Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(3); 141-150

ISSN: 0976 822X

Research Article

Comparison of Throat Sprays Containing Chlorhexidine Gluconate and Lidocaine Hydrochloride

Kapil L. Dubal[2], Vijay R. Ram[1], Govind J. Kher [2], Pragnesh N. Dave[1], Hitendra S. Joshi[2], Ekta Khosla[3]*

[1]*Department of Chemistry, KSKV Kachchh University, Bhuj-370 001, Gujarat, India.*
[2]*Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India.*
[3]*Department of Chemistry, Hans Raj Mahila Maha Vidyalaya, Jalandhar.144001India.*

Available Online:31st March, 2016

ABSTRACT
A novel UPLC method for determination of chlorhexidine gluconate and lidocaine hydrochloride in throat sprays has been presented as a tool suitable for quality monitoring of these compounds. The developed and validated UPLC method was compared with HPLC method. Experimental parameters like analysis time, sensitivity, mobile phase consumption, validation data, and demands on instrument equipment and operation were compared for two methods. UPLC and HPLC are important methods for routine monitoring of these drugs. In UPLC, Major degradation was found in photolytic condition that product was degraded up to 30 %. The major impurity peaks was found at 3.73 min. The concentration range was varied between 0.040-0.160 mg/mL, 0.016-0.064 mg/mL for chlorhexidine gluconate and lidocaine hydrochloride respectively. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for both the technique HPLC and UPLC fitted well. The RSD values for intraday precision study and interday precision study was < 2.0 % for chlorhexidine gluconate and lidocaine hydrochloride, which confirms that the method was precise. Recovery of chlorhexidine gluconate and lidocaine hydrochloride was determined at three different concentration levels. The mean recovery for chlorhexidine gluconate was 100.74-102.60 % and 99.07-101.11 % for lidocaine hydrochloride for HPLC and the mean recovery for chlorhexidine gluconate were 98.6-99.80 % and 99.17-99.52 % for lidocaine hydrochloride for UPLC. The appropriate method should be with less cost, high sensitivity, reproducibility, short analysis time and simplicity. All these necessities are met by both methods. In our work UPLC analysis took only seven minutes to complete a single run therefore UPLC method can be considered ideal for busy laboratories analyzing large numbers of samples.

Keywords: Ultra Performance Liquid Chromatography (UPLC), High Performance Liquid Chromatography (HPLC), Chlorhexidine Gluconate, Lidocaine Hydrochloride, Stability indicating assay, Method validation

INTRODUCTION

Throat sprays are much of interest now days in terms of stability parameters. Recently stability-indicating assays[1-3] by the approach of stress testing have been published in the International Conference on Harmonization (ICH) guideline Q1A (R2)[2]. Even this methodology has been extended to drug combinations[4,5] to evaluate precise and specific quantization of multiple drugs in presence of their degradation products and interaction product if any. Chlorhexidine gluconate is a chemical antiseptic[6] which has been known for antimicrobial activity. It is a component of throat sprays, oral rinses and skin cleansers. It has been marketed under the brand names Peridex, Medica (Throat spray), or Periogard Oral Rinse. In the UK it is chiefly marketed under the brand name Corsodyl. It kills both gram-positive and gram negative microbes. However, it has been found less active with some gram negative microbes[7]. Chemically, chlorhexidine gluconate (CHG) is 1,1'-hexamethylene bis(5-(p-chlorophenyl) biguanide) digluconate. Its molecular formula is $C_{22}H_{30}Cl_2N_{10}•2C_6H_{12}O_7$ having molecular weight 897.72 g/mole. The structures of the molecule has been shown in Figure 1(a). It is frequently used as an active ingredient in mouthwash intended to reduce dental plaque and oral bacteria. Chlorhexidine can therefore be used to improve bad breath[8]. It has been revealed to have an immediate bactericidal action and a prolonged bacteriostatic action due to adsorption onto the pellicle-coated enamel surface[9]. Chlorhexidine-based products are typically used to combat or prevent gum diseases such as gingivitis. According to Colgate[10], Chlorhexidine gluconate has not been proven to reduce sub gingival calculus and in some studies actually increased deposits. When combined with xylitol, a synergistic effect has been observed to enhance efficacy[11]. Lidocaine hydrochloride (LDC), the first amino amide-type local anesthetic, was first synthesized under the name Xylocaine by Swedish chemist Nils Lofgren in 1943. His colleague Bengt Lindquist

