To Design And Develop Cetylpyridium Chloride Combination With Azadirachta Indica Extract Antibacterial And Antimicrobial Effervescent Mouthwash

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Abstract:
Present work lead to the optimization of process for preparation of lyophilize Mouthwas solution and development of effervescent tablet comprising a solid water soluble excipient i.e. glycine, sorbitol, aspartame which dissolve in 160 seconds. Formulation being a solid dosage form, the predicted stability of Cetylpyridium Chloride and Azadirachta Indica is more, as Mouthwas solution is available in liquid form and possesses stability problems if not stored at low temperature. Further a fast dissolving tablet strip will always be preferred by an end user over a liquid mouthwash bottle. The said formulation will be widely useful for a traveler, tourist or a camper, as it is difficult to carry liquid mouthwash bottle with him because of weight, fragility or bulk hence offering patient compliance and also solving the problem of shelf space at the retail outlet and home..

Keywords: Cetylpyridium Chloride, Mouthwash, Dental plaque. HPLC.

1. INTRODUCTION

Typically employed as the cetylpyridinium chloride salt, this compound is commonly used as an active ingredient in various over-the-counter mouthwashes, toothpastes, lozenges, and mouth sprays where it is generally indicated for antiseptic actions, gingivitis and plaque prevention, as well as action or prevention against some other oropharyngeal bacterial infections Cetylpyridinium chloride is considered a cationic disinfectant with properties and uses similar to other such cationic surfactants. In particular, cetylpyridinium chloride has demonstrated a rapid bactericidal and fungicide effect on gram-positive pathogens and yeasts, respectively. Cetylpyridinium chloride is subsequently utilized in a variety of preparations for the local treatment of minor infections. Despite the variety of formulations in which cetylpyridinium chloride may appear as an active ingredient, it is generally accepted that it only elicits a local effect owing to the compound's relatively poor absorption by route of exposure
Mechanism of action

When incorporated into mouthwashes, toothpastes, lozenges, or mouth sprays, cetylpyridinium chloride is expected to elicit a mechanism of action that decreases new dental plaque growth, decreases or removes existing dental plaque, diminishes the growth of pathogenic bacteria, and inhibits the production of virulence factors. Cetylpyridinium chloride is a quaternary ammonium compound that demonstrates a broad spectrum anti-bacterial activity. It possesses a cationic surface active agent surfactant which can absorb readily to oral surfaces. The molecules of this agent have both hydrophilic and hydrophobic groups In action, the positively charged hydrophilic region of cetylpyridinium chloride molecules enables the compound to interact with microbial cell surfaces and even integrate into the bacterial cytoplasmic membrane. Consequently, there is a resultant disruption of bacterial membrane integrity causing a leakage of bacterial cytoplasmic components, interference with cellular metabolism, inhibition of cell growth, and ultimately - cell death 3. Moreover, cetylpyridinium chloride can also inhibit the synthesis of insoluble glucan by streptococcal glucosyl transferase, adsorb to pellicle-covered enamel, and inhibit co-adhesion of bacteria, and bind streptococcus mutans biofilms 3. This ability of cetylpyridinium chloride to be able to adsorb to pellicle covered enamel imparts substantivity to the compound molecules - that is retention in the mouth and continued antimicrobial activity for a period of time after rinsing 3. Taking these mechanisms into consideration, cetylpyridinium chloride may be considered an active ingredient that is effective in the treatment and prevention of bacterial or fungal disorders of the oropharyngeal cavity.

