

Confidence in the Daily Use of Antiseptic Alcohol

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ABSTRACT

Objective: To evaluate the active ingredient of 70% Alcohol in two most common daily usage patterns after container opening i.e. the long opening period without container lid closure and the short period opening with the lid opening twice a day.

Methods: 70 percent Alcohol was prepared in two common usages package sizes of 60 ml, and 250 ml, of our institution. We evaluated the quantity of ethyl alcohol that is the active ingredient in 70% alcohol in two aforementioned conditions to determine the time duration that the alcohol concentration remained greater than 60 percent in 60 ml, and 250 ml, package size. The alcohol concentration was quantified by a gas chromatography method for 3 batches and 5 samples per batch.

Results: The ethyl alcohol concentrations of 60 ml, and 250 ml, packages of 70 percent alcohol declined to lower than 60 percent in 18 and 32 days respectively in the long opening period condition, whereas the short period opening condition resulted in running out of volume in 24 and 49 days for 60 and 250 ml, package before the decline of the ethyl alcohol concentration to lower than 60 percent.

Conclusion: We can be confident that the 70 percent alcohol in our institution package sizes of 60 ml, and 250 ml, preserve their antiseptic properties for 18 and 32 days respectively when the lid is left open, whereas they maintain their antiseptic properties until the package is finished in the twice daily usage condition. These finding should be introduced into the practice guideline of the medical personnel and the patients.

Keywords: Active ingredient, ethyl alcohol, gas chromatography, percentage

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The alcohols such as ethyl alcohol and isopropyl alcohol, are used for disinfection and antiseptic purposes.¹ Ethyl alcohol is widely used as a disinfectant and antiseptic. Like many chemical disinfectants, alcohols are generally considered to be non-specific antimicrobials because of a multiplicity of toxic effect mechanisms. The predominant mode of action appears to stem from protein coagulation/denaturation. Associated disruptions of cytoplasmic integrity, cell lysis, and interference with cellular metabolism have been reported. Alcohol-induced coagulation of protein occurs at the cell wall, the cytoplasmic membrane and among the various plasma proteins. Coagulation of enzymatic proteins leads to loss of cellular functions. Protein coagulation occurs within concentration limits around an optimum alcohol level. In the absence of water, proteins are not denatured as readily as when

water is present.² This is the reason why absolute alcohol is less bactericidal than mixtures of alcohol and water. For this study, we consider only ethyl alcohol because Siriraj Hospital uses it to prepare 70% Alcohol (70% v/v) and Isopropyl alcohol may cause vasodilation of the skin when it is used on the skin before injection.^{3,4} Concentrations between 60 and 95 % are bactericidal and a 70% solution is usually employed for the disinfection of skin, clean instruments or surfaces.^{1,2,5}

We use 70% ethyl alcohol for cutaneous disinfection before insertion of an intravascular device, and for post insertion site care which can reduce the incidence of device-related infection substantially and use it for the composition of antiseptics.^{1,2,5} Ethanol, C₂H₅OH, (also called Ethyl Alcohol) is the second member of the aliphatic alcohol series.⁶ Ethyl alcohol is a volatile, flammable, clear colorless liquid with a pleasant smell. It is best known as the type of alcohol found in alcoholic beverages and in thermometers. In common usage, it is often referred to simply as alcohol. Ethyl alcohol has widespread use as a solvent of substances intended

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for human contact or consumption, including scents, flavorings, colorings, and medicines. In chemistry, it is both an essential solvent and a feedstock for the synthesis of other products. It has a long history as a fuel for heat and light and also as a fuel for internal combustion engines.^{7,8} From the volatile property, the quantity of ethyl alcohol may be lost when the container is opened and this is the reason to explain the question of why the effective bactericidal property of alcohol is decreases and the reason to explain the question why we determine the quantity of ethyl alcohol by gas chromatography.⁴