**Author for Correspondence*

performed the first injection anesthesia experiments on himself[12]. Lidocaine is a common local anesthetic and class IB (membrane-stabilizing) anti arrhythmic drug. It was first marketed in 1949. Lidocaine is used topically to relieve itching, burning and pain from skin inflammations, injected as a dental anesthetic or as a local anesthetic for minor surgery.Lidocaine is currently listed by the World Anti-Doping Agency as an illegal substance[13]. Lidocaine hydrochloride is chemically 2-(diethyl amino)-N-(2, 6-dimethylphenyl) acetamide hydrochloride . The molecular formula is $C_{14}H_{22}N_2O{\bullet}HCl$ having molecular weight 270.84 g/mole. The structures of the molecule has been shown in Figure 1(b). Lidocaine alters signal conduction in neurons by blocking the fast voltage gated sodium (Na^+) channels in the neuronal cell membrane, which are responsible for signal propagation.[14] With sufficient blockade, the membrane of the postsynaptic neuron will not depolarize and so fail to transmit an action potential, leading to its anesthetic effects. Careful titration allows for a high degree of selectivity in the blockage of sensory neurons, whereas higher concentrations will also affect other modalities of neuron signaling. Various research papers are available concerning determination method of Chlorhexidine gluconate and Lidocaine hydrochloride but most of the methods are applicable either to chlorhexidine gluconate or lidocaine hydrochloride in various pharmaceutical formulations or in biological fluids[15]. Spectrophotometric[16], ESI-MS[17], Polarographic[18], Gas chromatographic[19], hydrophobic interaction electro kinetic chromatography[20] and HPLC methods have been reported for determination of chlorhexidine[21-24]. Thin Layer chromatography[25], SPE-RP-HPLC[26], LC-MS-MS[27], HPLC methods are reported for the determination of lidocaine hydrochloride[28]. Only one HPLC method is reported simultaneous determination of chlorhexidine gluconate and lidocaine hydrochloride[29] but in this method two mobile phases were used: mobile phase A; comprised a mixture of methanol/water/triethyl amine (58:42:0.4) and the pH adjusted to 3 with phosphoric acid for analysing active ingredients, and mobile phase B; comprised a mixture of methanol/0.01 N ammonium acetate (70:30) for limit testing related substances. As far as our knowledge is concerned, no comparative stability-indicating HPLC and UPLC assay method is available for the determination of chlorhexidine gluconate and lidocaine hydrochloride in throat spray has been published. The previous published methods are not directly applicable for this issue and need more investigation for method development and validation. Consequently, the focus in the present study was to develop a comparative validated stability indicating HPLC and UPLC method for the combination, by degrading the drugs together under various stress conditions like acidic, alkali, oxidative, thermal and photolytic stress which is recommended by ICH guidelines.

Experimental

MATERIALS

Chlorhexidine gluconate and lidocaine hydrochloride standard of was provided by Hetero Drugs Ltd., Hyderabad (India). The sample of throat spray containing 20 mg chlorhexidine gluconate and 5 mg lidocaine hydrochloride per 10 mL of formulation and the inactive ingredients used in drug matrix were obtained from market. HPLC grade acetonitrile was obtained from Spectrochem Pvt. Ltd., Mumbai (India). MiliQ (Millipore India Ltd.) water purification system is used to obtain HPLC grade water and water is used as diluents for all the standard samples. Analytical grade sodium dihydrogen phosphate dihydrate, phosphoric acid, hydrochloric acid, sodium hydroxide pellets, triethylamine and 30 % v/v hydrogen peroxide solution were obtained from RANKEM, New Delhi (India). UV cabinet of Hally Instruments, Model: Mild Steel, Powder Coated was used for the UV light.

Instrumentation

HPLC

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photodiode array detector set at 215 nm and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu). Chromatographic analysis was performed on a SGE make SS Wakosil II RS column (150 mm x 4.6 mm i.d., 5 µm particle size) C8 column.

UPLC

The liquid chromatographic system of Waters Acquity UPLC, photodiode array detector set at 215 nm with Empower2 data processing system was used for this entire study and chromatographic separation was achieved by using Waters Acquity BEH C18, 2.4 x 50 mm, 1.7µ column as stationary phase with binary gradient mode.

Chromatographic conditions

Chromatographic conditions for HPLC

The flow rate of the mobile phase was adjusted to 1.0 mL/min with a gradient program used for the separation is shown below. Injection volume was 20 µL. Detection was performed on a Photodiode array detector at 215nm.The mobile phase consisted of acetonitrile – 50 mM sodium phosphate buffer pH 3.0 (Gradient Elution) (Table 1). To prepare the buffer solution, 7.8 gm sodium hydrogen phosphate monobasic dihydrate was weighed and dissolved in 900 mL HPLC grade water and 2.5 mL triethylamine was added then pH of the buffer is adjusted to 3.0 with orthophosphoric acid. The volume is then made up to 1000 mL with HPLC grade water. Mobile phase was filtered through a 0.45µm nylon membrane (Millipore Pvt. Ltd. Bangaluru, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

Chromatographic conditions for UPLC

Chromatographic analysis was performed on a Waters Acquity BEH C18, 2.4 x 50 mm, and 1.7µ column. The flow rate of the mobile phase was adjusted to 0.3 mL/min and the injection volume was 4 µL partial loop with

Table 1: Gradient program used for separation of chlorhexidine gluconate and lidocaine hydrochloride (HPLC)

Gradient Step	Time (Minutes)	Acetonitrile (%)	Buffer (%)
1	0	15	85
2	2	15	85
3	5	20	80
4	9	25	75
5	10	25	75
6	12	30	70
7	15	30	70
8	20	30	70
9	21	15	85
10	23	15	85

Table 2: Gradient program used for separation of chlorhexidine gluconate and lidocaine hydrochloride (UPLC)

Step	Time (minutes)	Flow (mL/min)	% Buffer	% Acetonitrile
1	Initial	0.3	85	15
2	2	0.3	85	15
3	4	0.3	70	30
4	6	0.3	70	30
5	7.5	0.3	85	15
6	8	0.3	85	15

needle overfill. Detection was performed at 215nm. The mobile phase consisted of acetonitrile – 20 mM sodium phosphate buffer pH 3.0 (Gradient Elution) (Table 2). To prepare the buffer solution, 3.12 g sodium dihydrogen phosphate was weighed and dissolved in 900 mL HPLC grade water, 2.5 mL triethylamine is added and pH was adjusted to 3.0 with orthophosphoric acid. The volume is made up to 1000 mL with HPLC grade water. Mobile phase was filtered through a 0.45 µm nylon membrane and degassed in an ultrasonic bath.

