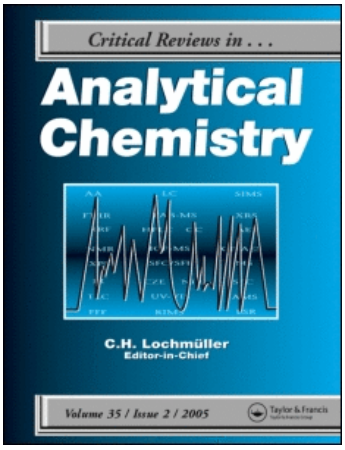

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

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Online publication date: 06 May 2010

To cite this Article Másquio Fiorentino, Flávia Angélica , Corrêa, Marcos Antonio and Nunes Salgado, Hérida Regina(2010) 'Analytical Methods for the Determination of Chlorhexidine: A Review', Critical Reviews in Analytical Chemistry, 40: 2, 89 — 101
To link to this Article: DOI: 10.1080/10408340903232020
URL: http://dx.doi.org/10.1080/10408340903232020

Critical Reviews in Analytical Chemistry, 40:89–101, 2010

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340903232020

Analytical Methods for the Determination of Chlorhexidine: A Review

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Chlorhexidine is a type of antiseptic belonging to biguanidic group and it is large, used in dentistry, human, and veterinary medicine because it is active against Gram-positive and Gram-negative microorganisms. It can be incorporated in several types of preparations and for this reason are necessary effective analytical procedures for quality control and pharmacodynamic and pharmacokinetic studies. Chlorhexidine can be analyzed by many types of assays, however the HPLC is the most used method. In this review, various methods to analyze chlorhexidine including spectrometry, chromatography, colorimetric reaction, solid phase extraction, and capillary electrophoresis are presented and the application of these methods for the determination of chlorhexidine in biological fluids and formulations are discussed.

Keywords review, chlorhexidine, methods of analysis.

INTRODUCTION

General Aspects of Chlorhexidine

Antiseptics are products which destroy microorganisms or inhibit their reproduction or metabolism (1). The antiseptics damage coagulation protoplasmatic or denaturation of proteins, cell lyses by structural change of the cell membrane, decrease of surface tension, inhibition of essential enzymes and they are used just to prevent infections because they destroy microorganisms in external surface and in the skin (2, 3). The use of antiseptics started in 1847 (4) and chlorhexidine, a type of antiseptic, was synthesized in the 1940s and commercialized in 1954 (5).

Chlorhexidine is an excellent cationic antiseptic belonging to biguanidic group, chemically known as 1,1′–hexamethylenebis {5–(*p*-chlorophenyl) biguanide} (Figure 1) (6, 7). It has a variety of salts, as digluconate (Figure 2), acetate (Figure 3), gluconate, and hydrochloride (Figure 4) (7, 8).

Chlorhexidine has many types of uses because is possible incorporate it into many types of products, for example, in soaps, oral rinse, mouthwash, solution of irrigation, gel, spray, and gum (9) and it has an extensive safety record, strong binding potential that results in effectiveness and low cost (10).

It is used in veterinary medicine as a disinfectant for cleansing wounds, skin, instruments, and equipment (11) and for treatment of dermatitis and piodermitis (12). In clinical practice, it is used for antisepsis of skin, hands, and mucous membranes and is currently recommended for treatment of wounds and burns (13, 14) and for preparation of patients in procedures (15). In dentistry it is used as a mouth antiseptic, for prevention of dental plaque formation and treatment of gingivitis, to eliminate oral pathogens, as an endodontic irrigant, and as an intracanal medication (11, 16).

The use of chlorhexidine has been suggested in spermicides to prevent the transmission of the HIV virus since it does not break the vaginal epithelium, has in vitro activity against the virus, and may prevent contraception because it spreads in the cervical mucus at concentrations of about 1 mg/mL and prevents the entry of sperm (17). Also, WHO recommends chlorhexidine for cleansing umbilical cords because it can markedly reduce the risk of omphalitis (10). Stevens and coworkers (18), Ha and Cheung (19), and Abad-Villar and collaborators (20) describe the use of chlorhexidine to preserve ophthalmic solution and to disinfect contact lenses because of its low toxicity.

