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Analytical Methods for Determination of Benzethonium Chloride: A Review

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Summary Benzethnoium chloride, Hyamine, is an anti-microbial agent in aid antiseptic. It can be found in cosmetics, toiletries, and food industry as hard surface disinfectant. It is also found in several grapefruit seed extract preparations. On the basis of its importance, different authors have published analytical methods for its determination. A chromatographic, spectral, and electrometric techniques were reported for this purpose. The final results, LOD and recovery for these methods were tabulated.

Contents

Introduction

Benzethonium chloride, is also known as Hyamine is a synthetic quaternary ammonium salt. This compound is an odorless white solid; soluble in water, has surfactant, and antimicrobial properties(1), and is used as a topical antimicrobial agent in aid antiseptics(2). Its antimicrobial activity is immediate upon contact with one log reduction within one minute (e.g., the log10 reduction values for *Escherichia coli* were 1.70+0.70) which reflect its fast action(3). Benzethonium chloride in combination with essentialoils is bactericidal (sustained 3 log10 reduction incfu/mL from the initial inoculums) against all strainsof methicillin-resistant *Staphylococcus aureus* by 6 hours.(4)

It is also found in cosmetics and toiletries such as mouthwashes, anti-itch ointments, and antibacterial moist towelettes (5). Benzethonium chloride is also used in the food industry as a hard surface disinfectant(6). It is available under trade names Salanine®, BZT®, Diapp®, Quatrachlor®, Polymine D®, Phemithyn®, Antiseptol®, Disilyn®, Phermerol®, and others(7). It is also found in several grapefruit seed extract preparations and can be used as a preservative(8), such as in the anesthetic Ketamine®(9). It was identified as a novel cancer-specific compound by using a cell-Based small-Molecule Screen,it reduce cell viability

through inducing apoptosis and activated caspases for cancer cells without interfering with cisplatin 5-fluorouracil,or γ-irradiation and ablating the tumor-forming ability of FaDu cells, delayed the growth of xenograft tumors, and combined additively with local tumor radiation therapy[10].

It is used inspermatocides but it can cause vaginal irritation with burning sensation and itching[11].Ingestion of benzethonium chloridecan cause vomiting, collapse, convulsion, and comma[12]and may be fatal when ingested by 1-3 gram[13,14].

Benzethonium chloride [($C_{27}H_{42}ClNO_2$) (CAS 121-54-0)] is Benzyldimethyl (2-{2-[4-(2, 4, 4-trimethylpentan-2-yl) phenoxy] ethoxy} ethyl) azanium chloride [15].

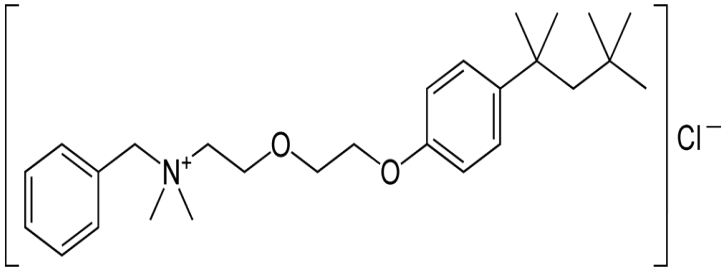

Fig. 1. The chemical structure of benzethonium chloride

Electrometric methods

Benzethonium chloride was determined by using a rugged, low resistance silver-silver sulphide solid-state electrode through potentiometric titration. Sodium tetraphenylborate, mercury (II) acetate and silver nitrate (0.01 mol L^{-1}) were worked as titrants[16].

Potentiometric sensors based on ion-pair (tetrahexadecylammonium dodecyl sulphate) as sensing materials were used for determination of benzethonium chloride. The response of the solid state surfactant sensitive electrode was investigated in the solutions of benzethonium chloride ion in the concentration range 1 μmol L^{-1} to 10 mmol L^{-1} with slope 51.10 mV per concentration decade based on a Teflonized graphite conducting substrate, coated with a PVC membrane containing sensing material. Sodium dodecylsulfatewas used as a titrant for determination of benzethonium chloride[17].

Another method was used for determination of benzethonium chloride depending on a capillary electrophoresis technique. A fused-silica capillary using a mixed 75 mmol L^{-1} phosphoric acid and 50% acetonitrile electrolyte at pH 2.5 was performed for the determination. Repeatability in migration times (RSD %) for benzethonium ion was 0.3

covering range 0.0125-0.400 mmol L^{-1} with minimum detection limits (signal-to-noise ratio =3) is 1.47µgmL^{-1}[18].