Standard solution preparation

Chlorhexidine gluconate standard stock solution containing 1000.70 µg/mL was prepared in a 100 mL volumetric flask by dissolving 509 mg of Chlorhexidine gluconate solution and then diluted to volume with diluent. Further 20 mL of this stock solution is taken in a 100 mL volumetric flask and made up to the mark with diluents water (this is the standard solution of 200.14 µg/mL). Lidocaine hydrochloride standard stock solution containing 250 µg/mL was prepared in a 100 mL volumetric flask by dissolving 25.0 mg of lidocaine hydrochloride and then diluted to volume with diluent. Further 20 mL of this stock solution is taken in a 100 mL volumetric flask and make the volume up to mark with diluent (this is the standard solution of 50.00 µg/mL). And two standard solutions were mixed up before injection.

Test sample preparation

Test sample preparation was prepared by taking 5 mL of the commercial throat spray sample in a 50 mL volumetric flask and about 30 mL of diluent was added and sonicated for a minimum 10 minute with intermittent shaking. Then content was brought back to ambient temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. The concentration obtained was 200.00 µg/mL of chlorhexidine gluconate and 50.00 µg/mL of lidocaine hydrochloride.

Method validation

Specificity study

The evaluation of the specificity of the method was determined against placebo and stress (forced degradation) application. The interference of the excipients of the claimed placebo present in the pharmaceutical formulation was derived from placebo solution. Further the specificity of the method toward the drug was established by means of the interference of the degradation products against drug during the forced degradation study by checking peak purity of individual drug.

Degradation study

The degradation samples were prepared by transferring commercial sample containing 10 mg chlorhexidine gluconate and 2.5 mg lidocaine hydrochloride into a 50 mL round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to ambient temperature and diluted with diluents to attain 0.20 mg/mL of chlorhexidine gluconate and 0.05 mg/mL of lidocaine hydrochloride concentration. Specific degradation conditions were described as follows.

Acidic condition

Acidic degradation study was performed by dissolving the drug in 0.1 N HCl at ambient temperature for 1 h and mixture was neutralized.

Alkaline condition

Alkaline degradation study was performed by taking the drug content in 0.1M NaOH at ambient temperature for 1 hour and mixture was neutralized.

Oxidative condition

Oxidation degradation study was performed by taking the drug content in 30 % v/v H_2O_2 at ambient temperature for 0.5 h.

Thermal condition

Thermal degradation was performed by exposing formulation at 75˚ C for 72 h.

Photolytic condition

Photolytic degradation study was performed by exposing the drug content in UV light for 72 h.

Linearity

For linearity seven points calibration curve were obtained in a concentration range from 0.040-0.160 mg/mL for chlorhexidine gluconate and 0.016-0.064 mg/mL for Lidocaine hydrochloride. The peak areas versus concentration data were evaluated by linear regression analysis.

LOD and LOQ

The limit of detection and limit of quantification were evaluated by serial dilutions of Chlorhexidine gluconate

Table 3: Results of precision study (HPLC)

Set	chlorhexidine gluconate (%Assay)		lidocaine hydrochloride (%Assay)	
	Intraday (n = 6)	Interday (n = 6)	Intraday (n = 6)	Intraday (n = 6)
1	100.7	99.4	100.0	99.5
2	99.4	100.6	99.1	99.3
3	99.6	103.1	99.2	99.1
4	101.2	99.6	99.0	99.2
5	100.4	99.6	99.4	99.2
6	100.7	101.5	99.4	99.4
Mean	100.3	100.6	99.4	99.3
Standard deviation	0.70	1.47	0.36	0.15
% RSD	0.70	1.46	0.36	0.15

Table 4: Results of precision study (UPLC)

Set	chlorhexidine gluconate (%Assay)		lidocaine hydrochloride (%Assay)	
	Intraday (n = 6)	Interday (n = 6)	Intraday (n = 6)	Intraday (n = 6)
1	100.5	100.8	99.7	99.5
2	99.8	99.8	100.1	99.4
3	100.6	100.7	99.7	99.7
4	100.2	100.4	100.3	99.8
5	99.9	101.0	99.9	99.5
6	99.6	99.7	99.3	98.9
Mean	100.2	100.4	99.9	99.5
Stdev	0.42	0.53	0.33	0.33
% RSD	0.42	0.53	0.33	0.33

and lidocaine hydrochloride stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ.

Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of chlorhexidine gluconate and lidocaine hydrochloride test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

Accuracy

An accuracy study was performed by adding known amounts of Chlorhexidine Gluconate and Lidocaine hydrochloride to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate.

Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (± 0.1 mL/min), buffer pH (± 0.2 pH) and using different lot of LC column.