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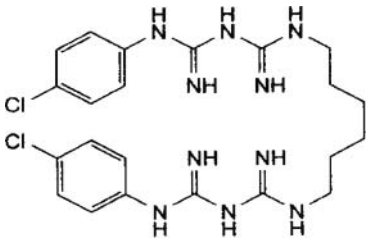

FIG. 1. Chemical structure of chlorhexidine. CAS: 55-56-1.

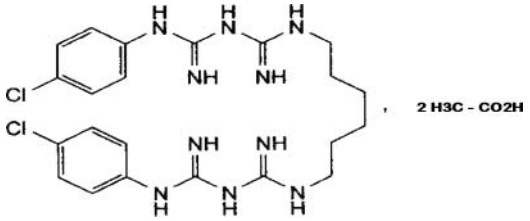

FIG. 3. Chemical structure of chlorhexidine acetate. CAS: 56-95-1

Chlorhexidine is active against Gram-positive bacteria and less effective for Gram-negative bacteria, fungi, and species of *Proteus*; it has activity only for certain types of enveloped viruses, including hepatitis virus, herpes simplex, HIV, cytomegalovirus, influenza, and respiratory syncytial virus. For mycobacteria, chlorhexidine presents minimal activity; against endospores and cysts of protozoan the activity is nil (3, 8, 9, 17, 21). The anti-microbial activity against spores is much discussed. Some authors say it has low activity (21, 22), while others say it does not (8, 11, 23). Martindale (17) and Bambace and coworkers (24) complement the chlorhexidine is effective against spores only in high temperatures.

Chlorhexidine acts on the cell membrane causing disruption and its consequent loss of intracellular material, respiratory inhibition, and cytoplasmic coagulation (9, 25). The links in the cell membrane probably occur between the positive charge of chlorhexidine with the negative charge of carboxyl groups available for proteoglycans and the phosphate groups of teicoic and lipoteicoic acids in bacterial inner wall. Regarding the metabolism, chlorhexidine inhibits the enzyme complex responsible for the incorporation of glycosis in the bacterial cell (5). The action of chlorhexidine is inhibited by nicotinic acid, but it the exact mechanism of this inhibition is not known; it is believed to occur by blocking the receptors of chlorhexidine by acid (26).

Side effects are significant discoloration of the teeth, restorations, and back of the tongue, flaking, and oral sensitivity; there is also a bitter taste and interference with the taste for a few hours after the mouthwash (27). When it is associated with soap, it can cause local or systemic toxicity; change in the normal flora of the skin predisposes it to the colonization by Gram-negative bacteria (28). Contact with strong chlorhexidine solution in eyes and sensitive tissue can cause irritation, when ingested, it can cause gastrointestinal irritation, vomiting, and dizziness, while systemic toxicity is rare (17, 21).

ANALYTICAL METHODS FOR DETERMINATION OF CHLORHEXIDINE

Development and validation of analytical methods is very important for the pharmaceutical industry to guarantee the quality of the commercialized products. Several techniques have been developed for the determination of chlorhexidine and the official method for salts of chlorhexidine described in the literature is an aqueous titration using 0.1 *M* perchloric acid (6, 29) and high performance liquid chromatography (HPLC) (7). For irrigation solution and mouthwash with chlorhexidine, HPLC is the described method in the European and British Pharmacopoeias (6, 29). The United States Pharmacopoeia (7) describes HPLC to analyze oral rinse with chlorhexidine gluconate. All these official compendiums describe HPLC to analyze related substances. The United States Pharmacopoeia (7) describes HPLC to analyze *p*-chloroaniline and the European and British Pharmacopoeias (6, 29) describe a colorimetric method. For analysis of chlorhexidine and its salts, gas chromatography (GC), (30) and HPLC; are the methods presented in the literature (31) to analyze impurities and related substances, catalytic oxidizer and HPLC (18), GC (32), and HPLC (33) are the methods mentioned. The parameters used in these analyses are presented in Tables 1 and 2.