2. MATERIALS AND METHODS

2.1 Azadirachta indica

Azadirachta indica L. (neem) shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. A proper method has to be carried out while formulating the antimicrobial Mothwash,

1. Selection of active
2. Collection and Authentication

3. Extraction Method
4. Preparation

2.1.1 Methods:-

1) Selection of active

Azadirachta indica commonly known as Neem, is an evergreen tree. Since time immemorial it has been used by Indian people for treatment of various diseases due to its medicinal properties. It possesses anti-bacterial, anti-cariogenic, anti-helminthic, anti-diabetic, anti-oxidant, astringent, anti-viral, cytotoxic, and anti-inflammatory activity. Nimbidin, Azadirachtin and nimbinin are active compounds present in Neem which are responsible for antibacterial activity. Neem bark is used as an active ingredient in a number of toothpastes and toothpowders. Neem bark has antibacterial properties; it is quite useful in dentistry for curing gingival problems and maintaining oral health in a natural way. Neem twigs are used as oral deodorant, toothache reliever and for cleaning of teeth.

2) Collection and Authentication

Herb authentication is a quality assurance process that ensures the correct plant species and plant parts are used as raw materials for herbal medicines. The proper authentication of herbal raw materials is critically important to the safety and efficacy of herbal medicines.

Azadirachta indica were purchased from local market and authenticated in botanical department by botanist.

3) Extraction Method

a. Grinding Mill:-

A mill is a device that breaks solid materials into smaller pieces by grinding, crushing, or cutting. Such comminution is an important unit operation in many processes. There are many different types of mills and many types of materials processed in them.

b. Soxhlet Extraction:-

Soxhlet extraction is a continuous solid / liquid extraction. A solid which contains the material to be extracted is placed in what is called a thimble. A thimble is made out of a material which will contain the solid but allow liquids to pass through. A lot like filter paper. The thimble containing the material is placed in the Soxhlet extractor. An organic solvent is then heated at reflux. As it boils its vapors rise up and are condensed by a condenser

2.2 Preparation of antimicrobial Mouthwash

To make mouthwash, cetylpyridium Chloride and Azadirachta Indica was taken and added to the water while mixing at appropriate speed.

Composition used for mouthwash is as follows: Sodium Bicarbonate, Citric Acid (Monohydrate),Tartaric Acid,PEG 6000,GlycineSodium Benzoate, Manitol, Menthol Aspartame brilliant blue, Orange Peel

2.3 Lyophilize the Mouthwash solution

Mouthwash solution (IP) was dried using freeze dryer (lyophilizer). Take a Mouthwash solution 100 ml and add sorbitol 80 gm. as bulking agent. These mixture thoroughly mix then transfer into a lyophilization tray. This lyophilization tray placed in a lyophilizition shelf chamber also temperature probe was kept in that lyophilization trays properly. The main stage in the lyophilization is set a proper lyophilization cycle and ramping rate. In the first step in the lyophilization is freezing the cetylpyridium Chloride and Azadirachta Indica and sorbitol mixture below at -40^{o}C. Then freezing material placed under vacuum and increase the temperature gradually to deliver enough energy for the ice to sublimate. Finally allows the higher vacuum for the extraction of bound water at above zero temperature. These lyophilization cycle carried out in a five segment. Each segment set all parameter like temperature, pressure and ramping rate.

Overall lyophilization process set a ramping rate at 0.5^{0}C/min.

TABLE 1: LYOPHILIZED MIXTURE COMPOSITION

Sr. No.	Lyophilized mixture composition	Quantity
1	Mouthwash solution (20%)	100 ml.
2	Sorbitol	80 gm.

2.4 Characterization and Evaluation of lyophilized mixture

2.4.1 Fourier Transform Infra-Red Spectroscopy

FTIR spectrum of lyophilized mixture was obtained by scanning over a range of 4000- 400cm^{-1} and spectrum was recorded.

2.4.2 Content analysis (Assay %)

The analysis of content of the lyophilized mixture of Mouthwash solution was carried out utilizing IP assay of Mouthwash solution by High Performance Liquid Chromatography method (HPLC).