Solution stability

The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2 - 8˚ C and tested at interval of 12 h, 24 h, 36 h and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

RESULTS

Development and optimization of the HPLC method

In the present work, an analytical method based on LC using UV detection was developed and validated for assay determination of Chlorhexidine gluconate and Lidocaine hydrochloride in liquid formulation. The analytical conditions were selected, keeping in mind the different chemical nature of chlorhexidine gluconate and lidocaine hydrochloride. The development trials were carried out by using the degraded sample of each component was done, by keeping them in various extreme conditions. The column selection has been done on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between Chlorhexidine gluconate and Lidocaine hydrochloride peak. After evaluating all these factors, SGE make SS Wakosil II (150 mm x 4.6 mm i. d., 5 µm particle size) C8 column was found to be giving satisfactory results. The selection of buffer based on chemical structure of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Considerably good results were obtained with 50 mM sodium phosphate buffer, pH 3.0 with orthophosphoric acid solution. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. To improve the shape the peaks of chlorhexidine gluconate and lidocaine hydrochloride, 0.25 % Triethylamine was added in the buffer. Finally, by fixing 50 mM sodium hydrogen phosphate monobasic dihydrate pH 3.0 and mobile phase composition consisting of a mixture of acetonitrile and sodium hydrogen phosphate monobasic dihydrate pH 3.0- (85:15, v/v). During the development stage it was found that by using isocratic elution, the retention time of chlorhexidine gluconate was around 35 minutes. Therefore, the gradient elution mode is selected to shorten the runtime. Optimized proportion of mobile phase has shown good resolution between chlorhexidine gluconate and lidocaine hydrochloride and also for degradation product which is generated during force degradation study. Figure 2 represent the chromatograms of standard preparation.

Development and optimization of the UPLC method

Proper selection of the methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its solubility and polarity. Chlorhexidine gluconate and lidocaine hydrochloride is soluble in polar solvent therefore Reversed phase UPLC was selected to estimate them. To develop a rugged and suitable UPLC method for the quantitative determination of chlorhexidine gluconate and lidocaine hydrochloride, the analytical condition were selected after testing the different parameters such

Table 5: Results of accuracy study (HPLC)

	Level (%)	Theoretical concentration[a] (µg/mL)	Observed concentration[a] (µg/mL)	% Recovery	% RSD
Chlorhexidine gluconate	50	98.69	99.42	100.74	0.65
	100	196.99	197.71	100.36	0.76
	150	296.74	304.45	102.60	0.40
Lidocaine hydrochloride	50	25.07	25.09	100.12	1.22
	100	50.20	50.05	99.07	0.65
	150	75.00	75.83	101.11	0.33

[a]Each value corresponds to the mean of three determinations

Table 6: Results of accuracy study (UPLC)

	Level (%)	Theoretical concentration[a] (µg/mL)	Observed concentration[a] (µg/mL)	% Recovery	% RSD
Chlorhexidine gluconate	50	97.78	97.70	99.80	0.63
	100	199.35	196.55	98.60	0.34
	150	294.11	292.01	99.29	0.25
Lidocaine hydrochloride	50	24.90	24.73	99.31	0.99
	100	50.20	49.96	99.52	0.52
	150	75.07	74.45	99.17	0.90

[a]Each value corresponds to the mean of three determinations

Table 7: Evaluation data of solution stability study (HPLC)

Intervals (hours)	% Assay for test solution stored at 2 -8˚ C		% Assay for test solution stored at ambient temperature	
	chlorhexidine gluconate	lidocaine hydrochloride	chlorhexidine gluconate	lidocaine hydrochloride
Initial	--	--	100.8	99.2
12	100.4	98.6	100.4	98.9
24	100.6	97.8	99.5	97.7
48	100.6	98.0	100.9	97.6

as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our initials trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. A method, which is developed using HPLC system is transferred to UPLC by considering the dimensions of column, diameter of particles. A software provided by the Waters Corporation suggested possible UPLC method parameters by entering current HPLC analysis parameters. Software suggested various parameters, like optimum flow rate, injection volume, mobile phase composition, gradient program etc. A binary mobile phase system of 20 mM sodium phosphate, pH 3.0 and acetonitrile. After evaluating all these factors, Waters Acquity BEH C18, 2.4 x 50 mm, 1.7µ column was found to be giving satisfactory results. The selection of buffer based on chemical structure of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Considerably good results were obtained with 20 mM sodium hydrogen phosphate monobasic dihydrate pH 3.0 with orthophosphoric acid solution. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. To improve the shape the peaks of chlorhexidine gluconate and lidocaine hydrochloride, 0.25 % Triethylamine was added in the buffer. Finally, by fixing 20 mM sodium hydrogen phosphate monobasic dihydrate pH 3.0 and mobile phase composition consisting of a mixture of acetonitrile and sodium dihydrogen phosphate dihydrate pH 3.0- (85:15 v/v). During the development stage it was found that by using isocratic elution, the retention time of chlorhexidine gluconate was around 13 minute and peak shape was not good. Therefore, the gradient elution mode is selected to shorten the runtime. Optimized proportion of mobile phase has shown good resolution between chlorhexidine gluconate and lidocaine hydrochloride and also for degradation product which is generated during force degradation study.