The criteria standard of 70% alcohol for antiseptic property mainly considers the concentration of ethyl alcohol that is not less than 60%.⁵ We evaluate the active ingredient of 70% Alcohol in two most common daily usage patterns i.e. the long opening period without container lid closure and the short period opening with the lid opening twice a day and dip the cotton ball in the 70% alcohol for cutaneous disinfection before injection of the insulin or any medicine that the patients must use everyday at home. For all usage patterns we mimic the situation by using 3 batches to compare the difference of batches and 5 samples per batch to measure the stability.⁹⁻¹² The result of this study is the number of days that the remaining quantity of ethyl alcohol is in the standard criteria after the container has been opened. The reason we chose a long opening period by opening for 24 hours daily is because the true practice use at the ward is not the same period of opening i.e. some wards have a daily usage patterns with a long opening period in the morning and a short opening period in the afternoon, but not all wards are the same. From this reason we simulated the worst situation by opening 24 hours daily.

MATERIALS AND METHODS

1. Chemicals and reagents

- Ethyl alcohol, AR grade
- Isopropanol, AR grade
- Sterile water
- National Institute of Standard and Technology (NIST) Standard Reference Material[®]
- 70% Alcohol 60 ml, 250 ml, in the PET (Poly ethylene terephthalate) bottle, 3 batches and 5 samples per batch.

2. Instrumentation

Gas Chromatography AutoSystemXL, Perkin Elmer: Headspace sampler Turbo Matrix 40. Chromatography was performed on GC chromatography by the method Headspace Gas Chromatography for the purified sample.

Gas chromatography¹³⁻¹⁴ specifically gas-liquid chromatography - involves a sample being vapourised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase (nitrogen gas). The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. The type of alcohol compared with the retention time of the sample and the standard. The quantity of the sample was compared with the peak area of the sample and the standard (Ethyl alcohol Standard Solution is 70 % Ethyl alcohol, AR grade and Internal Standard Solution 2.5% v/v Isopropanol, AR grade). All of the solutions were kept in a refrigerator at 4-8°C and the standard material

is National Institute of Standard and Technology (NIST) Standard Reference Material[®].

Samples were prepared from 70% Alcohol produced by the Pharmacy Department, Siriraj Hospital. The sample size is 3 batches in the package sizes 250 and 60 ml.; 5 samples per batch. The samples were transferred 100 μ l. to a 10-ml, volumetric flask diluted to volume with sterile water, and mixed and then internal standard samples were transfer 100 μ l. to sample vials and the rubber closer was fastened and kept at room temperature. After that the samples were transferred 500 μ l. to sample vials and the rubber closers were fastened and tightened with Aluminum crimper.

The standard solution preparation was prepared for the samples with standard ethyl alcohol standard solution.

Gas Chromatography (GC): capillary column, Flame Ionization Detector (FID) by Perkin Elmer and the operating conditions as follows:

Column : Rtx[®] BAC-1
 Injector : Temperature 200°C
 Oven : Temperature 40°C, Isothermal
 Detector : FID (Flame Ionization Detector) Temperature 200°C, H₂ flow = 35 ml./min
 Air flow = 350 ml./min, Carrier Flow : N₂ = 11.0 psi

Headspace Sampler by Perkin Elmer : model TurboMatrix 40 Calculation by calculated program : Perkin Elmer Turbochrom Workstation Version 6.3 Workstation by calibration type : internal standard, the program used the average value that used the response factor of an eight level standard (R-squared = 0.9989) and then plotted the graph to calculate the quantity of sample from the standard curve.

3. Methods

Part 1: Evaluate the concentration of ethyl alcohol of 70% initial alcohol in the long opening period without container lid closure (24 hours daily) at room temperature and measure the concentration of ethyl alcohol by GC (Gas-liquid Chromatography)⁴ every 2-5 days until the percentage of ethyl alcohol is less than 60%.

Part 2: Evaluate the concentration of ethyl alcohol in the situation after a short period of opening with the lid opening twice a day. The period opening is not more than 25 seconds then use the cotton ball (weight 0.47 grams) and soak with 70% alcohol. We measure the concentration of ethyl alcohol by GC every 2-5 days until the final volume or the percentage of ethyl alcohol is less than 60%.

