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Development and Validation of a precise method for determination and quantitative evaluation of Benzalkonium Chloride in General purpose Disinfectant product.

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Abstract:*Benzalkonium chloride is a quaternary ammonium antibacterial and disinfectant with properties and applications comparable to cationic surfactants. Because of their broad-spectrum antimicrobial activities against bacteria, fungi, and viruses, benzalkonium chlorides (BKCs) are widely used compounds. The goal of this work was to create an effective high-performance liquid chromatography (HPLC) method for determining and quantifying benzalkonium chloride in a cleaning solution quickly and accurately. On a Waters Spherisorb® CN (4.6x150 mm) column packed with 5 m particles, the analyte was chromatographed. The mobile phase was a 40:60 (v/v) mixture of Sodium acetate buffer (pH 5.05) and acetonitrile, pumped at a flow rate of 1.0 mL/min while maintaining column temperature at Room temperature, as determined by an experimental design. At 254 nm, the maximum UV detection was attained. In terms of specificity, linearity, repeatability, and method accuracy, the method was validated. Without interference from common excipients, the approach was effectively used to determine and quantify BKC in a general-purpose disinfectant cleaning solution. According to ICH criteria, all validation parameters were within the acceptable range. Apart from direct dilution, the approach is straightforward and does not necessitate any sophisticated sample preparation.*

Keywords*(Benzalkonium chloride, Detection, Test method Validation, General purpose Disinfectant cleaner, Isocratic High performance liquid chromatography, Mobile phase)*

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I. Introduction

Benzalkonium Chloride is predominantly utilized as a preservative and antibacterial agent, with surfactant usage coming in second. It kills bacteria and prevents them from growing in the future. Cleaning solutions are compounds or mixes of substances used to combat pathogens that are hazardous to human or animal health. Quaternary ammonium salts, such as benzalkonium chloride (BKC), Didecyl Dimethyl Ammonium Chloride (DDAC), and others, have antibacterial properties. Benzalkonium chloride (BKC) is a cationic surfactant used in personal hygiene, cosmetics, and skin disinfection products that belongs to the quaternary ammonium compounds (QAC) class. [1]. BKC is a mixture of alkylbenzyldimethylammonium chlorides [C6H5CH2N(CH3)2RCl], with R = C12, C14, C16, C18 (CAS#8001-54-5) as the major compounds and antibacterial properties provided by the alkyl chain length and quaternary ammonium groups. [1,2].

Because of the ubiquitous use of BKC, it is a critical requirement to determine and quantify the BKC component. 3M™ P2 General Purpose Cleaner and Disinfectant is a concentrated disinfectant/cleaner that contains the active component anti-microbial BKC (Benzalkonium chloride) and is perfect for cleaning hard surfaces such as floors, platforms, and tiles quickly and affordably. Al-kyldimethylbenzylammonium chloride is another name for benzalkonium chloride (ADBAC). It is made up of alkylbenzyldimethylammonium chlorides with different even-numbered alkyl chain lengths. This substance belongs to the qua-ternary ammonium group and is a nitrogenous cationic surface-acting agent. It has three primary applications in the chemical industry: as a biocide, cationic surfactant, and phase transfer agent. It has a wide range of applications, from disinfectant formulations to microbial corrosion inhibition in the oilfield.It's one of the safest synthetic biocides on the market, with a proven track record of effectiveness. Its overall safety is demonstrated by its usage as a preservative in cosmetics such as eye and nasal sprays. This work describes a simple, precise, and accurate liquid chromatographic method for quantifying BKC in a disinfection product for general use. As a result, the goal of the current study is to design and validate a simple, precise, and accurate HPLC-UV method for quantifying benzalkonium chloride (BKC).

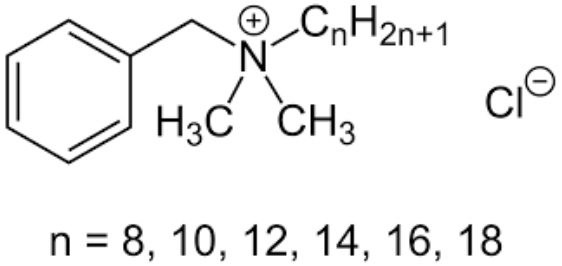

Figure 1. Structure of benzalkonium chloride (BKC).