System suitability

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less than 40000 for chlorhexidine gluconate and 2000 for lidocaine hydrochloride and % RSD of peak area not more than 2.0, were fulfilled during all validation parameter

Table 8: Evaluation data of solution stability study (UPLC)

Intervals hours	% Assay for test solution stored at 2 – 8 ˚C		% Assay for test solution stored at ambient temperature	
	chlorhexidine gluconate	lidocaine hydrochloride	chlorhexidine gluconate	lidocaine hydrochloride
Initial	--	--	100.2	99.6
12	100.1	99.5	100.1	99.9
24	100.2	99.8	100.0	99.2
36	100.2	99.1	100.4	99.0
48	100.2	98.7	100.3	98.7

Table 9: Evaluation data of robustness study of chlorhexidine gluconate (HPLC)

Robust conditions	% Assay	System suitability parameters		
		Theoretical plates	Asymmetry	Resolution
Flow 0.9 mL/min	99.1	64832	1.29	27.01
Flow 1.1 mL/min	101.9	66345	1.27	28.26
Buffer pH 3.5	101.6	64509	1.13	26.21
Buffer pH 2.5	98.5	56877	1.49	27.35
Column change	101.5	49547	1.24	28.44

Table 10: Evaluation data of robustness study for lidocaine hydrochloride (UPLC)

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.2 mL/min	99.4	3328	1.36
Flow 0.4 mL/min	100.0	3964	1.31
Buffer pH 3.2	99.4	3785	1.34
Buffer pH 2.8	98.6	3458	1.29
Column change	99.1	3013	1.30

Specificity

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method were eluted by checking the peak purity of Chlorhexidine gluconate and Lidocaine hydrochloride during the force degradation study. The peak purity of the Chlorhexidine Gluconate and Lidocaine hydrochloride was found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak.

Forced degradation study

In HPLC, there is major degradation (15-42 %) is observed in photolytic condition. Retention time of major degradation product is at 17.1 minute and in UPLC, Major degradation was found in photolytic condition that product was degraded up to 30 %. The major impurity peaks was found at 3.73 min (Figure 3), and in thermal condition around 20-25 % of the drug degraded, in alkaline condition around 5-10 % of the drug degraded, in oxidative degradation, it was found that around 2-3 % of the drug degraded and in acidic condition around 10-17 % of the drug degraded (Table 7 and Table 8**)**.

Linearity for HPLC

For linearity seven points calibration curve were obtained in a concentration range from 0.040-0.160 mg/mL for chlorhexidine gluconate and 0.016-0.064 mg/mL for lidocaine hydrochloride. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for chlorhexidine gluconate was y = 58207663.48x+ 855859.63 with correlation coefficient 0.9983 and for lidocaine hydrochloride was y = 45959789.29x + 6293.39 with correlation coefficient 0.9998. Where x is the concentration in mg/mL and y is the peak area in absorbance unit.

Linearity for UPLC

For linearity seven points calibration curve were obtained in a concentration range from 0.040-0.160 mg/mL for chlorhexidine gluconate and 0.016-0.064 mg/mL for lidocaine hydrochloride. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for chlorhexidine gluconate was y = 29516386.61x - 210798.46 with correlation coefficient 0.9997 and for lidocaine hydrochloride was y = 28773953.57x – 5344.25 with correlation coefficient 0.9994.

LOD and LOQ for HPLC

The limit of detection and limit of quantification were evaluated by serial dilutions of chlorhexidine gluconate and lidocaine hydrochloride stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD values for chlorhexidine gluconate and lidocaine hydrochloride were found to be 0.02 µg/mL and 0.05 µg/mL, respectively and the LOQ value 0.10 µg/mL and 0.09 µg/mL, respectively.

3.5.1. LOD and LOQ for UPLC

The LOD values for chlorhexidine gluconate and lidocaine hydrochloride were found to be 0.02 µg/mL and 0.01 µg/mL, respectively and the LOQ value 0.04 µg/mL and 0.25 µg/mL, respectively.

Table 11: Comparison of chromatographic conditions

	UPLC	HPLC
Stationary phase	Bridged ethylsiloxane/silica, Hybrid C18 particles, 1.7µ	C8, 5µ
Column dimensions	2.4 x 50mm	4.6 x 150mm
Flow rate (mL/min)	0.3	1
Retention time in minutes		
Chlorhexidine gluconate	4.5	19.1
Lidocaine hydrochloride	1.3	6.2
Total analysis time (Minutes)	7	35
Solvent consumption (mL per run)	2.1	35

Table 12: Comparison of validation data for chlorhexidine gluconate and lidocaine hydrochloride

Parameter	chlorhexidine gluconate		lidocaine hydrochloride	
	UPLC	HPLC	UPLC	HPLC
Area repeatability(%RSD)	0.24	0.77	0.31	0.56
Retention time repeatability (%RSD)	0.002	0.01	0.001	0.02
Accuracy (%RSD)	0.35	0.76	0.52	0.65
Calibration range (ppm)	0.08-0.32	0.08-0.32	0.02-0.08	0.02-0.08
Correlation coefficient	0.9997	0.9984	0.9994	0.9998
LOD (ppm)	0.02	0.02	0.01	0.05
LOQ (ppm)	0.04	0.1	0.25	0.09