RESULTS

Part 1

The GC method is suitable to determine the percentage of ethyl alcohol in pharmaceutical preparations, so we use this method to measure in this study. Three batches of 70% alcohol were prepared to compare the differences of each batch and 5 samples per batch. All samples were prepared to measure the percentage of ethyl alcohol when open 24 hours at the room temperature, and measure the concentration of ethyl alcohol as the schedule time until the concentration of ethyl alcohol was less than 60%. From Fig 1 the results for package size 250 ml, was 32, 33, 34 days for batch

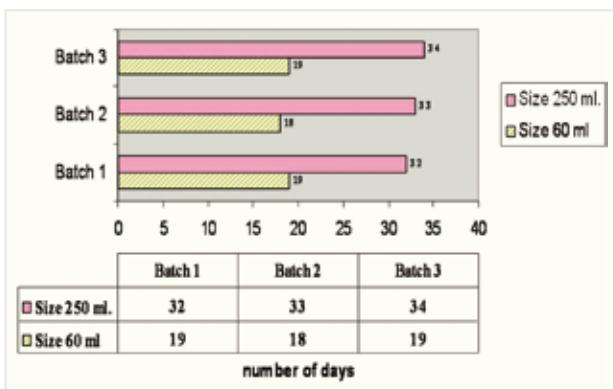


Fig 1. Number of days of 70% alcohol that remain the quantity of ethyl alcohol $> 60\%$ after open 24 hours daily.

1, 2, 3 respectively and the results for package size 60 ml, was 19, 18, 19 days for batch 1, 2, 3 respectively. After that the percentage of ethyl alcohol was less than 60%.

Part 2

Three batches of 70% alcohol were prepared to compare the differences of each batch and 5 samples per batch to measure the percentage of ethyl alcohol when we dip the cotton ball in the alcohol for cutaneous disinfection before injection 2 times daily at the room temperature and measure the percentage of ethyl alcohol at the scheduled time until 70% alcohol was out of volume or the concentration of ethyl alcohol was less than 60%. From Fig 2 the period that the percentage of ethyl alcohol was not less than 60% in the package size 250 ml, was 52, 50, 49 days for batch 1, 2, 3 respectively and the package size 60 ml, was 24, 25, 24 days for batch 1, 2, 3 respectively.

DISCUSSION

The GC method is suitable to determine the percentage of ethyl alcohol in pharmaceutical preparations, so we use this method to measure in this study. Three batches of 70% alcohol were prepared to compare the differences of each batch and 5 samples per batch to measure the percentage of ethyl alcohol when open 24 hours at the room temperature and measure the percentage of ethyl alcohol at the scheduled time until the concentration of ethyl alcohol was less than 60%. The

criteria of this study was determined by the concentration of ethyl alcohol that was not less than 60% after open 24 hours and we determined from the minimum time duration that the alcohol concentration remained greater than 60% for 3 batches and 5 samples per batch. From the results of package size 60 ml, the first and the third batch declined to lower than 60% in 19 days but the second batch declined to lower than 60% in 18 days so we determined 70% alcohol in package size 60 ml, preserved its antiseptic property for 18 days. For the package size 250 ml, the results of batch 1, 2 and 3 declined to lower than 60% in 32, 33 and 34 days respectively so we determined 70% alcohol in package size 250 ml, preserved its antiseptic property for 32 days.

To measure the percentage of ethyl alcohol when we dip the cotton ball in the alcohol for cutaneous disinfection before injection 2 times daily at room temperature and measure the percentage of ethyl alcohol at the scheduled time until the concentration of ethyl alcohol was less than 60%. The criteria of this study was the concentration of ethyl alcohol that was not less than 60% until the final volume and we determined from the minimum time duration that the alcohol concentration remained greater than 60% for 3 batches and 5 samples per batch. From the results of package size 60 ml, the first and the third batches were running out of volume in 24 days but the second batch was running out of volume in 25 days so we determined 70% alcohol in package size 60 ml, preserved its antiseptic property until the final volume in 24 days. For the package size 250 ml, the results of batches 1, 2 and 3 were running out of volume in 52, 50 and 49 days respectively, so we determined 70% alcohol in package size 60 ml, preserved its antiseptic properties until the final volume in 49 days.