2.4.3 HPLC assay method:
Mobile phase: Prepare by dissolving 2 gm. of sodium octane sulphanate in a mixture of 120 ml of glacial acetic acid, 270 ml of water and 730 ml of methanol.
Solution (1): Dilute accurately about 5 gm. of sample with mobile phase to make 100 ml solution in a volumetric flask.
Solution (2): Dissolve 0.1 gm. of cetylpyridium Chloride and Azadirachta Indica in the water to make 50 ml. pipette out 2 ml and dilute to 50 ml with mobile phase in another volumetric flask.

2.5 Formulation studies
2.5.1 Selection of process
Method of direct compression was selected for the preparation of reconstituted fast dissolving tablet.

2.5.2 Mixing and Blending
All the components were weighed and triturated in glass mortar in ascending order of their quantities and were taken in an air tight sealable poly bag and mixed for 5-6 minutes.

2.5.3 Evaluation of tablet blend
The evaluation of tablet blends for different flow properties study as given below all the components were weighed and triturated in glass mortar in ascending order of their quantities and were taken in an air tight sealable poly bag and mixed for 5-7 minutes.

2.5.3.1 Angle of repose
The frictional forces in a loose powder or granules can be measured by the angle of repose. Angle of repose was determined by funnel method. The blend was poured through a funnel which raise vertically until a maximum cone height (h) was obtained. Radius of the heap (r) measured and angle of repose (θ)

TABLE 2: RELATIONSHIP BETWEEN ANGLE OF REPOSE (Θ) AND FLOWABLITY

Angle of repose (θ)	Flowablity
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very Poor

2.5.3.2 Bulk density (Db)
It is the ratio of total mass of the powder to the bulk volume of powder. It was measured by pouring the weight powder (passed through standard sieve # 44) into a measuring cylinder and initial volume is called the bulk volume.

2.5.3.3 Tapped density (Dt)
It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 100 times and the tapped volume was noted if the difference between these two volumes is less than 2%. If it is more than 2%, tapping is continued for some times and tapped volume was noted. Tapping was continued until the successive volume is less than 2% (in a bulk density apparatus).

2.5.3.4 Carr's index
The simplest method of measurement of free flow of powder is the compressibility, an indication of the ease with which material can be induced to flow is given by compressibility index (I)

TABLE 3: FLOW PROPERTIES ACCORDING TO CARR'S INDEX AND FLOWABLITY

Carr's index (%)	Type of Flow	Hausner's Ratio
≤10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Extremely poor	>1.60

2.5.3.5 Hausner's ratio
This is an indirect ratio for ease of powder flow.

2.5.4 Tableting
The resulting uniform blends of composition per tablet were compressed on 12 stations compression machine using 8 mm flat faced tooling.

2.5.5 Tablet batches

The effect of glycine was studied at three levels (-1, 0, +1), amount of rest of the ingredients were fixed. At the level of (-1), glycine concentration of 2%, at level (0), Glycine concentration 4%, at level (+1), Glycine at concentration of 6% of that of the tableting mass were taken.

2.5.5.1 Preformulation

Firstly, the formulas were made up in the different stoichiometric ratios from tartaric acid, citric acid and sodium bicarbonate based on below reactions. According to materials of each formulation were weighed and then mg of Active was added to each formulation. Finally, after preparation of appropriate mixture, the lubricants including 30 mg of PEG 6000 and 10 mg of sodium benzoate were added the mixture and then the tablets compressed by using a single-punch press machine. For next stages, the better stoichiometric ratios were selected with regard to three factors: solubility, effervescence time

TABLE 4: COMPOSITION OF PRELIMINARY FORMULATIONS (RATIO) WITH THEIR EFFERVESCENCE TIME, PH AND SOLUBILITY (MEAN ± SD).