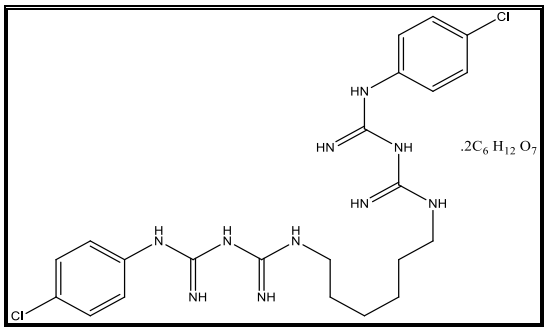

Figure 1(a): Molecular structure of Chlorhexidine gluconate

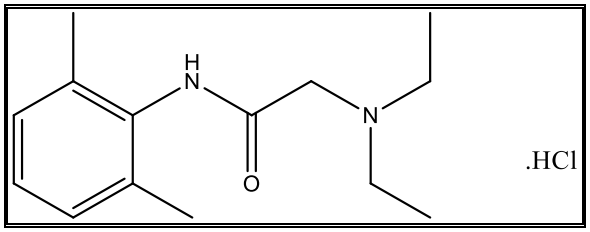

Figure 1(b): Molecular structure of Lidocaine hydrochloride

Precision

Data obtain from precision experiments are given in Table 3 and Table 4 for intraday and interday precision study for both chlorhexidine gluconate and lidocaine hydrochloride. The RSD values for intraday precision study and interday precision study was < 2.0 % for chlorhexidine gluconate and lidocaine hydrochloride, which confirms that the method was precise.

Accuracy for HPLC

Recovery of chlorhexidine gluconate and lidocaine hydrochloride was determined at three different concentration levels. The mean recovery for chlorhexidine gluconate was 100.74-102.60 % and 99.07-101.11 % for lidocaine hydrochloride (Table 5). The result indicating that the method was accurate.

Accuracy for UPLC

Recovery of chlorhexidine gluconate and lidocaine hydrochloride was determined at three different concentration levels. The mean recovery for chlorhexidine gluconate was 98.6-99.80 % and 99.17-99.52 % for lidocaine hydrochloride (Table 6). The result indicating that the method was accurate.

Solution stability study

Table 7 and Table 8 shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2 - 8˚ C and ambient temperature with the consideration of < 2.0 % in % assay value difference against interval value.

Robustness

The result of robustness study of the developed assay method was established in Table 9 and Table 10. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

DISCUSSION

UPLC has been proved to be an effective technique for the analysis of chlorhexidine gluconate and lidocaine hydrochloride in throat spray sample analysis. Total analysis time was 7.0 min and the retention times of chlorhexidine gluconate and lidocaine hydrochloride were 1.3 min and 4.5 min, respectively. Performance data for UPLC are discussed below in the critical comparison of UPLC analysis with use of HPLC. Use of classical HPLC, with C8 columns, for this purpose has been developed earlier. The conditions used for each type of chromatography are compared in Table 11. Sodium

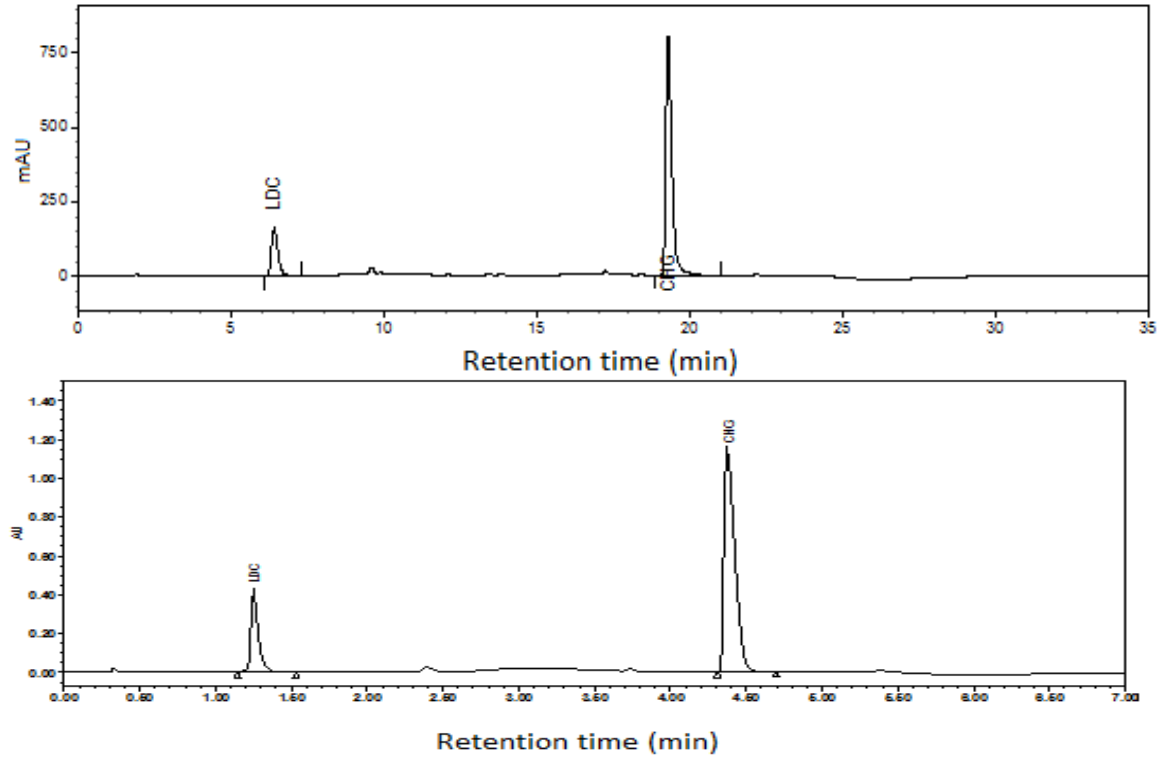

Figure 2: Comparison chromatogram of Standard (HPLC VS UPLC)

