



Edition: BP 2025 (Ph. Eur. 11.6 update)

Benzalkonium Chloride Solution



[General Notices](#)

(Ph. Eur. monograph 0371)

Action and use

Antiseptic.

Ph Eur

DEFINITION

Aqueous solution of a mixture of alkylbenzyltrimethylammonium chlorides, the alkyl groups mainly having chain lengths of C₁₂, C₁₄ and C₁₆.

Content

475 g/L to 525 g/L of alkylbenzyltrimethylammonium chlorides, calculated using the average relative molecular mass (see Tests). The solution may contain ethanol (96 per cent).

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

It froths copiously when shaken.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry ([2.2.25](#)).

Test solution Dilute 0.3 mL to 100.0 mL with [water R](#).

Spectral range 220-350 nm.

Absorption maxima At 257 nm, 263 nm and 269 nm.

B. Examine the chromatograms obtained in the test for average relative molecular mass and ratio of alkyl components.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to the principal peaks in the chromatogram obtained with the reference solution.

C. To 0.05 mL add 2 mL of [water R](#), 0.1 mL of [glacial acetic acid R](#) and, dropwise, 1 mL of [sodium tetraphenylborate solution R](#). A white precipitate is formed. Filter. Dissolve the precipitate in a mixture of 1 mL of [acetone R](#) and 5 mL of [ethanol \(96 per cent\) R](#), heating to not more than 70 °C. Add [water R](#) dropwise to the warm solution until a slight opalescence forms. Heat gently until the solution is clear and allow to cool. White crystals separate. Filter, wash with 3 quantities, each of 10 mL, of [water R](#) and dry *in vacuo* ([2.2.32](#)) at a temperature not exceeding 50 °C. The crystals melt ([2.2.14](#)) at 127 °C to 133 °C.

D. To 5 mL of [dilute sodium hydroxide solution R](#) add 0.1 mL of [bromophenol blue solution R1](#) and 5 mL of [methylene chloride R](#) and shake. The methylene chloride layer is colourless. Add 0.05 mL of the solution to be examined and shake. The methylene chloride layer becomes blue.

E. To 0.05 mL add 1 mL of [dilute nitric acid R](#). A white precipitate is formed which dissolves on the addition of 5 mL of [ethanol \(96 per cent\) R](#). The solution gives reaction (a) of chlorides ([2.3.1](#)).

TESTS

Solution S

Dilute 2.0 g to 100 mL with [carbon dioxide-free water R](#).

Appearance of solution

Solution S is clear ([2.2.1](#)) and not more intensely coloured than reference solution Y₆ ([2.2.2, Method II](#)).

Acidity or alkalinity

To 50 mL of solution S add 0.1 mL of [bromocresol purple solution R](#). Not more than 0.1 mL of [0.1 M hydrochloric acid](#) or [0.1 M sodium hydroxide](#) is required to change the colour of the indicator.

Average relative molecular mass and ratio of alkyl components

Liquid chromatography ([2.2.29](#)).

Test solution Determine the density ([2.2.5](#)) of the solution to be examined. Dilute a quantity of the solution to be examined equivalent to about 0.400 g of benzalkonium chloride to 100.0 mL with [water R](#).

Reference solution Dissolve the contents of a vial of [benzalkonium chloride for system suitability CRS](#) in 5 mL of [water R](#).

Column:

— size: $l = 0.25$ m, $\varnothing = 4.6$ mm;

— stationary phase: [end-capped cyanosilyl silica gel for chromatography R](#) (5 μ m).

Mobile phase Mix 45 volumes of [acetonitrile R](#) and 55 volumes of a 13.6 g/L solution of [sodium acetate R](#) previously adjusted to pH 5.0 with [glacial acetic acid R](#).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 μ L.

Identification of homologues Use the chromatogram supplied with [benzalkonium chloride for system suitability CRS](#) and the chromatogram obtained with the reference solution to identify the peaks due to homologues C₁₂, C₁₄ and C₁₆.

Relative retention With reference to C₁₂ homologue (retention time = about 6 min): C₁₄ homologue = about 1.3;
C₁₆ homologue = about 1.7.

— [resolution](#): minimum 1.5 between the peaks due to the C_{12} and C_{14} homologues.

Calculate the average relative molecular mass of the sample by summing the products for each homologue, using the following expression:

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A = area of the peak due to the given homologue in the chromatogram obtained with the test solution;
 B = sum of the areas of the peaks due to all homologues in the chromatogram obtained with the test solution;
 W = relative molecular mass for the given homologue: 340, 368 and 396 for the C_{12} , C_{14} and C_{16} homologues, respectively.

Calculate the percentage of each homologue, using the following expression:

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C = product of the relative molecular mass of the given homologue and the area of the corresponding peak in the chromatogram obtained with the test solution;
 D = sum of the C values for all homologues quantified.