II. Materials and Methods

Materials

All experiments were performed using 'A class' volumetric glassware, Benzalkonium chloride standard(Certified reference material Sigma-Aldrich make), General purpose Disinfectantcleaner product(3M India Limited manufactured),Acetic acid glacial, (Analytical grade, Merck Life Sciences Private Ltd, India), Sodium acetate, LiChropur^{TM}(Sigma-Aldrich), Acetonitrile for chromatography(Merck Life Sciences Private Ltd, India) were employed for preparation of mobile phase, standard and sample preparation. The highly pure HPLC grade Milli Q water was used as diluent solvent in sample and standard aliquots. The mobile phase was filteredthrough 0.45 µm nylon-membrane filter (3M Purification Inc) and degassed under vacuum, prior to use. The sample 3M^{TM} P2 Benzalkonium Chloride Cleaner Solution declaring to contain 2.25% (w/v) which is general purpose cleaner cum disinfectant solution were obtained from M/s 3M India Limited for analysis.

Instrumentation and ChromatographyThe liquid chromatograph consisted of a Waters alliance, equipped with Waters e2695 separation module and a 2998 PDA detector. Data collection and interpretation was done usingEmpower software.

Methods

Buffer preparation:

Preparation of Dilute glacial acetic acid: Dilute 12 g of glacial acetic acid to 100 ml with water.

Buffer preparation: Dissolve and dilute 8.2 g of sodium acetate anhydrous into 900 ml with water. Adjust to the pH 5.05± 0.05 with dilute glacial acetic acid, to produce 1000 ml with water.

Mobile phase preparation: Mix Buffer and Acetonitrile in the ratio of 40: 60 v/v. Filter through 0.45µ-membrane filter, sonicate to degas. The liquid chromatography was performed using anAnalytical column, Waters Spherisorb CN, (4.6 mm x 150 mm) column packed with 5µm particles as stationary phase and a mobile phase consisting of buffer preparation and acetonitrile (40:60 v/v),which was filteredthrough 0.45 µm nylon-membrane filter (3M Purification Inc.) and degassed under vacuum, prior to use. The mobile phase was delivered at a flow rate of 1.0 mL/min. The UV detection and quantification were done at 254nm by injecting 50 µL of sample & standard, with the above chromatographic conditions and after partition equilibration, well-shapedsymmetric peak was eluted.

Stock standard, System suitability standard solution& sample solutions

200 mg of benzalkonium chloride (Sigma Aldrich) of high purity and known potency was weighed and transferred to a 100 mL volumetric flask, about 60 mL of HPLC grade Milli Q water was added and sonicated to dissolve, diluted up to volume with same solvent and mixed. Further 2.5 mL of this solution was diluted to 10 mL with Milli Q water and mixed (250 mcg/mL). The suitability of system and stability of the solution was checked over the period by injecting this solution.

Sample solution

The P2 solution was accurately weighed to 1.1 gms in 100 ml volumetric flask and diluted up to volume with milli Q water mixed which was assayed & quantified.

Validation parameters:

1. Specificity/Selectivity

The specificity of the assay method will be investigatedby injecting of the extracted placebo to demonstrate theabsence of interference with the elution of analyte.

The excipient compounds must not interfere with theanalysis of the targeted analyte.Specificity of an analytical method is its ability to measure accurately and specifically theconcentration of analyte of interest without interference from otheringredients of the samples, diluent, mobile phase.

1. Diluent
2. Placebo solution
3. Placebo spiked with BKC at target concentration
4. Standard solution
5. Sample solution

Above mentioned each solution was injected to the liquid chromatograph, equippedwith photo diode array detector. Chromatograms were recorded and interference of mobilephase and placebo with analyte's peak was observed.

Preparation of Placebo spike with Analyte (BKC)

Transferred accurately 25 ml of BKC standard stock solutionin to a 100mL volumetricflask, and about 1.1 gm of Placebo solution was added and make up to 100 mlwith HPLC grade Milli Q water and mixed. This was injected as placebo spike.

2. Linearity

Standard solutions were prepared at six concentrations,25, 50, 75, 100, 125, and 150% of targetconcentration. Three individually prepared replicates ateach concentration will be analyzed. The method ofstandard preparation and the number of injections willbe same as used in the final procedure.