An ion-pair was prepared by complexation of highly lipophilic 1,3-didecyl-2-methyl-imidazolium cation and a tetraphenylborate and used to construct sensor. Sensor show a Nernstian response 56.8 mV/decade with linear range $4.0x10^{-6}$ and $4.0x10^{-4}$ mol L^{-1} and low detection limit 2.9×10^{-6}mol L^{-1}. Sodium tetraphenyl borate used as titrant for determination of benzethonium chloride[19].

Table 1. Electrometric methods for determination of benzethonium chloride.

Method	Slope mV/ decade	Range	LOD mol L^{-1}	Recovery(%)±SD	Ref.
Poteniometric titration using Ag/Ag_2S electrode					
	……	2-6 mg	…….	100.40±0.70	16
Ion selective electrode using tetrahexadecylammonium dodecyl sulphate as sensing material					
	51.10	$1.00x10^{-6}$-$1.00x10^{-2}$mol L^{-1}	$4.50x10^{-6}$	99.33±0.06	17
Electrophoresis using a mixed phosphoric acid and acetonitrile electrolyte					
	.....	$1.25x10^{-5}$-$4.00x10^{-4}$mol L^{-1}	$3.28x10^{-6}$	98.00-102.00	18
Ion selective electrode using 1,3-didecyl-2-methyl-imidazolium cation and a tetraphenylborate as sensing material					
	56.80	$4.00x10^{-6}$-$4.00x10^{-4}$ mol L^{-1}	$2.90x10^{-6}$	98.36±0.57	19

Spectral Methods

Spectrophotometric determination of benzethonium chloride in pure form was carried outbased on the solvent extraction of the ion-pair formed between benzethonium chloride and colored tetrabromophenolphthalein ethyl ester, into 1,2-dichloroethane. The extract has a maximum absorbance at wavelength 615 nm, and follows Beer's law with precision up to $4.0x10^{-6}$mol L^{-1} in aqueous solution. The effect of pH and organic cations on the absorbance of the extract was investigated[20].

Ion-associates were prepared by complexation of benzethonium chloride with bromocresol green (BCG) and quinine in 1,2-dichloroethane and used to determine benzethonium chloride ($1.0\text{-}5.0\times10^{-6}$mol L^{-1}). At pH 8.2, Benzethonium chloride was extracted quantitatively after the formation of a new type of ion-associate with BCG and quinine in an aqueous solution. The ion-associate shows an absorption maximum at 630 nm[21].

Anotherspectrophotometric method reported based on the reaction of quinine cations with bromophenol blue (BPB) to form complex anion, which react with benzethonium chlorideat pH 6.7 then extracted into chloroform as an ion pair. Benzethonium was extracted in large amounts into chloroform with BPB only when quinine, in a pH 6.7 buffered aqueous solution, co-existed with them. In addition, when this showed a strong ion associate ability, then benzethonium reacts with BPB in the organic phase to give a blue product. The absorbance of the ion pair in chloroform was measured at 610 nm. Calibration graphs were linear in the range $0.5x10^{-6}$-$3.0x10^{-6}$ mol $L^{-1}$with molar absorptivitiy3.14×10^{4}L $mol^{-1}cm^{-1}$[22].

Table 2.Spectrophotometric methods for determination of benzethonium chloride.

Reagent used	λ_{max}, nm	pH	Range, mol L^{-1}	LOD, mol L^{-1}	Recovery%	Ref.
Tetrabromophenolphthaleinethyl ester						
	615	7-11	up to $4.0x10^{-6}$	$4.00x10^{-6}$	---	20
Bromocresol green and quinine						
	630	8.2	1.0×10^{-6}-5.0×10^{-6}	---	100.50-100.90	21
Bromophenol blue and quinine						
	610	6.7	0.5×10^{-6}-3.0×10^{-6}	---	100.00±2	22

Chromatographic Methods

Benzethonium chloride was identified as the main constituent in the extract which obtained from commercial grapefruit seed. This extract was dissolved in chloroform and evaporated and the the resulting solid was subsequently analyzed by high-performance liquid chromatography, electrospray ionization mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, and elemental analysis (by proton-induced X-ray emission [PIXE] analysis). Benzethonium chloride comprised 8.03% (n = 2) of the liquid grapefruit seed extract sample[23].

