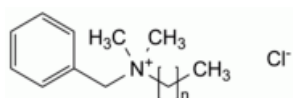Edition: BP 2025 (Ph. Eur. 11.6 update)

Benzalkonium Chloride

[General Notices](#)

(Ph. Eur. monograph 0372)

8001-54-5

Action and use

Antiseptic.

Ph Eur

DEFINITION

Mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups mainly having chain lengths of C_{12}, C_{14} and C_{16}.

Content

95.0 per cent to 104.0 per cent of alkylbenzyldimethylammonium chlorides (anhydrous substance) calculated using the average relative molecular mass (see Tests).

CHARACTERS

Appearance

White or yellowish-white powder or gelatinous, yellowish-white fragments, hygroscopic. On heating it forms a clear molten mass.

Solubility

Very soluble in water and in ethanol (96 per cent). An aqueous solution 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   Dissolve 80 mg in _water R_ and dilute to 100.0 mL with the same solvent.

Spectral range   220-350 nm.

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

Shoulder   At about 250 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 2 mL of solution S (see Tests) add 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 0.1 mL of solution S and shake. The methylene chloride layer becomes blue.

E.   To 2 mL of solution S 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

Dissolve 1.0 g in _carbon dioxide-free water R_ and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (_2.2.1_) and not more intensely coloured than reference solution Y_6 (_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   Dissolve 0.400 g of the substance to be examined in _water R_ and dilute to 100.0 mL with the same solvent.

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, Ø = 4.6 mm;

— *stationary phase*: _end-capped cyanosilyl silica gel for chromatography R_ (5 µm).

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 µL.

Identification of homologues Use the chromatogram supplied with <u>benzalkonium chloride for system suitability CRS</u> and the chromatogram obtained with the reference solution to identify the peaks due to C_{12}, C_{14} and C_{16}.

Relative retention With reference to C_{12} homologue (retention time = about 6 min): C_{14} homologue = about 1.3; C_{16} homologue = about 1.7.

System suitability Reference solution:

— <u>resolution</u>: 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:

$$ — $$

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:

$$ — $$

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 (<u>2.2.29</u>). *Prepare the solutions immediately before use*.

Test solution Dissolve 0.500 g of the substance to be examined in <u>methanol R</u> and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 25.0 mg of <u>benzyl alcohol CRS</u> (impurity A) in <u>methanol R</u> and dilute to 100.0 mL with the same solvent.

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

Reference solution (c) Dilute 1 mL of reference solution (a) to 10 mL with <u>methanol R</u>.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: <u>end-capped octadecylsilyl silica gel for chromatography R</u> (5 μm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dissolve 1.09 g of <u>sodium hexanesulfonate R</u> and 6.9 g of <u>sodium dihydrogen phosphate monohydrate R</u> in <u>water for chromatography R</u>; adjust to pH 3.5 with <u>phosphoric acid R</u> 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

Dissolve 5.0 g with heating in 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 substance 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 *dimethyldecylamine R* in *2-propanol R* before the titration. If the titration curve after addition of 12.0 mL of the titrant shows only 1 point of inflexion, the substance to be examined does not comply with the test.

Water (*2.5.12*)

Maximum 10 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.00 g in _water R_ and dilute to 100.0 mL with the same solvent. 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_.
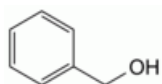
1 mL of _0.05 M potassium iodate_ is equivalent to —— mg of benzalkonium chloride where _x_ is the average relative molecular mass of the sample.

STORAGE

In an airtight container.

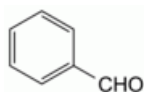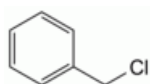## IMPURITIES

Specified impurities   A, B, C.

A.   benzyl alcohol,

B.   benzaldehyde,

C.   (chloromethyl)benzene.

Ph Eur