The linearity study verifies and approves that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. Standard solutions of six concentration levels of BKC, from 25 to 150% of target concentration were prepared in triplicate at each level to perform the study. (25, 50, 75, 100, 125 and 150 %). The results from experimental study were graphically plotted, obtained a calibration, Calculate the mean, standarddeviation, and Relative Standard Deviation (RSD)for each concentration. Plot concentration (x-axis) versusmean response (y-axis) for each concentration. Calculatethe regression equation and coefficient of determination(r^2).

3. Accuracy (by recovery method)

Spiked samples will be prepared at three concentrations over the range of 80 to 120% of the target concentration. Three individually prepared replicates at each concentration will be analyzed.

Accuracy of a method is defined as the closeness of the measured value to the true value for the samples. The recovery method was studied by spiked samples that will be prepared at three concentrations over the range of 80 to 120% of the target concentration of BKC.

Transferred accurately weighed 200 mg of benzalkonium chloride in to a 100 mL volumetric flask, about 60 mL of HPLC grade Milli Q water was added and sonicated to dissolve, diluted up to volume with same solvent and mixed. This recovery stock solution was suitably further spiked with placebo to get desired different concentrations over the range of 80 to 120% of BKC target concentration.

4. Precision - Repeatability

The precision is the parameter that expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analysis of the same homogenous sample under the prescribed conditions.

Method precision (Repeatability)

This experiment was conducted to prove the repeatability of the assay results obtained by this quantification methodology. For six consecutive times, a same system suitability standard solution (refer Method section) was injected and percentage standard deviation and relative standard deviation (R.S.D.) were observed. Method repeatability study can be found successful when, the R.S.D. value is lesser or equal than 1.5% for area of principal peak in each of the six sets.

5. System suitability (Instrumental precision)

The purpose of this experiment was to demonstrate the suitability of the HPLC system prior to start actual analysis of samples. Prepared the system suitability standard solution as mentioned (Refer method section) and injected six replicate injections of this solution. By recording chromatograms, measured the peak responses for interested analyte peak, and instrumental precision was demonstrated in terms of percentage relative standard deviation for area of analyte peak. Other parameters, like tailing factor, retention time and column efficiency in terms of theoretical plates of analyte peak was also observed.

III. Results And Discussion

Method development

To optimize the simple and precise method following parameters wereexamined; theamount of acetonitrile and phosphate buffer in the mobile phase, mobile phase flow rate was examined. WatersSpherisorb® CN,

(4.6 mm x 150 mm), 5µm column and a mobile phase constituted from amixture of foresaid buffer and acetonitrile (40:60) as organic modifier was foundappropriate to obtain an adequate peak shape and shorter analysis time. The aim ofdevelopment and validation study were to have a simple, specific, and reproducibleLC chromatographic method for quantification of BKC in Cleaner solution.

1.Specificity / selectivity

The observations from the chromatogram of BKC specificity study was that the componenteluted at retention time of about 3.6 minutes. Nointerferences with the analyte peaks due to placebo or mobile phase have been observed. Based on the study and above-mentioned observations and results, the method established as specificfor the qualitative and quantitative analysis of BKC in the said P2 cleaner formulation.