Formulations	Tartaric acid	Citric acid	Na bicarbonate	Effervescent time(s)	pH	*Solubility
S_1	-	0.5	0.5	105 ± 2.08	5.9 ±0.05	3
S_2	-	0.5	1	40 ± 1.52	6.2 ± 0.1	3
S_3	1	0.5	1	39 ± 1.51	6.1 ±0.04	1
S_4	0.5	1	1	36 ± 2	6.1 ±0.05	2
S_5	-	1	1	50 ± 2.13	5.9 ±0.06	5
S_6	1	1	1	48 ± 2.01	6.1 ±0.06	2
S_7	1.5	0.5	1	52 ± 1.8	6.1 ± 0.1	2
S_8	2	0	1	55 ± 1.83	6.1 ±0.08	1
S_9	-	1	1.5	43 ± 1.51	6.1 ± 0.7	4
S_{10}	-	1	0.5	30 ± 3.11	5.6 ± 0.4	4
S_{11}	-	1.5	1.5	25 ± 2.13	5.6 ±0.05	5
S_{12}	-	1.5	1	49 ± 1	5.6 ±0.04	4
S_{13}	-	2	2	20 ± 2.07	5.5 ±0.06	4
*Solubility was defined by Likert Scale from 1= very poor, 2 = poor, 3 = average, 4 = good and 5 = excellent						

TABLE.5 FORMULATION DESIGN FOR FAST DISSOLVING TABLET.

Batch	Lyophilized mixture	Sorbitol	Aspartame	Glycine	Menthol	Orange peel	BB	PEG 600	Total wt. (mg)
F1	146.41	14.64	0.57	2.92	0.44	1.46	0.0025	1.46	168
F2	146.41	14.64	0.58	5.85	0.44	1.46	0.0025	1.46	171
F3	146.41	14.64	0.58	8.78	0.44	1.46	0.0025	1.46	174

2.5.5.2 Methods of Anti-Microbial Effervescent Tablets Production

a. Direct Compression

According to Table 2, raw materials of each formulation were weighed and were mixed in a tumbling cubic blender for 15 minutes.

TABLE 6: DIFFERENT COMPONENTS OF PREPARED TABLETS FROM THE DIRECT COMPRESSION (D) AND FUSION (F) METHODS.

Ingredients (mg)			Formulations			
	F_1	F_2	F_3	F_4	F_5	F_6
K citrate	2700	2700	2700	2700	2700	2700
Citric acid	570	850	850	850	850	850
Na bicarbonate	500	750	750	750	750	750
Mannitol	-	-	60	120	-	60
Sorbitol	-	-	-	-	60	-
Aspartame	-	-	-	-	-	1.5

After the preparation of the primary powder mixtures, sweeteners including aspartame, sorbitol, mannitol and fruit flavoring agents were passed through the appropriate mesh and were added to the powders and these were mixed altogether for 5 minutes. Finally, the selective lubricants including sodium benzoate (10 mg) and PEG 6000 (30 mg) were added and again mixed for about 2-5 minutes with other material Then, the powders were compressed into tablets by using a singlepunch press machine with 25 mm punch set. Weight of each tablet was considered about 4.5 g. At the end, the tablets were dried in an oven with air circulation at 54°C for 1 hr and after cooling were packed in plastic tubes.

b. Fusion Method

According to the formulations which are shown in Table 2, amounts of citric acid, sodium bicarbonate, Active and mannitol (sorbitol) were weighted accurately and were mixed for about 15 minutes in a tumbling cubic blender. Then, the obtained mixture was placed in an oven at 54 °C. The powder was mixed regularly until the crystallization water of citric acid was released as binder factor (approximately 30 minutes). After obtaining an appropriate pasty mass, this wet mass was passed through sieve No. 20 and the obtained granules were dried in an oven at 54 °C for 1 hr. After drying, for second times the granules were passed through sieve No. 20. In the next stage, sweeteners and flavors were added with the granule mass and mixed for 5 minutes with other material.