In biological fluids, chlorhexidine is determined by several methods. For studies or the retention of chlorhexidine in the mouth is determined by radiolabelled chlorhexidine (^{14}C) (34), by direct UV spectroscopy (35), by fluorometry (36), by HPLC (37–39), and by solid phase microextraction (40) in saliva; by

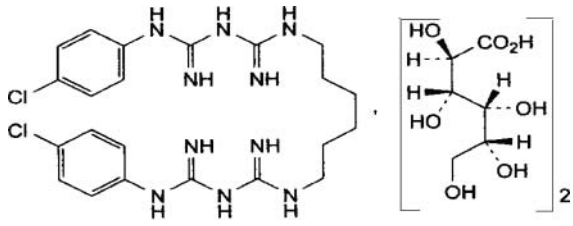

FIG. 2. Chemical structure of chlorhexidine digluconate. CAS: 18472-51-0.

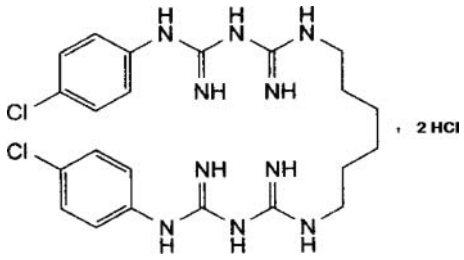

FIG. 4. Chemical structure of chlorhexidine hydrochloride. CAS: 3697-42-5.

TABLE 1
Parameters described in the literature to analyze chlorhexidine and its salts.

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
USP (2008)	HPLC	239	Solution A: sodium phosphate buffer pH 3.0 with triethylamine in water, mixing with acetonitrile Solution B: acetonitrile	L1		Gluconate
EP (2005)	Titration		Perchloric acid			Salts
EP (2005)	Titration		Perchloric acid			Salts
Siefert et al. (1975)	Gas chromatography		^{63}Ni detector	Supelco	50–200 ppm	Acetate
Doub et al. (1996)	Gradient HPLC	230	Phase A: ammonium acetate pH 5.0 Phase B: acetonitrile	Nucleosil C_{18}	10 μg/mL to 10 mg/mL	Gluconate

HPLC in urine (41, 42), in urine and serum, (43–45) and in serum (46), by liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) in hemolyzed blood (47), and determination of the elimination lifetime of chlorhexidine residues in milk of cow (25). The parameters used in these analyses are presented in Table 3.

In pharmaceutical products with chlorhexidine, HPLC is the method most used to analyze this antiseptic (7, 11, 19, 48–55); solid-phase extraction (SPE) with UV spectrophotometry (56), gas-liquid chromatography (57), liquid chromatography (6, 29), capillary electrophoresis (CE) (20, 58), flow injection extraction-spectrophotometry (59, 60); and voltametry (DPV) (61) also are used to assess it. The parameters used in these analyses are presented in Table 4.

Chlorhexidine has been incorporated in products to have controlled release delivery in the skin and in the mouth to prevent the biofilm formation and for the treatment of periodontal disease. HPLC is the method frequently used to analyze the liberation of chlorhexidine (62–67); however, spectrophotometry also can be used (68). The parameters used in these analyses are presented in Table 5.

The percutaneous absorption of chlorhexidine digluconate solution was assessed by reverse phase adsorption chromatography (69). The parameters used in this analyze is presented in Table 6.

DISCUSSION AND CONCLUSION

There are many methods described to analyze chlorhexidine, its degradation products, and impurities in the literature. However, HPLC is the most used. The physico-chemical properties of chlorhexidine indicate that HPLC with UV detection should be the analytical technique of choice (41). The method related in the European and British Pharmacopoeias (6, 29), colorimetric reaction, is loss-sensitive and accurate; the methods proposed by Revelle and coworkers (33) and by Doub et al. (31) only separate and identify the impurities of chlorhexidine, but do not quantity them.

The spectroscopic method proposed by Jensen and Christensen (35) to analyze chlorhexidine in biological fluids is no, specific for chlorhexidine because recovery compounds of saliva, but is a simple method and easy to carry out. However, fluorescence described by Vries and coworkers (36) is an easy and exact method to determine chlorhexidine in aqueous solution and in saliva. The method proposed by Bosnevoll and coworkers (34) assesses the quantity of chlorhexidine retained in the mouth 24 hours after the use of 0.2% chlorhexidine digluconate aqueous solution.