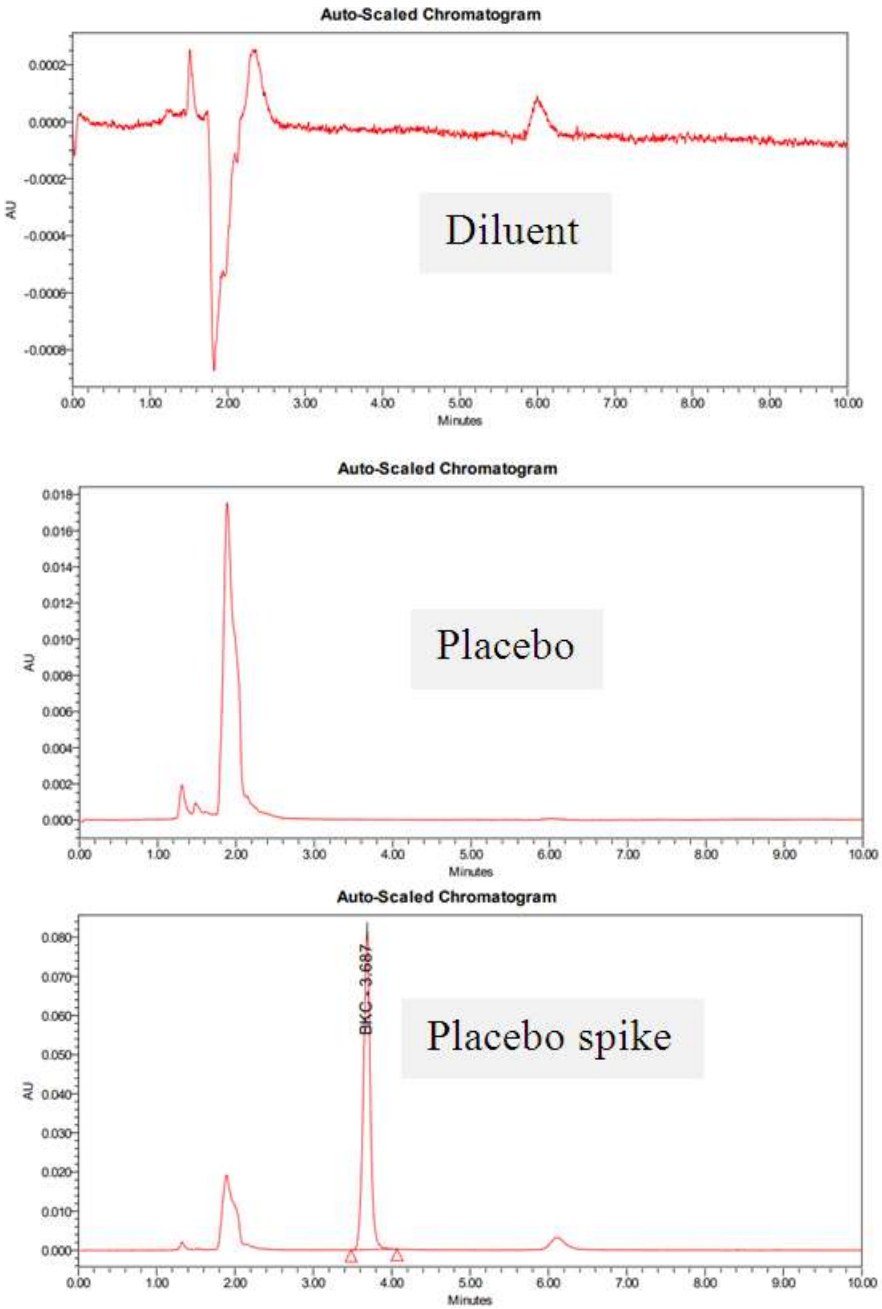

Figure 2. Chromatogram of Diluent, Placebo and Placebo spike.

2. Linearity

The linearity was determined as linear regression with least square method on standardsolution. Concentration levels were 0.4, 5, 25, 50, 75, 100, 125 and 150% of the claimed analyteconcentration, corresponding to the range of about 1–375 mcg/mL. The calibration curvesobtained by plotting the BKC peak area versus the concentration of standard solution and the result wasfound linear in the mentioned concentration range of 25% to 150%. For acceptance oflinearity, correlation coefficient of linearity curve shall not be less than 0.990 and Yinterceptbias cannot be within ±2.0 % of 100% linearity level response. The resultsindicated that the method is linear up to the specified range of concentrations.

Table 1. Data of linearity study

	Benzalkonium chloride		
Sl no.	**Concentration in %**	**Concentration in µgm/ml**	**Peak Area**
1	0.4	1.0	1149
2	5	12.7	22918
3	25	63.3	119779
4	50	126.5	246004
5	75	189.8	371952
6	100	253.1	492682
7	125	316.4	628507
8	150	379.6	751496
	Correlation coefficient (R^2)		**0.9999**

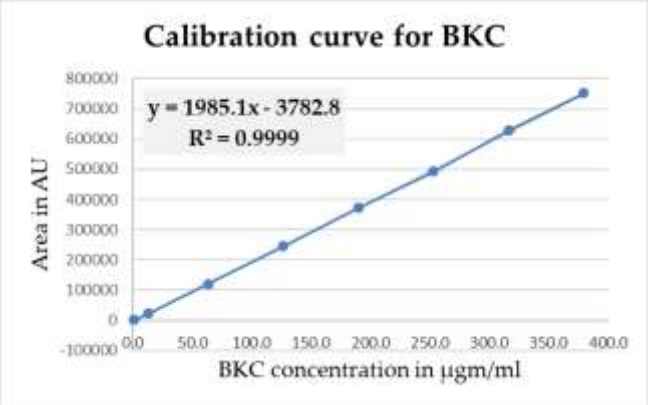

Figure 3. Linearity curve of Benzalkonium chloride (BKC).