At last, the lubricants including sodium benzoate (10 mg) and polyethylene glycol 6000 (30 mg) were added and mixed for 2-5 minutes with other material. The granule mixtures compressed into tablets by a single-punch press machine with 25 mm punch set. Finally, they were dried and packed with the previous methods.

c.Wet granulation Method

Wet granulation was performed on F5 and F6 formulations. First, citric acid and sodium bicarbonate and Active were milled by using miller so that all powders were passed through sieve No. 35 and were blended for 10 minutes. Then 9.5 % w/v PVP solution in absolute ethanol was added with the mixture, so that white pasty mass was formed. This wet mass was passed through sieve No. 20 and the granules were dried in an oven at 54 °C for 75 minutes. So, the dried mass was passed through sieve No. 20 and the other ingredients were added to them like as fusion method. The granule mixtures were compressed into tablets by using a single-punch press machine with 25 mm punch set. Prepared tablets were dried in an oven with air circulation at 54 ° C for 90 minutes, then were wrapped in Aluminum foil and were packaged in plastic tubes.

2.5.6 Evaluation of tablet

2.5.6.1 Appearance and shape

The general appearance of the tablet includes the morphological characteristics like size, shape, color, odor, etc.

2.5.6.2 Uniformity of thickness and diameter

The uniformity of the diameter and thickness was measured using Vernier caliper. The average thickness of the 20 tablet was calculated. The test was positive if none of the individual thickness value deviated by ±5% of the average.

2.5.6.3 Hardness

Hardness of the tablet was tested by Monsanto Hardness tester which measures the diametrical crushing strength of the tablets. The tablet to be tested was placed in between the fixed and movable jaw after adjusting the reading to zero. By moving the screw knob the force on the tablet was gradually increased until the tablet breaks. The pressure required in kg to break the tablet was noted from the scale on the tester. The hardness of the tablet depends on weight of the material used and compression force applied during compression.

2.5.6.4 Friability

Tablets require certain amount of strength or hardness and resistance to friability. It is necessary or important to withstand mechanical shocks of handling while manufacturing, packaging and shipping. This test was performed by using Roche Friabilator. Six tablets were weighed and tumbled at rate of 25 rpm for 4 min.

2.5.6.5 Disintegration test

The disintegration test was performed by placing one tablet in 15 ml water. The time required for the complete disintegration noted as disintegration time.

2.6 Content analysis (Assay %)

The analysis of content of the batch F1, F2 and F3 reconstituted solution of cetylpyridium Chloride and Azadirachta Indica tablet was carried out utilizing IP assay of cetylpyridium Chloride and Azadirachta Indica solution by High Performance Liquid Chromatography method (HPLC).

2.6.1 HPLC Method

Mobile phase: Prepare by dissolving 2 gm. of sodium octanesulphanate in a mixture of 120 ml of glacial acetic acid, 270 ml of water and 730 ml of methanol.

Solution (1): Dilute accurately about 5 gm. of sample with mobile phase to make 100 ml solution in a volumetric flask.

Solution (2): Dissolve 0.1 gm. of cetylpyridium Chloride and Azadirachta Indica WS in the water to make 50 ml. pipette out 2 ml and dilute to 50 ml with mobile phase in another volumetric flask

3. RESULT AND DISCUSSION

3.1 Lyophilize the Mouthwash solution

Mouthwash solution and Sorbitol were taken a 100ml (20%), 80 gm. at an optimized ratio respectively. Mix properly this mixture then transferred into tray and loaded into a lyophilizer chamber. Set a five segment in the lyophilizer in each segment set a different time and temperature for a lyophilization purpose (Table 13, 14, 15). After the completion of lyophilization cycle (figure 4) obtained the final lyophilized product.