The chromatographic methods to analyze chlorhexidine in saliva are laborious, requiring lengthy and complicated extraction processes; however, they are more sensitive and specific than other techniques such as colorimetric, titration, fluorescence, and spectrophotometric methods. The solid-phase micro extraction allows one to assess several aspects such as connection and stability of chlorhexidine in the saliva and its pharmacokinetic effects after use of chlorhexidine solution. However, it requires many steps for extraction and because of this, it is laborious and requires more analysis time.

The method presented by Usui and coworkers (47) is more sensitive and selective than the HPLC-UV method and it also supplies more information about the identification of chlorhexidine and its impurities.

To determine chlorhexidine in urine, HPLC is the most used method. The method described by Wainwright and Cooke (42) allows detecting *p*-chloroaniline in the urine, but only chlorhexidine was quantified. The HPLC technique associated with SPE, described by Below and collaborators (45), allows one to determine the quantification limit of chlorhexidine in biological fluids and in pharmaceutical products and also verify the presence of *p*-chloroaniline and 1-chloro-4-nitrobenzene, but its quantification is not exact.

TABLE 2
Parameters described in the literature to analyze impurities and degradation products of chlorhexidine

Reference	Method	Λ(nm)	Mobile phase or solvent	Column	Linearity	Product found
USP (2008)	HPLC	239	Solution A: sodium phosphate buffer pH 3.0 with triethylamine in water, mixing with acetonitrile Solution B: acetonitrile	L1		Related substances and *p*-chloroaniline
EP (2005), BP (2005)	Gas chromatography and colorimetric reaction		Nitrogen	OV – 225		4- chloroaniline
EP (2005), BP (2005)	HPLC	254	Sodium octanesulphonate: glacial acetic acid: water: methanol	Nucleosil C_{18}		Related substances
Gavlick, Davis (1994)	Gas chromatography		Helium	J&W Scientific DB-1	0.956–10 ppm	*p*-chloroaniline
Revelle et al. (1993)	Isocratic HPLC with diode array detector, HPLC-MS*, NMR**	PHLC and HPLC-in: 230 RMN: 299.92 MHz	HPLC and HPLC-MS: buffer acetate pH 5.0: methanol NMR: D_2O ou CD_3OD	HPLC and HPLC in Zorbax CN		11 impurities called: B, B1, C, D, D1, D2, E, F, G, G1, H
Stevens et al. (1986)	HPLC-UV detector Catalytic oxidizer (CO)	220	H_3PO_4: sodium 1-heptanesulfonate: CH_3CN, pH 2.2	Regis Chemical C_6 Oxygen 300 mL/min for CO	1–80 μg/mL	*p*-chloroaniline *p*-chlorophenyl biguanide and phenyl-biguanide

MS*—mass spectroscopy; NMR**—nuclear ressonance magnetic.

In pharmaceutical preparations, HPLC is the most used method; however, it is necessary to extract the chlorhexidine of the products and in some cases this process is lengty. The method described by Miribel and coworkers (57) needed several steps. Moreover, mass spectroscopy and nuclear resonance magnetic are carried out to verify the chemical structure of chlorhexidine. The methods proposed by Gavlick (54) and Bauer and coworkers (50) allow the quantification of chlorhexidine and chloroaniline. The method proposed by Abad-Villar and coworkers (20) was successful and the detection limit for chlorhexidine is comparable with the best value reported for HPLC with UV-detection; this method is useful in ophthalmic drug penetration studies due to its simplicity, sensitivity, and limited requirement on sample volume. The other method of CE described by Okamoto and coworkers (58) also was useful to separate and to quantify seven compounds in an ointment.

Calatayud and coworkers (59) described a method that uses the precipitation of chlorhexidine with thymol blue; this precipitation is evaluated by flow injection. This method was fast and simple. The method proposed by Pérez-Ruiz and coworkers (1999) (60) might be applicable for many types of pharmaceutical preparations and also has instrumental simplicity when compared with HPLC.