3. Accuracy (by recovery method)

The accuracy of the method was determined by measuring the analyte recovery and by study of stock recovery solution. The study was performed to determine eventual positive or negative interferences produced by the excipients in the formulation. The results obtained for the accuracy study in the samples ranging a BKC concentration between 80, 100 and 120 % and being the 100% corresponding to 250µgm/mL (n=3 for 50%, 80% and 120%) indicated that the recovery percentage was between 99.5% and 101.0% of injected. The results were found within the acceptance criteria with acceptable %RSD of within 2.0% at each level. The recovery at each level were between 98.0 to 102.0% which indicated that the method is appropriate to produce accurate estimation of BKC preservative analogue in said formulation.

Table 2. Data of accuracy by recovery study

Sl no.	**Recovery Level**	**% Recovery**	**Average**	**%RSD**
1	Level 80%-1	101.1		
2	Level 80%-2	100.9	**101.0**	**0.1**
3	Level 80%-3	101.1		
4	Level 100%-1	99.8	**99.9**	**0.1**

5	Level 100%-2	100.0		
6	Level 100%-3	99.8		
7	Level 120%-1	99.4		
8	Level 120%-2	99.6	**99.5**	**0.1**
9	Level 120%-3	99.6		

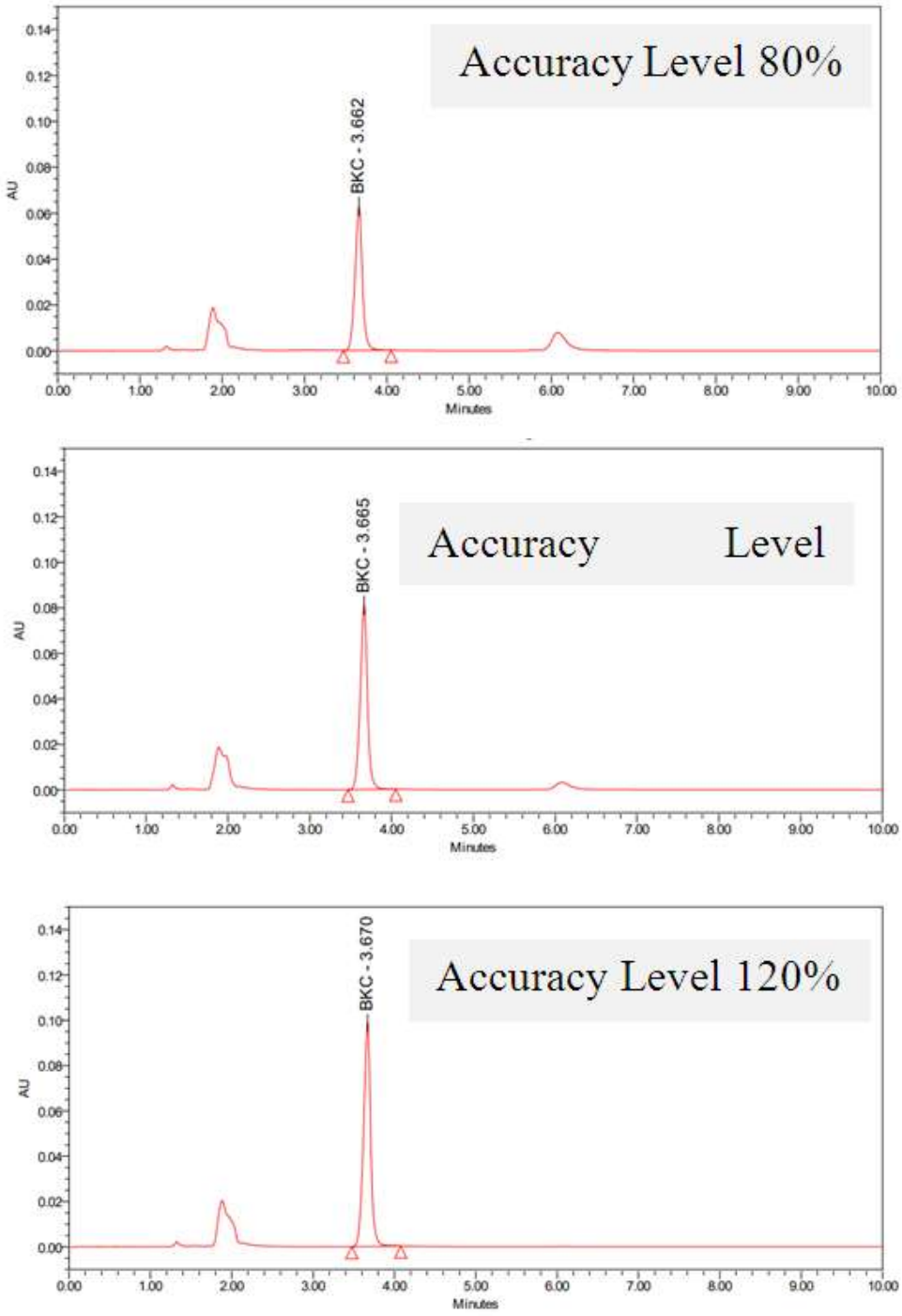

Figure 4. Chromatogram of Accuracy Level 80 to 120%

4. Precision - Repeatability

The method precision was conducted to prove the repeatability of the assay results obtained by this quantification methodology. For six consecutive times, a same system suitability standard solution (Refer Method section) was injected and percentage standard deviation and relative standard deviation (R.S.D.) were observed. Method repeatability study can be found successful when, the R.S.D. value is lesser or equal than 1.5% for area of principalpeak in each of the six sets.

An RSD of 0.2% was obtained in this study, by injecting six sets of sample preparation solution. %RSD for percentage assay results of six sample preparation should be not more than 2.0 for acceptance of repeatability. The very low %RSD was observed for the six assay resultshence it concluded that the method is precise & reproducible for the analysis of said BKC content in this cleaner formulation.

Table 3. Data for Method repeatability for BKC content

Sl no.	Sample preparation	% Assay
1	Sample prearation-1	99.1
2	Sample prearation-2	99.5
3	Sample prearation-3	99.6
4	Sample prearation-4	99.4
5	Sample prearation-5	99.3
6	Sample prearation-6	99.2
	Average	**99.4**
	%RSD	**0.19**