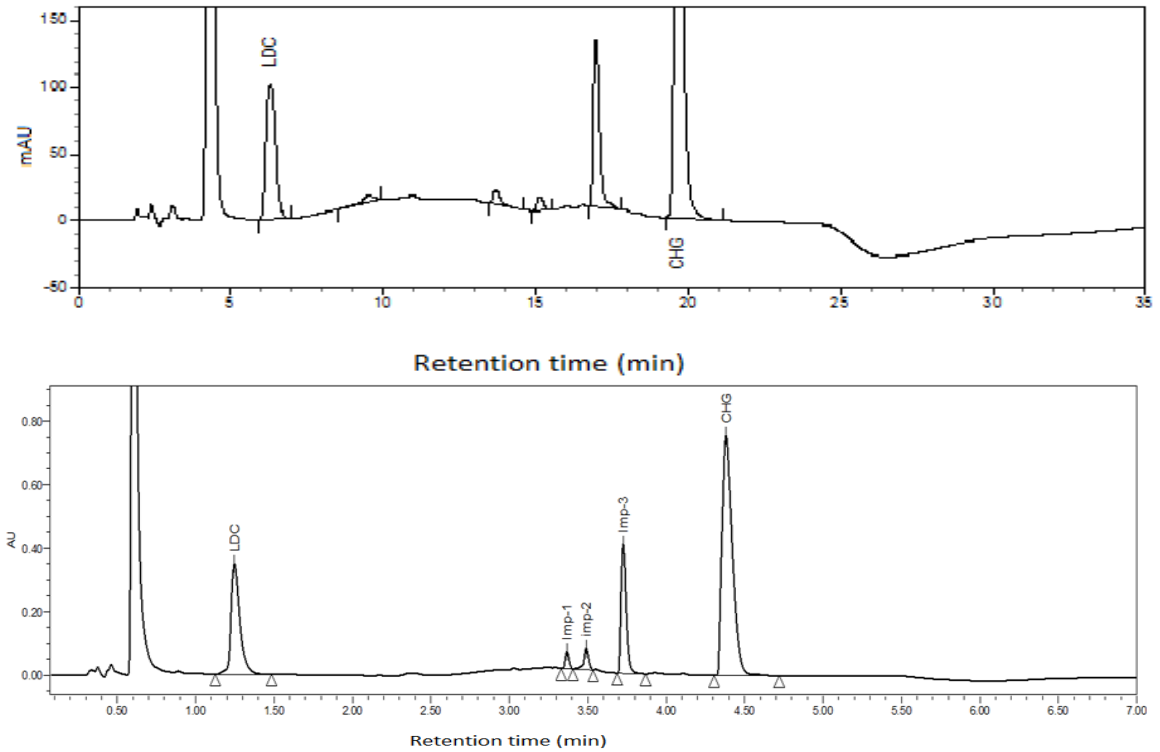

Figure 3: Comparison chromatogram of UV-light degradation study (HPLC VS UPLC)

phosphate Buffer solution: Acetonitrile was always used as mobile phase (MP) and all the stationary phases were based on octadecylsilica; there were, however, significant differences between other properties of the columns used, especially particle size, porosity, internal diameter, and column length. As would be expected, analysis time was longest for the conventional HPLC particulate column. Better resolution, speed, and sensitivity were obtained by use of the UPLC column packed with 1.7-µm particles, but at higher back-pressure, which the UPLC system can resist. Because of the high separation efficiency, small particle size, and narrow column internal diameter, UPLC uses low mobile phase flow rates and analysis times are short. In summary, use of

UPLC columns results in time saving of approximately 47 h for analysis of one-hundred samples. Mobile phase consumption per minute in UPLC is approximately one third that in HPLC, which is economically important to laboratories processing large numbers of samples. When the different internal diameters of the columns are taken into account (4.6 mm for the HPLC column), this effect on solvent consumption can, nevertheless, be regarded as insignificant.

CONCLUSION

Comparison of validation data for chlorhexidine gluconate and lidocaine hydrochloride in Table 12. In all aspects like Analysis time, solvent consumption etc. We can conclude that UPLC method is superior than HPLC method. Hence UPLC method can be considered as best method for routine use in the quality control laboratories.

