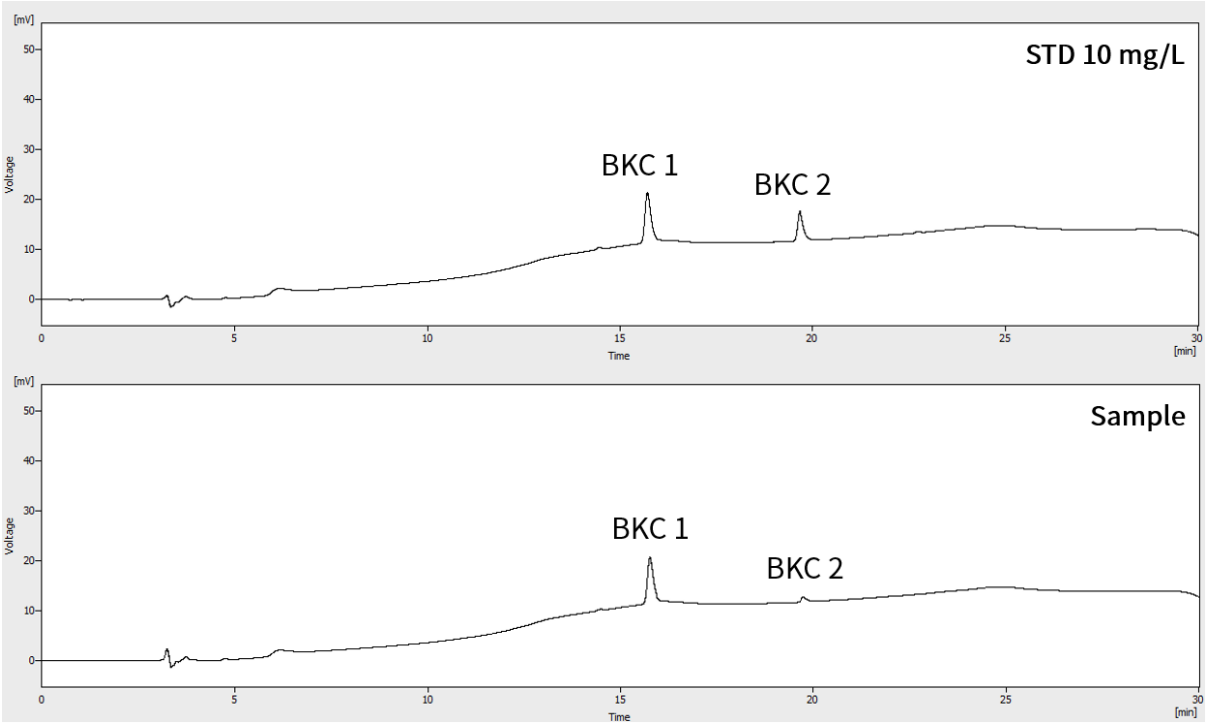


Figure 4. Chromatogram of Benzalkonium chloride Standard & Sample

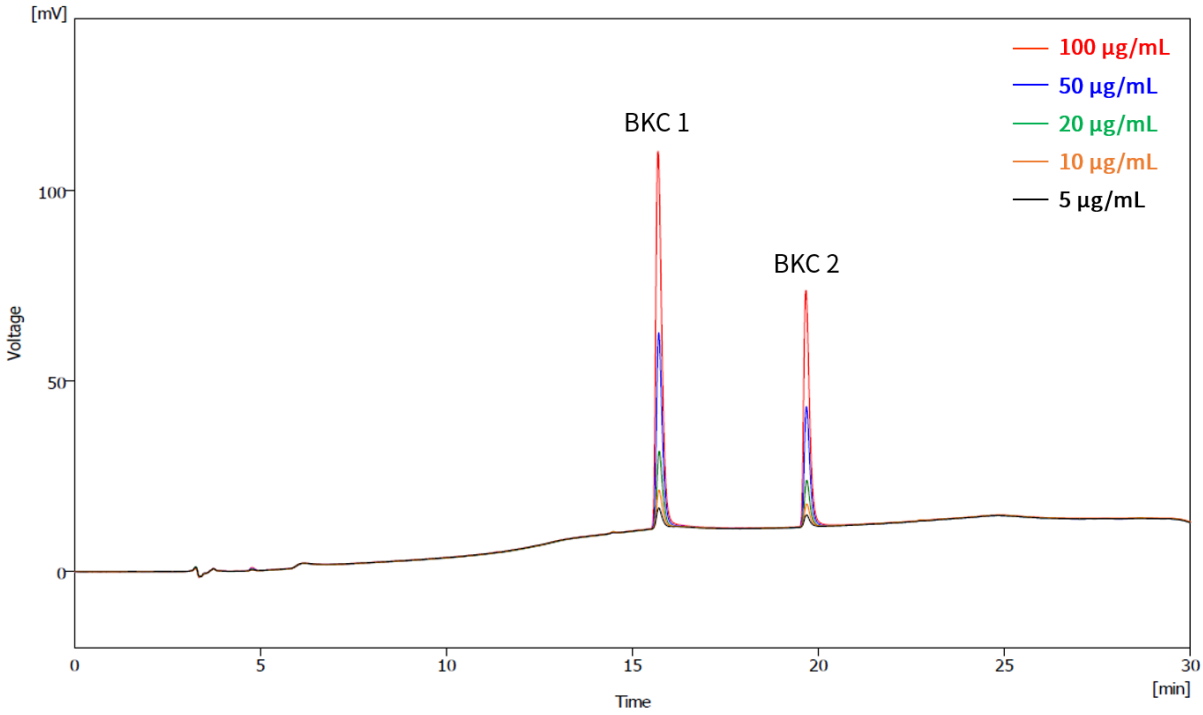
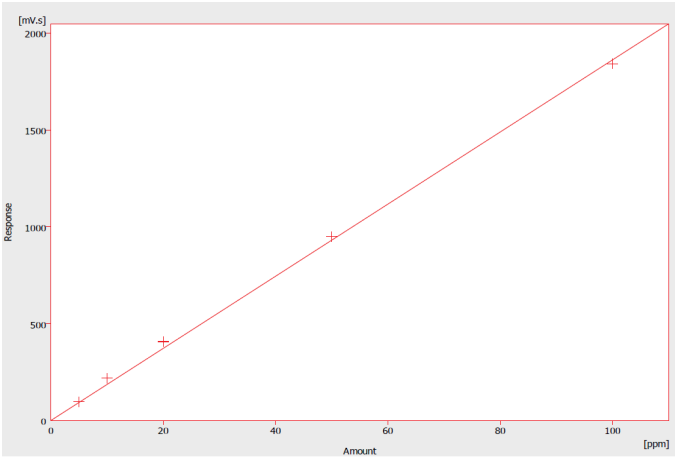


Figure 5. Chromatogram Overlay of Benzalkonium chloride Standards



STD	Standard Concentration (µg/mL)	Area (mV.s)
1	5	97.1190
2	10	218.7178
3	20	407.1826
4	50	949.2359
5	100	1841.6945
Linearity		0.9996826

Figure 6. Verification of Calibration curve

Table 1. Validity of Test Method

	MDL (mg/kg)	Linearity (R^2)	RSD (%)	Accuracy (%)
Regulation by Products of Risk Concertn	50	> 0.98	≤ 30	70 ~ 130
Result	0.13	0.99968	0.043	100.30
	Pass	Pass	Pass	Pass

Calculation

$$Amount\ of\ Benzalkonium\ Chloride\ in\ the\ Sample(\mathrm{mg/kg}) = \frac{(C_1 - C_0)}{W_d} \times f \times V$$

C_1 = Concentration of BKC in the Sample (mg/L)

C_0 = Concentration of BKC in Method Blank (mg/L)

f = Dilution Rate (In case of out of linear range)

V = Volume of Extracted Sample (mL)

W_d = Amount of Sample(g)

Ex. $\frac{(6.242 - 0)}{1\mathrm{g}} \times 1 \times 50 = 312.1\ \mathrm{mg/kg}$

Promising Solution for the Test Method of Product of Risk Concenrns by the Ministry of Environment

Instrument Conditions & Chromatogram

ChroZen HPLC system	
Mobile phase	1% $NaClO_4$: MeOH = 35 : 65
Column	CN (4.6 mm x 150 mm x 5 µm)
Flow rate	1.0 mL/min
Temperature	30°C
Injection volume	20 µL
Detection	UV/Vis detector 220 nm