The determination of benzethonium chloride in anthrax vaccine was carried out by using a novel and sensitive HPLC method. Adjuvant AL hydrogel was removed by syringe filter after a simple sample pretreatment - acidification prior to injection. Chromatography was performed by isocratic reverse phase separation with methanol/262 mmol L^{-1} ammonium acetate (80/20, v/v) on an end capped C18 column with diode array detector (DAD). The method showed recovery (100±1.5%) with limit of detection 0.5 µg$L^{-1}$and the limit of quantitation (LOQ) of 1.5 µg $L^{-1}$with dynamic range up to 100 µg L^{-1}[24].

Ganzera et al.[25]proposed an HPLC method for the determination of benzethonium chloride in grapefruit seed extract. This method was developed allowing the baseline separation of all compounds within 40 min. Optimum results were obtained with a C-8

stationary phase and a solvent system comprising aqueous trifluoroacetic acid, acetonitrile, and 2-propanol.

Another HPLC method [26] was developed for determination of benzethonium chloride in various samples of grapefruit seed extract. The best results were obtained with a Phenomenex Gemini C18 column using gradient mobile phase of water (0.1% acetic acid) and acetonitrile (0.1% acetic acid) with a flow rate of 1.0 mL min^{-1}. The detection wavelength was 275 nm for benzethonium chloride covering the concentrations range from 0.29–21.84%.

High performance liquid chromatography–ultraviolet (HPLC–UV) detection methodwas developed to determine benzethonium chloridewhichis characterized by fast, accurate and sensitive outcomes. Detection was done using Phenomenex Gemini column [C18 (250 × 4.6 mm, 5 μm)] and the mobile system was methanol and 0.01 mol L_{-1} potassium phosphate (monobasic) in water (pH = 3.3)[27].

Another HPLC method was studied through the possibility of using mobile phases with ion-pairing reagents for determination of benzethonium chloride in eye drops. The most suitable mobile phases among those studied were acetonitrile:aqueous tetrabutylammonium hydrophosphate (5 mmol L^{-1}) (65:35) and acetonitrile:aqueous tetrabutylammonium hydrophosphate (2.5 mmol L^{-1}) and sodium heptanesulfonate (2.5 mmol L^{-1}) (65:35). The used columns were Zorbax SB C8 (150 × 4.6 mm, 3.5 μm) and Diaspher-110-Phenyl (150 × 4.6 mm, 5 μm)[28].

Gas chromatography-mass spectrometry (GC/MS) was used for determination of benzethonium chloride in commercial grapefruit seed extracts. Presence of benzethonium chloridein the range of 0.14-22.2% was found in 4 of 5 analyzed commercial samples (one of them - especially developed for children). The presence of benzethonium chloride was additionally confirmed by high-performance liquid chromatography with diode-array detection (HPLC/DAD) and direct infusion electrospray ionization mass spectrometry (ESI-MS). This methodclarifies that GC/MS represents a simple, rapid, selective and sensitive alternative approach for qualitative and quantitativeanalysis for benzethonium chloride in commercial grapefruit seed extracts[29].

Table 3. Chromatographic Methods for determination of benzethonium chloride.

Method	Sample used	Solvent	Column used	LOD μg L^{-1}	Recovery± SD%	Ref.
HPLC	Grapefruit seed extract	Methanol/ water	Phenomenex LUNA C18	**----**	**----**	23

HPLC	Anthrax vaccine	Methanol/ ammonium acetate	end capped C18	0.5	100±1.5	24
HPLC	Grapefruit seed extract	Trifluoroacetic acid, acetonitrile, and 2-propanol.	C-8 stationary phase	$12.1x10^{-3}$	96.1±4.5	25
HPLC	Grapefruit seed extract	Acetonitrile[1]	Phenomenex Gemini C18	1.5	101.5±2.8	26
HPLC–UV	Benzethonium chloride	Methanol/ potassium phosphate	Phenomenex Gemini C18	**----**	99.4±0.76	27
HPLC	Eye drops	Acetonitrile:	Zorbax SB C8/Diaspher-110-Phenyl	**----**	**----**	28
GC/MS	Grapefruit seed extract	Methanol	Ultra-inert fused silica capillary column DB-5ms UI	1.0	**----**.	29

[1]acetonitrile/tetrabutylammonium hydrophosphate ortetrabutylammonium hydrophosphate and sodium heptanesulfonate

Conclusion

Benzethonium chloride was determined by different analytical techniques. The electrometric methods are based on construction of silver-silver sulphide electrode and ion selective electrodes with tetrahexadecylammonium dodecyl sulphate, or 1,3-didecyl-2-methyl-imidazolium tetraphenylborate as sensing materials. Also capillary electrophoresis technique was reported. Spectrophotometric methods involve the use of different reagents based on tetrabromophenolphthaleinethyl ester, bromocresol green with quinine, and bromophenol blue with quinine. Also chromatographic methods were studied involving the use of HPLC and gas chromatography with mass spectrometry.