REFERENCES

1. Singh S, Singh B, Bahuguna R, Wadhwa L, Saxena R. Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay. Journal of pharmaceutical and biomedical analysis. 2006 Jun 7;41(3):1037-40.
2. Mohammadi A, Haririan I, Rezanour N, Ghiasi L, Walker RB. A stability-indicating high performance liquid chromatographic assay for the determination of orlistat in capsules. Journal of Chromatography A. 2006 May 26;1116(1):153-7.
3. Ivana I, Ljiljana Z, Mira Z. A stability indicating assay method for cefuroxime axetil and its application to analysis of tablets exposed to accelerated stability test conditions. Journal of Chromatography A. 2006 Jun 30;1119(1):209-15.
4. ICH, Q1A (R2) Stability testing of new drug substances and products, in: International Conference on Harmonization, IFPMA, Geneva, 2003.
5. Grosa G, Del Grosso E, Russo R, Allegrone G. Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup. Journal of pharmaceutical and biomedical analysis. 2006 Jun 7;41(3):798-803.
6. Chlorhexidine Official FDA information, side effects and uses. Drug information Online.Drugs.com. Revised November. (2006). http://www.drugs.com/pro/chlorhexidine.html. (Retrieved October 10, 2007)
7. The most common topical antibiotics, 1998. who.int/reproductive-Health/publications/MSM_98_4/MSM_98_4_annex.en.html.
8. http://www.freshbreath.ca/fbcpubs.html.
9. Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. Journal of clinical periodontology. 1988 Aug 1;15(7):415-24. Colgate PerioGard Chlorhexidine Gluconate oral rinse 0.12% (Rx)". https://secure.colgateprofessional.com/app/ColgateProfessional/US/EN/Products/ProductItems/ColgatePerioGard/ProductSpecifics.cvsp.
10. Decker H. The Holiday Card. Plath Profiles: An Interdisciplinary Journal for Sylvia Plath Studies. 2008 Jun 21; 1:17-29.
11. Löfgren N. Studies on local anesthetics: Xylocaine: a new synthetic drug. Solarcaine topical gel. (July 27, 2009).
12. http://www.wada-ama.org/Documents/World anti doping program/WADP prohibited list.
13. Cage SA, Rupp KA, Castel JC, Saliba EN, Hertel J, Saliba SA. Relative Acoustic Transmission of Topical Preparations Used with Therapeutic Ultrasound. Archives of physical medicine and rehabilitation. 2013 Nov 30;94(11):2126-30.
14. Havlíková L, Matysová L, Nováková L, Hájková R, Solich P. HPLC determination of chlorhexidine gluconate and p-chloroaniline in topical ointment. Journal of pharmaceutical and biomedical analysis. 2007 Feb 19;43(3):1169-73.
15. Pérez-Ruiz T, Martínez-Lozano C, Tomás V, Sanz A, Sahuquillo E. Flow-injection extraction-spectrophotometric method for the determination of ranitidine in pharmaceutical preparations. Journal of pharmaceutical and biomedical analysis. 2001 Nov 30;26(4):609-15.
16. Nakayama F, Nakagaki M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications. 1980 Sep 12;183(3):287-93.
17. Jacobsen E, Glyseth B. Polarographic determination of chlorhexidine in pharmaceutical preparations. Talanta. 1975 Dec 31;22(12):1001-4.
18. Gavlick WK, Tomkins DF. An updated liquid chromatographic assay for the determination of glyphosate in technical material and formulations. Journal of AOAC International. 2008 Jan 1;91(1):1-4.
19. Okamoto H, Uetake A, Tamaya R, Nakajima T, Sagara K, Ito Y. Simultaneous determination of ingredients in an ointment by hydrophobic interaction electrokinetic chromatography. Journal of Chromatography A. 2001 Sep 21;929(1):133-41.
20. Lam YF, Chan DC, Rodriguez SY, Lintakoon JH, Lam TH. Sensitive high-performance liquid chromatographic assay for the determination of chlorhexidine in saliva. Journal of Chromatography B: Biomedical Sciences and Applications. 1993 Jan 29;612(1):166-71.
21. Barbin LE, Saquy PC, Guedes DF, Sousa-Neto MD, Estrela C, Pécora JD. Determination of para-chloroaniline and reactive oxygen species in chlorhexidine and chlorhexidine associated with calcium hydroxide. Journal of endodontics. 2008 Dec 31;34(12):1508-14.
22. Medlicott NJ, Ferry DG, Tucker IG, Rathbone MJ, Holborow DW, Jones DS. High performance liquid chromatographic (HPLC) assay for the determination of chlorhexidine in saliva film. Journal of Liquid Chromatography & Related Technologies. 1994 Apr 1;17(7):1605-20.

23. Kudo K, Ikeda N, Kiyoshima A, Hino Y, Nishida N, Inoue N. Toxicological analysis of chlorhexidine in human serum using HPLC on a polymer-coated ODS column. Journal of analytical toxicology. 2002 Mar 1;26(2):119-22.
24. Zivanovic L, Agatonovic-Kustrin S, Vasiljevic M, Nemcova I. Comparision of high-performance and thin-layer chromatographic methods for the assay of lidocaine. Journal of pharmaceutical and biomedical analysis. 1996 Jun 30;14(8):1229-32.
25. Xue Y, Tang M, Hieda Y, Fujihara J, Takayama K, Takatsuka H, Takeshita H. High-performance liquid chromatographic determination of chlorhexidine in whole blood by solid-phase extraction and kinetics following an intravenous infusion in rats. Journal of analytical toxicology. 2009 Mar 1;33(2):85-91.
26. Dal Bo L, Mazzucchelli P, Marzo A. Highly sensitive bioassay of lidocaine in human plasma by high-performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 1999 Aug 27;854(1):3-11.
27. Salas S, Talero B, Rabasco AM, González-Rodríguez ML. Development and validation of a reverse-phase liquid chromatographic method for the assay of lidocaine hydrochloride in alginate-Gantrez® microspheres. Journal of pharmaceutical and biomedical analysis. 2008 Jul 15;47(3):501-7.
28. Xu Y, Wong GY. Simultaneous determination of lignocaine hydrochloride, chlorhexidine gluconate, and triamcinolone acetonide in suspension by reversed-phase HPLC. Journal of liquid chromatography & related technologies. 1999 Jan 1;22(13):2071-91.
29. D. Root, Techniques and strategies for transferring methods from HPLC to UPLC, 7200250EN, Waters Corporation (2008)