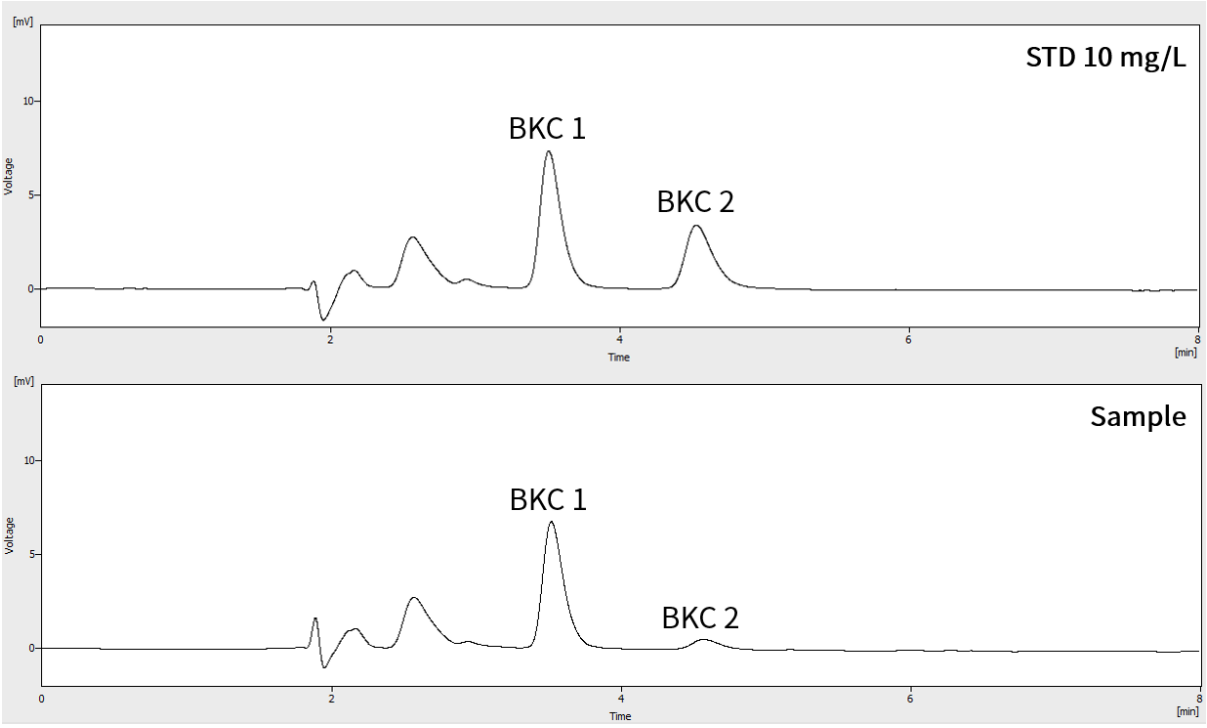


Figure 7. Chromatogram of Benzalkonium chloride Standard & Sample

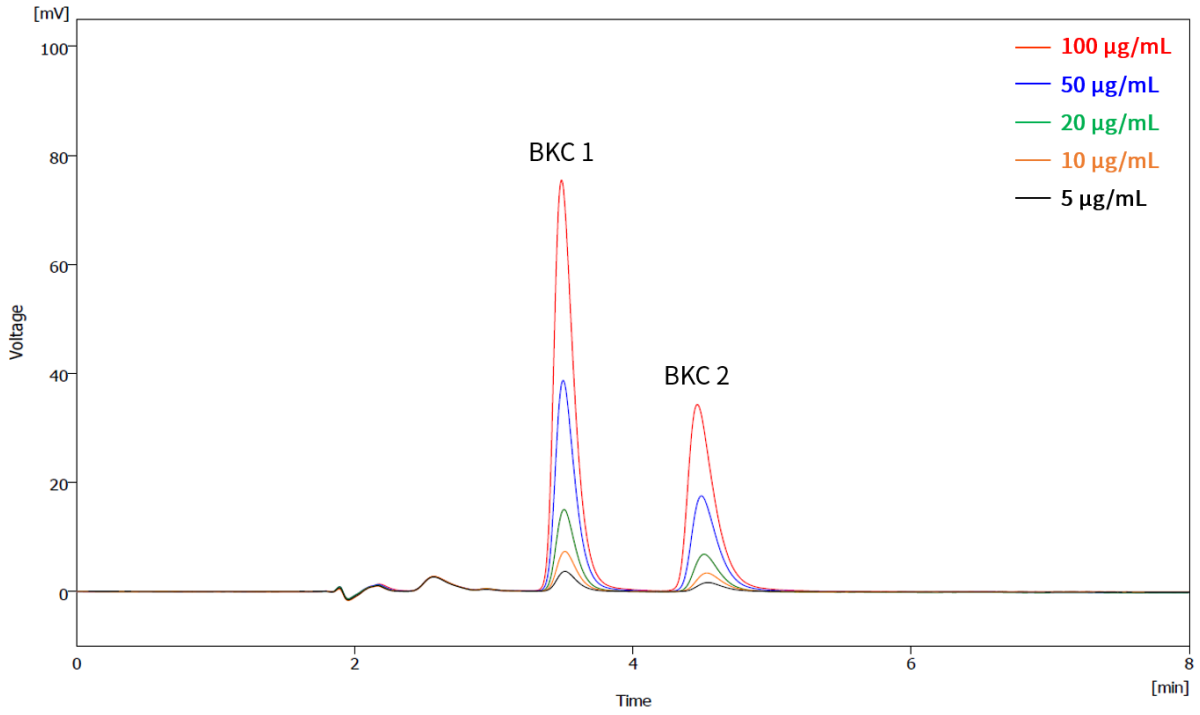
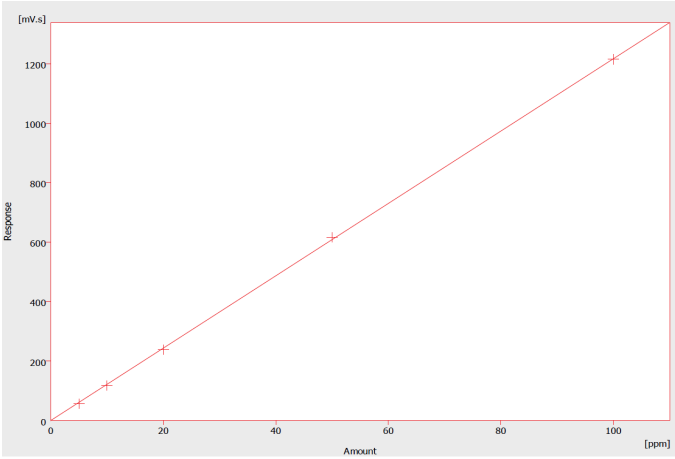


Figure 8. Chromatogram Overlay of Benzalkonium chloride Standards



STD	Standard Concentration (µg/mL)	Area (mV.s)
1	5	56.3969
2	10	117.2638
3	20	238.2750
4	50	616.2953
5	100	1215.1720
Linearity		0.9999506

Figure 9. Verification of Calibration Curve

Table 2. Validity of Test Method

	MDL (mg/kg)	Linearity (R^2)	RSD (%)	Accuracy (%)
Regulation by Products of Risk Concertn	50	> 0.98	≤ 30	70 ~ 130
Result	0.63	0.99995	0.346	95.98
	Pass	Pass	Pass	Pass

Conclusion

In this study, the determination of Benzalkonium Chloride was conducted by YoungIn Chromass's new HPLC, ChroZen HPLC, referring to several Korean standard methods. The linearity for each analyte was evaluated to verify the validity of the analysis results. The method detection limit (MDL) and precision (RSD%) were calculated as indicated in the quality control methods.

The linearity for all 3 test methods resulted in greater than 0.999. The test results referring to the test methods by the Korea ministry of environment satisfy the regulated standards which precision is 0.043% and the accuracy is 100.30% as indicated in [Fig 6] & [Table 1].

The promising solution which shortens the analysis time to 5 times shorter than the one in the standard method approves the results; 0.346 % for precision and 95.98 % for accuracy which of both fully comply with the regulated level.

The analytical condition described in this application can be recommended depending on the sample matrix and the impurities presence to optimize the test results.

Reference

- Act on the Safety Management of Household Chemicals and Biocides'
- General test methoods & preservative test methods by Korean Pharmacopoeia
- Evaluation for products of risk concerns for safety by the Korean ministry of environment
- Guidelines for the safe use of COVID-19 sterilization and disinfection products
- Study on the Respiratory Toxicity of Biocide, Benzalknonium Chloride

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