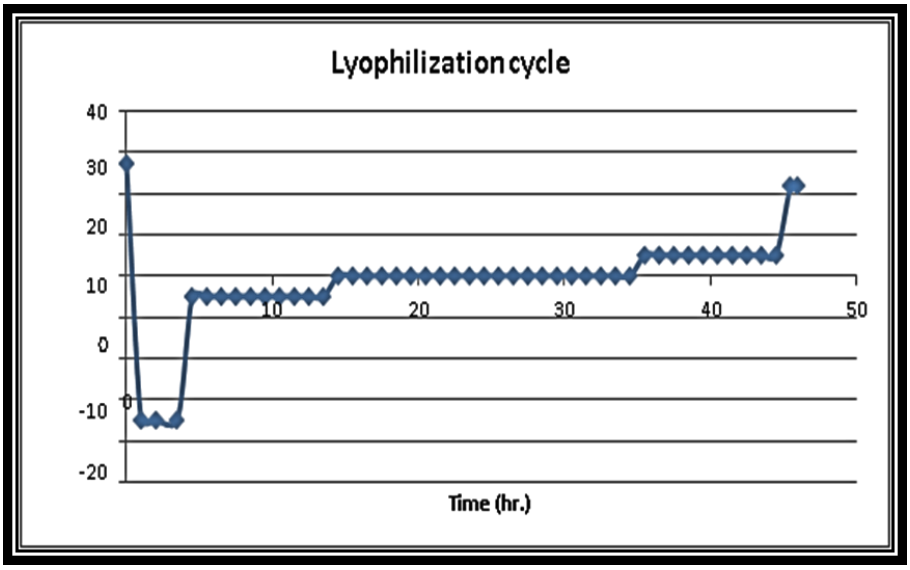

FIG.NO 1: LYOPHILIZATION CYCLE OF MOUTHWASH SOLUTION

TABLE 7: TIME AND TEMPERATURE FOR SEGMENT 1 AND SEGMENT 2

Parameter		Segment 1							Segment 2					
Time(hr.)	0	1	2	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5
Temp.(^{0}C)	27	-35	-35	-35	-5	-5	-5	-5	-5	-5	-5	-5	-5	-5

TABLE 8: TIME AND TEMPERATURE FOR SEGMENT 3

Parameter	Time (hr.)	Temperature (^{0}C)
Segment 3	14.5	0
	15.5	0
	16.5	0
	17.5	0
	18.5	0
	19.5	0
	20.5	0
	21.5	0
	22.5	0
	23.5	0
	24.5	0
	25.5	0
	26.5	0
	27.5	0
	28.5	0
	29.5	0
	30.5	0
	31.5	0
	32.5	0
	33.5	0
	34.5	0

TABLE 9: TIME AND TEMPERATURE FOR SEGMENT 4 AND SEGMENT 5

Parameter					Segment 4						Segment 5	
Time(hr.)	35.5	36.5	37.5	38.5	39.5	40.5	41.5	42.5	43.5	44.5	45.5	46
Temp.(^{0}C)	5	5	5	5	5	5	5	5	5	5	22	22

3.2 Characterization and evaluation of lyophilized mixture

3.2.1 Fourier Transform Infra-Red Spectroscopy

IR spectrum of Mouthwash solution lyophilized mixture showing following characteristics peaks confirming its structure