TABLE 3
Parameters described in the literature to analyze chlorhexidine in biological fluids

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
Bosnevoll et al. (1974)	Liquid scintillation spectrometer				Retention of chlorhexidine 6.9 ± 1.4 mg	Saliva
Jensen, Christensen (1971)	UV	250	Chloroform		5–25 μg/mL; 25–200 μg/mL	Saliva
Vries et al. (1991)	fluorescence	541	Dye eosin-Y		6.7–20 μg/mL	Saliva
Lam et al. (1993)	HPLC with UV detector	260	Acetonitrile: socium acetate buffer: heptanesulfonic acid pH 5.0	Bekman Ultrasphere ODS C_{18}	0.05–20 μg/mL	Saliva
Medlicott et al. (1994)	HPLC with dual detector	254	Acetronitrile: glacial acetic acid: sodium laurylsulphate	Exsil ODS-B C_{18}	2–30 μg/mL	Saliva
Pesonem et al. (1995)	HPLC with dual detector	260	Acetonitrile: buffer (disodium hydrogen phosphate, 1-heptanesulfonic acid, triethylamine) pH 2.5	LiChrospher 100 RP-18	0.5–100 μg/mL	Saliva
Musteata, Pawliszyn (2005)	Solid phase micro extraction		HPLC-MS and *ESI-MS: acetonitrile: water (acidificated with formic acid pH 3.2)	Zorbax Eclipse C_{18}	0.05–40 μg/mL	Saliva
Gaffney, Cooke (1984)	HPLC with UV detector	260	Methanol: sodium acetate buffer pH 5.0	μBondapak C_{18}	1–10 μg/mL	Urine
Wainwright, Cooke (1986)	HPLC with UV detector	260	Methanol: ammonia solution: ammonium nitrate	Partisil silica	50–200 μg/mL	Urine

(Continued on next page)

TABLE 3
Parameters described in the literature to analyze chlorhexidine in biological fluids *(Continued)*

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
Huston et al. (1982)	HPLC with UV detector	238	Toluene-4-sulphonic acid in methanol: water	ODS Waters	1–20 μg/mL	Urine and blood
Brougham et al. (1986)	HPLC with UV detector	260	Methanol: water with sodium heptanesulfonate	Bondapak C_{18}	20.2–808 ng/mL	Serum and urine
Below et al. (2004)	Gradient HPLC with UV, combined with solid phase extraction	250	Acetonitrile: buffer solution (ammonia solution and acetic acid) pH 8.7 (eluant A) Acetonitrile: acetic acid (eluant B)	Separation column Luna C_{18}	Detection at 2.5 mg/L and 25 mg/L	Serum and urine
Kudo et al. (2002)	HPLC with UV detector	260	Acetonitrile: water with 0.05% trifluoroacetic acid, 0.05% heptafluorobutyric acid and 0.1% de triethylamine	Capcell Pak C_{18}	0.05–50 μg/mL	Serum
Usui et al. (2006)	HPLC-ESI-MS* HPLC-MS: isocratic HPLC		LC-MS: acetonitrile: water: trifluoroacetic acid	LC-MS: TSK gel ODS 100 V	0.1–11 μg/mL	Hemolyzed blood
Hebert et al. (2003)	HPLC with photodiode array detector	258	Acetate buffer (pH 3.6): acetonitrile	Supelco Discovery RP-Amide C_{16}	1–100 μg/mL	Saliva

*Liquid chromatography—elestrospray ionization and mass spectrophotometry.

TABLE 4
Parameters described in the literature to analyze chlorhexidine in pharmaceutical products