References

1. Susan Budavari, The Merck Index, 11th Ed., Merck & Co., Inc. publisher, Rahway, NJ, USA, p. 1084 (1989).

2. M. S. Shintre, T. A. Gaonkar, S. M. Modak, Int. J. Hyg. Envir. Heal.,**209**(5), 477-487 (2006).

3. L. Jimenez, M. Chiang, AJIC: Amer. J. Infect. Control, **33**(5) , e41-e42 (2005)

4. D. T. Bearden, G. P. Allen, and J. M. Christensen, J AntiMicrob.Chemother.,**62**(4), 769-772 (2008).

5. R. L. Elder,J. Amer. Acad.Dermat.,**11**(6),1168-1174 (1984).

6. D. T Bearden, G. P. Allen, J. M. Christensen, J Antimicrob. Chemother.,**62**, 769-772 (2008).

7. National Toxicology Program,NTP Toxicology and Carcinogenesis Studies ofBenzethonium Chloride (CAS No. 121-54-0) in F344/N Rats and B6C3F1 Mice (Dermal Studies), Nat. Toxicol Program Tech RepSer., **438**,1-220 (1995).

8. G.Takeoka, L. Dao, R. Y. Wong, R. Lundin andN. Mahoney, Agri. Food Chem.,**49**(7), 3316-3320 (2001).

9. K. M. Coates andP. Flood, Brit. J.Pharmacol., 871-879 (2001).

10. K. W. Yip , X. Mao, P. Y. Au , D. W. Hedley, S. Chow ,S.Dalili, J. D. Mocanu, C. Bastianutto, A. Schimmer, F. F Liu, Clin. Cancer Res., **12**(18):5557-5569 (2006).

11. L. S. Goodman and A. Gilman (eds.), The pharmacological Basis of therapeutics, 5th ed., New York, Macmillan publishing Co., Inc., P. 1002 (1975).

12. S.Budavari, The Merck Index, 11th Ed., Merck & Co., Inc. publisher, Rahway, NJ, USA, p. 167 (1989).

13. R. H. Dreibach, Handbook of poisoning, 9thed., Los Altos. California: Lange medical publication, p.357 (1977).

14. K. Verschueren, Handbook of environmental data of organic chemicals, 2nd ed., New York, NY: Van Nostrand Reinhold Co., p.244 (1983).

15. J. L. Rodríguez, R. M. Minardi,E. P. Schulz,O.Pieroni andP. C. Schulz, j. SurfactantDeterg., **15**(2), 147-155 (2012).

16. S. Pinzauti, a, G. Papeschib, E. La Portaa. J. Pharm. Biomed. Anal.,**1**(1), 47-53(1983).

17. R.Matešic-Puac, M.Sak-Bosnar, M.Bilic,B.S. Grabaric. Electroanal.,**16**, 843-851 (2004).

18. N. Öztekin, F. B.Erim, Journal of Pharmaceutical and Biomedical Analysis,**37**, 1121-1124 (2005).

19. D.Madunić-Čačića, M.Sak-Bosnarb, O.Galovićb, N.Sakačb, R.Matešić-Puačb. Talanta, **76**, 259-264 (2008).

20. M.Tsuboughi, Bull.Chem.Soc.Japn.,**44**, 1560-1562 (1971).

21. T. Sakai,Anal.Chim.Acta, **147**, 331-337 (1983).

22. T. Sakai, Analyst, **108**, 608-614, (1983)

23. G.Takeoka, L. Dao, R. Y. Wong, R. Lundin, N. Mahoney J. Agric. Food Chem., 49 (7), 3316-3320(2001).

24. H.Wang, A. V. Del Grosso, J. C. May. Biologicals, 34(4), 257-263 (2006).

25. M.Ganzera, A.Aberham, H.Stuppner, J. Agric. Food Chem.,**54**(11), 3768-3772 (2006).

26. D.Avula, I. Khan; Pharmazie, **62(**8) 593-596 (2007).

27. J. G. Lee, S. Shin, S. B. Han, Y.O. Kim, J. Pharm. Invest., **42**, 47-50 (2012).

28. A. Osipov, E. Orlov, Pharm. Chem. J.,**46**(11),684-686 (2013).

29. D. V. Nedeltcheva-Antonova, K. S. Tsandeva, R. D. Dimitrova, Bulgarian Chem.Commun., **46**, 94-99 (2014).