Limits:

— C_{12} homologue: minimum 40 per cent;
— C_{14} homologue: minimum 20 per cent;
— sum of C_{12} and C_{14} homologues: minimum 70 per cent.

Impurities A, B and C

Liquid chromatography ([2.2.29](#)). Prepare the solutions immediately before use.

Test solution Determine the density ([2.2.5](#)) of the solution to be examined. Dilute a quantity of the solution to be examined equivalent to 2.5 g of benzalkonium chloride to 50.0 mL with [methanol R](#).

Reference solution (a) Dissolve 25.0 mg of [benzyl alcohol CRS](#) (impurity A) in [methanol R](#) and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 75.0 mg of [benzaldehyde CRS](#) (impurity B) in [methanol R](#) and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with [methanol R](#).

Reference solution (c) Dilute 1 mL of reference solution (a) to 10 mL with [methanol R](#).

Column:

— size: $l = 0.15$ m, $\varnothing = 4.6$ mm;
— stationary phase: [end-capped octadecylsilyl silica gel for chromatography R](#) (5 μ m);
— temperature: 30 °C.

Mobile phase:

— mobile phase A: dissolve 1.09 g of [sodium hexanesulfonate R](#) and 6.9 g of [sodium dihydrogen phosphate monohydrate R](#) in [water for chromatography R](#); adjust to pH 3.5 with [phosphoric acid R](#) and dilute to 1000 mL with the same solvent;
— mobile phase B: [methanol R2](#);

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 10	80	20
10 - 14	80 → 50	20 → 50
14 - 35	50	50
35 - 36	50 → 20	50 → 80
36 - 55	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm for impurities A and C, and at 257 nm for impurity B.

Injection 20 µL.

Relative retention With reference to impurity A (retention time = about 10 min): impurity B = about 1.3; impurity C = about 2.4.

System suitability At 210 nm:

- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c);
- *symmetry factor*: minimum 0.6 for the peak due to impurity A in the chromatogram obtained with reference solution (a).

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity C by 1.3;
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *impurity C*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Amines and amine salts

Mix 10.0 g, while heating, with 20 mL of a mixture of 3 volumes of [1 M hydrochloric acid](#) and 97 volumes of [methanol R](#) and add 100 mL of [2-propanol R](#). Pass a stream of [nitrogen R](#) slowly through the solution. Titrate with up to 12.0 mL of [0.1 M tetrabutylammonium hydroxide](#) and record the potentiometric titration curve ([2.2.20](#)). If the curve shows 2 points of inflexion, the volume of titrant added between the 2 points is not greater than 5.0 mL. If the curve shows no point of inflexion, the solution to be examined does not comply with the test. If the curve shows 1 point of inflexion, repeat the test but add 3.0 mL of a 25.0 g/L solution of [dimethyldodecylamine R](#) in [2-propanol R](#) before the titration. If the titration curve after the addition of 12.0 mL of the titrant shows only 1 point of inflexion, the solution to be examined does not comply with the test.

Sulfated ash ([2.4.14](#))

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Determine the density ([2.2.5](#)) of the solution to be examined. Dilute 4.00 g to 100.0 mL with [water R](#). Transfer 25.0 mL of the solution to a separating funnel, add 25 mL of [methylene chloride R](#), 10 mL of [0.1 M sodium hydroxide](#) and 10.0 mL of a freshly prepared 50 g/L solution of [potassium iodide R](#). Shake well, allow to separate and discard the methylene chloride layer. Shake the aqueous layer with 3 quantities, each of 10 mL, of [methylene chloride R](#) and discard the methylene chloride layers. To the aqueous layer add 40 mL of [hydrochloric acid R](#), allow to cool and titrate with [0.05 M potassium iodate](#) until the deep-brown colour is almost discharged. Add 5 mL of [methylene chloride R](#) and continue the titration, shaking vigorously, until the methylene chloride layer no longer changes colour. Carry out a blank titration on a mixture of 10.0 mL of the freshly prepared 50 g/L solution of [potassium iodide R](#), 20 mL of [water R](#) and 40 mL of [hydrochloric acid R](#).

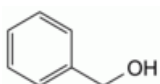
1 mL of [0.05 M potassium iodate](#) is equivalent to — mg of benzalkonium chloride where x is the average relative molecular mass of the sample.

LABELLING

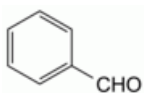
The label states the content of ethanol (96 per cent), if any.

IMPURITIES

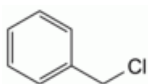
Specified impurities A, B, C.



A. benzyl alcohol,



B. benzaldehyde,



C. (chloromethyl)benzene.

Ph Eur