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
Bailey et al. (1975)	HPLC with UV detector	254	Acetonitrile: 0.02 *N* sulphuric acid in water	Partisil	10–600 μg/mL	Antiseptic solution, talc, lotion, oral gel, obstetric cream
Bauer et al. (1984)	HPLC with variable – wavelength detector	294	Acetonitrile: potassium dihydrogen phosphate buffer solution with tetrabuthylammonium hydrogen sulphate pH 5.9	μBondapck C_{18}	0.64–2.56 μg/mL	Pastille
Bauer et al. (1983)	HPLC with variable – wavelength detector	262	Buffer solution with sodium acetate pH 3.5: acetonitrile with lauryl sulphate sodium	μBondapack CN_{10}	0.6–1.4 μg/mL	Pastille
Gagliard et al. (1985)	Gradient HPLC with UV detector	264	Acetonitrile: water with sodium perchlorate and TMAB*	Erbasil C_{18}	0.02–6 μg/mL	Cream
Hu et al. (1991)	HPLC with UV detector	254	Potassium dihydrogen phosphate buffer solution pH 3.5: methanol	Nucleosil C_{18}	2.72–436 μg/mL	Solutions for soft contact lenses
Gavlick (1992)	Gradient HPLC with photodiode-array detector	239	Phase A: acetonitrile Phase B: buffer TFA pH 2.0	Suples pkb- 100	19.1–146 ppm	Handwash product
Há, Cheung (1996)	Gradient HPLC with UV-Vis detector and photodiode array detector	235	Phase A: acetonitrile: ammonium acetate pH 5.0 Phase B: phosphate buffer: acetonitrile	Hamilton PRP-1	Solution with 30 ppm of chlorhexidine	Eye-drop solution
Richard et al. (1984)	Gradient HPLC with UV detector	254	Phase A: sodium acetate buffer solution pH 4.0: acetic acid: methanol: sodium heptanesulphonate solution (92.1 0.2: 4.8: 2.9) Phase B: iqual phase A, but in proportion 4.8: 0.4: 91.9: 2.9	μBondapck C_{18}	0.312–10 mg/100 mL	Eye-drop, ophthalmic preparation and contact lens solutions

(Continued on next page)

TABLE 4
Parameters described in the literature to analyze chlorhexidine in pharmaceutical products *(Continued)*

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
Havlíková et al. (2007)	HPLC with UV-Vis detector	239	Acetonitrile: sodium phosphate buffer solution containing triethylamine pH 3.0	Zorbax SB Phenyl column	51.2–178.5 μg/mL	Veterinary ointment
Bonazzi et al. (1995)	Solid-phase extration (SPE) and UV spectroscopy (conventional and derivative)	256 (UV) 248 (^{1}D) 241 (^{2}D)	Solvent: methanol-phosphate buffer solution pH 4.5	SPE C_{18} (para SPE)	7.3–36.5 μg/mL	Creams
Izumoto et al. (1997)	HPLC with UV detector and gas chromatography (GC)	270	2-propanol: phosphate buffer pH 3.0	Zorbax RX-C_8(for HPLC) Chromosorb W (for GC)	60–140% of the assay concentration	Ointment
Miribel et al. (1983)	Gas liquid chromatography, mass spectrometry and nuclear magnetic resonance		Nitrogen (for gas chromatography)	3% OV-101 on Chromosorb W HP (gas)	0.2–2 mg/mL	Cream and ointments
Calatayud et al. (1987)	Flow injection with turbidimetric detection	610	Thymol blue		10.5–63.0 μg/mL	Solution
Pérez-Ruiz et al. (1997)	flow injection extraction spec-trophotometric	422	Bromophenol blue		57840–5784 μg/mL	Tablets, solution, toothpaste
Okamoto et al. (2001)	Capillary electrophoresis	230	80 nM TDA, 20 nM ammonium chloride, 20 nM triethylamine and 40 mM acetic acid in acetonitrile: water (80:20)	Fused silica capillaries of 50 μm i.d. and 375 μm o.d.	25–75 μg/mL	Ointment

Abad-Villar et al. (2006)	Capillary electrophoresis		2.3 *M* acetic acid containing tween 20	Fused silica capillaries of 25 μm i.d. and 375 μm o.d.	10 μg/mL – 20 mg/mL	Eye drops
Wang, Tsai, 2001	Voltametry HPLC	240	Three-electrode system: working electrode (Hg^{2+}/GCE and Hg^{2+}/Au), platinum counter and saturated calomel electrode Methanol:water (80:20 and 90:10) containing phosphate or acetate buffer	μBondapack C_{18}	5–40 ppm 5–40 ppm	Antiperspirant toothpaste, gargle mounthwash, antiseptic liquid
BP (2005), EP (2005)	HPLC	254	Sodium octanesulphonatein mixture of glacial acetic acid, water and methanol	Nucleosil C_{18}		Irrigation solution and mouthwash
USP (2008)	Gradient and isocratic HPLC	239	Solution A: sodium phosphate buffer solution with triethylamine pH 3.0: acetonitrile Solution B: acetonitrile	L1		Oral rinse
BP (2005), EP (2005)	HPLC	254	Sodium octanesulphonatein mixture of glacial acetic acid, water and methanol	Nucleosil C_{18}		Irrigation solution and mouthwash

*TMAB is effective in deactivating residual negatively-charged silanol in sites of the bonded-phase.