5. System suitability (Instrumental precision)

The purpose of this experiment was to demonstrate the suitability of the HPLC system prior to start actual analysis of samples. Prepared the system suitability standard solution as mentioned (Refer Method section) and injected six replicates' injections of this solution. By recording chromatograms, measured the peak responses for interested analyte peak, and instrumental precision was demonstrated in terms of percentage relative standard deviation for area of analyte peak. Other parameters, like tailing factor and column efficiency in terms of theoretical plates of analyte peak was also observed.

Consecutive six replicate injections of system suitability standard solution revealed that, by analyzing this method the desired suitability of HPLC instrument can be achieved through out the analysis. The % relative standard deviation of replicate injections were 0.1%. The effective ness of selected column was observed by getting average theoretical plates above 8000 and the tailing observed was about 1.0 for analyte peak.

Table 4. Data of System suitability

Injection No	Standard RT	Peak response	USP Tailing	USP plate count
1	3.64	492056	1.00	8504
2	3.64	492712	1.00	8476
3	3.64	492808	1.00	8441
4	3.64	492388	1.00	8428
5	3.64	492723	1.00	8474
6	3.64	491639	1.00	8495
Average	**3.64**	**492388**	**1.00**	**8470**
%RSD	**0.0**	**0.1**	**0.0**	**0.4**

IV. Conclusion

A reversed phase liquid chromatographic analytical quantification methodology of benzalkonium chloride (BKC) content in General purpose cleaner disinfectantproductis developed and validated successfully. This method has significant advantages, in terms of shorter analysis time, selectivity and accuracy than previously reported. The simple dilution method of sample preparation consistent and reproducible recovery for analyte from formulated preparation, with no interference and matrix suppression. The validation study indicates that method can be considered suitable for carrying out quality control & routine use for quantification of foresaid preservative component with said formulation.

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ABBREVIATIONS
HPLC: High Performance Liquid Chromatography, BKC: Benzalkonium chloride, RSD: Relative Standard Deviation, UV: Ultraviolet, ICH: International Council for Harmonization of Technical Requirements for Pharmaceuticals for human use.

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