The results shown that 70% alcohol was prepared in two common usage package sizes of 60 ml, and 250 ml, in our institution. The ethyl alcohol concentration of 60 ml, and 250 ml, packages of 70% alcohol declined to lower than 60% in 18 and 32 days respectively in the long opening period condition whereas the short period opening condition resulted in running out of volume in 24 and 49 days for 60 and 250 ml, packages before the decline of ethyl alcohol concentration to lower than 60%.

The number of days from these results can only be used for the practice use of the patient and the health related worker who uses 70% ethyl alcohol in the PET package that consists of ethyl alcohol, Edicol blue color and purified water in the formula. Other formulas such as the formula that contains other substances were not included in this study, so that in the practical use in the hospital or the patient who uses 70% alcohol that is produced in the hospital or contains the composition the same as this formula should be aware of the bactericidal properties of alcohols that have been studied on a scientific concentration in the standard range 60-75% aqueous solution. The period of time for effective use of 70% ethanol is shown as the number of days after opening the container which should not be longer than the period times explained above, otherwise the germicidal effects that we need to use for disinfection and antiseptic properties are decreased which leads to the disadvantages to use before injection or insertion of a catheter.

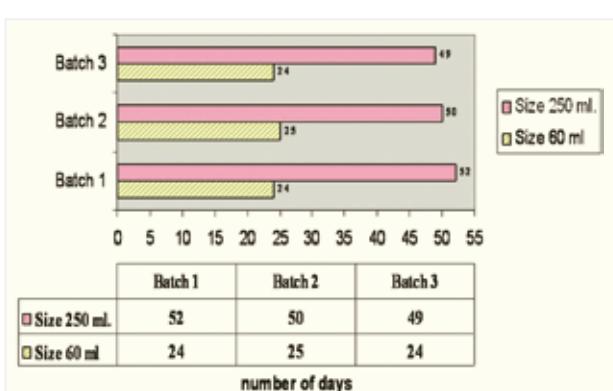


Fig 2. Number of days of 70% alcohol that remain the quantity of ethyl alcohol $> 60\%$ until the final volume when dip the cotton ball in 70% alcohol.

CONCLUSION

70% alcohol (active ingredient is ethyl alcohol 70%) was prepared in the package size 60 ml, and 250 ml, for use in the hospital for inpatients and outpatients, but we never know the percentage of ethyl alcohol after opening the container. To evaluate the active ingredient of 70% alcohol in two most common daily usage patterns after container opening i.e. the long opening period without container lid closure and the short period opening with the lid opening twice a day. We evaluate the quantity of ethyl alcohol that is the active ingredient in 70% alcohol in two aforementioned conditions, to determine the time duration that the alcohol concentration remained greater than 60% in 60 ml, and 250 ml, package sizes. The alcohol concentration was quantified by gas chromatography method for 3 batches and 5 samples per batch. The results shown that 70% alcohol was prepared in two commonly usage package size of 60 ml, and 250 ml, of our institution. The ethyl alcohol concentration of 60 ml, and 250 ml, package of 70% alcohol declined to lower than 60% or ran out of volume in 18 and 32 days respectively in the long opening period condition, whereas the short period opening condition resulted in the strength falling below the 60% minimum in 24 and 49 days for 60 and 250 ml, packages respectively, before the decline of ethyl alcohol concentration. We can be confident that the 70% alcohol in our institution package size of 60 ml, and 250 ml, preserved its antiseptic property or ran out of volume within 18 and 32 days when the lid is left open, whereas it maintains its antiseptic property for 24

and 49 days for 60 and 250 ml, package sizes respectively, in twice daily usage condition. These finding should be introduced into to the practice guideline of the medical personnel and the patients.

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