TABLE 5
Parameters described in the literature to analyze the liberation of chlorhexidine in pharmaceutical products

Reference	Method	λ (nm)	Mobile phase or solvent	Column	Linearity	Sample
Medlicott et al. (1996)	UV spectroscophy HPLC with dual detector	254	Sodium citrate/sodium hydroxide buffer (for UV) Acetonitrile: glacial acetic acid: sodium laurylsulphate (for HPLC)	C_{18}ODS-B Exsil	1 – 60 μg/mL	Poly(ε-caprolactone)
Yue et al. (2004)	HPLC with UV-Vis detector	280	Methanol: water pH 3.0	C_{18} Nova-Pak	Theorical loading of 10 – 40%	Biodegradable poly(dl-lactic acid—co—glycolic acid) microspheres by complexation with cyclodextrins
Paler et al. (2004)	RP HPLC	270	Propanol (40%):0.05M NaH_2PO_4 (40%) and sodium dodecyl sulphate (0.4%), pH 3.0	C_{18}Jones chromatography		Chlorhexidine acetate in glass ionomer cements
Rasimick et al. (2008)	HPLC with UV-Vis detector	288		C_{18} Waters X Bridge	10–150 ppm chlorhexidine	Precipitate of chlorhexidine and EDTA
Farkas et al. (2007)	UV spectroscopy	255			1–20.0 μg/mL	Chlorhexidine and its salts in crystalline structures
Medlicott et al. (1999)	HPLC with dual detector	254	Acetronitrile: glacial acetic acid: sodium laurylsulphate	Exsil ODS-B C_{18}	2–30 μg/mL	Saliva
Giunchedi et al. (2002)	RP- HPLC	257	Acetonitrile:buffer (0.1 M disodium hydrogen phosphate, 0.005 M 1-heptanesulfonic acid and 0.05 M triethylamine) (35:65	Nucleosil RP-18		Saliva

TABLE 6
Parameters described in the literature to analyze the liberation of chlorhexidine in the skin

Reference	Method	λ(nm)	Mobile phase or solvent	Column	Linearity	Sample
Lafforgue (1997)	RP adsorption chromatography	254	Acetonitrile: 50 mM acetate buffer (50:50), pH 3.15		0–20 mg/mL	Intact and stripped skin

The voltametric method described by Wang and Tsai (2001) (61) to analyze chlorhexidine in pharmaceutical preparations might be performed without separation from the fatty constitutents and simple extraction procedure, different than the HPLC method compared by these authors.

The method presented by Palmer and coworkers (66) was useful to demonstrate that chlorhexidine might be incorporate, in glass ionomer cements because it is released into solution. Rasimick and coworkers (67) describe a method that was useful to quantify chlorhexidine and EDTA in the precipitate formed between the association of these products. These results demonstrate that it can be used in dentistry with efficiency. The spectroscopic method describe by Farkas and coworkers (68) demonstrated that different liquid crystalline structures might be used to control the release of chlorhexidine and its salts. The authors concluded that this method is more simple than HPLC methods.

The methods described by Medlicott and coworkers (63) and Giunchedi and coworkers (64) were also useful to analyze chlorhexidine in the controlled system delivery.

The method described by Lafforgue and coworkers (69) was simple and important to demonstrate that when the skin is damaged the amount of chlorhexidine absorbed and stored is larger then the skin is intact.

Pharmaceutical products have to obey the law and ensure their efficacy without a raise in risk of the life of the consumer. It is necessary for the routine quality control of pharmaceutical products to employ well-characterized and fully validated analytical methods to yield reliable results that can be satisfactorily interpreted. The analytical methods are constantly undergoing changes and improvements, and in many instances, they are at the cutting edge of the technology (70). This review is important because it presents several methods to analyze chlorhexidine and their advantages with technological improvement.

ACKNOWLEDGMENTS

The authors acknowledge to CAPES (Brasília, Brazil) and CNPq (Brasília, Brazil).

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