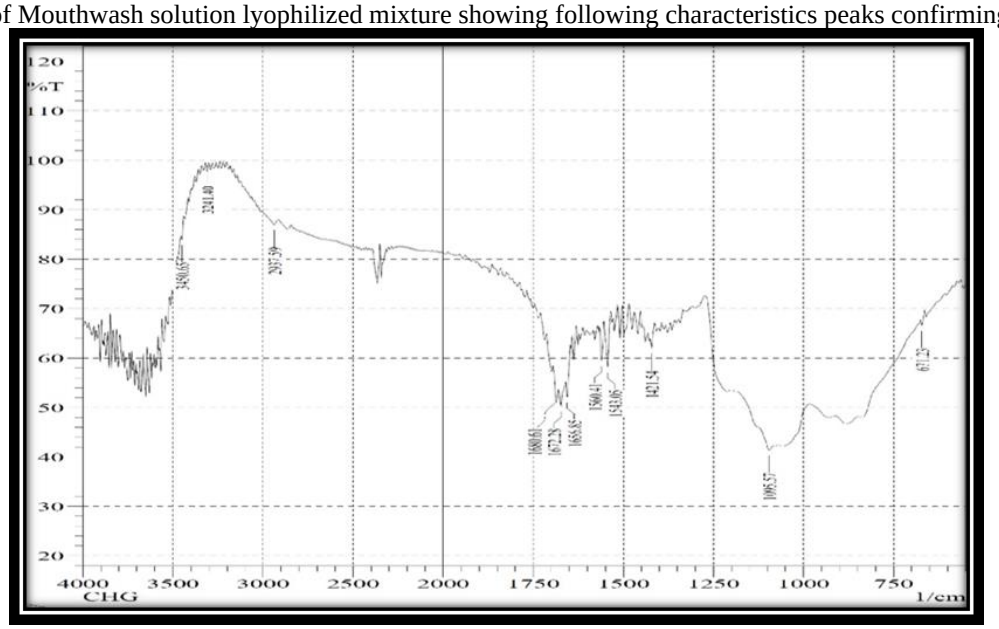

FIG. NO 2: FT-IR SPECTRUM OF MOUTHWASH SOLUTION LYOPHILIZED MIXTURE.

TABLE 10: FT-IR ASSIGNMENT FOR MOUTHWASH SOLUTION

Sr. no.	Particular	Standard range(cm^{-1})	Observed value(cm^{-1})
1.	2º Amine (–NH-)	3200-3400	3241.40
2.	Hydroxyl group (-OH)	3300-3500	3450.65
3.	Chlorine group (–Cl)	600-800	671.23
4.	Carbonyl group (>C=O)	1680-1740	1680.61
5.	Aromatic C-H (stretching)	2900-3100	2937.29
6.	Aromatic C-H (bending)	1475-1575	1543.05

3.2.2 Content analysis (Assay %)

Content analysis of the Mouthwash solution lyophilized mixture by using HPLC assay IP method.

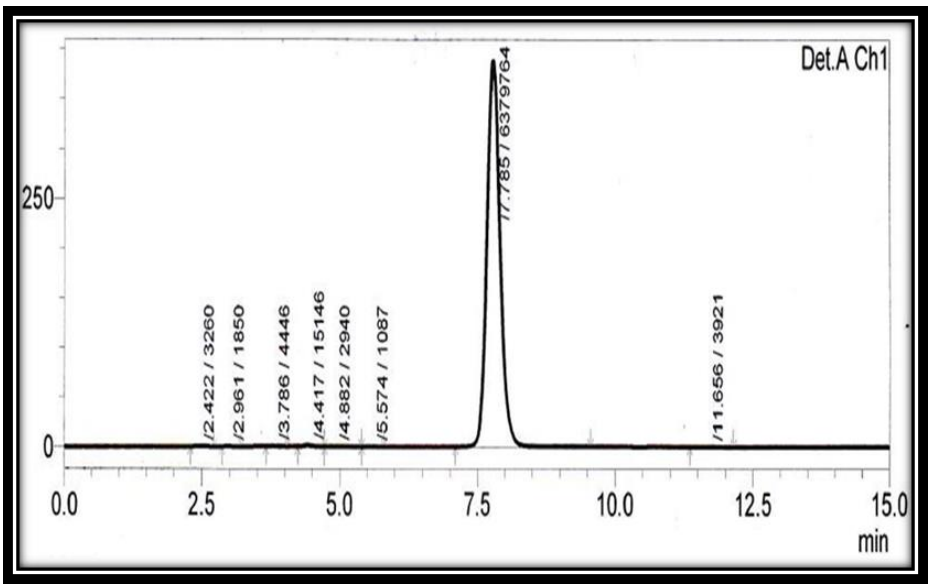

FIG. NO 3: HPLC CHROMATOGRAM OF STANDARD MOUTHWASH SOLUTION

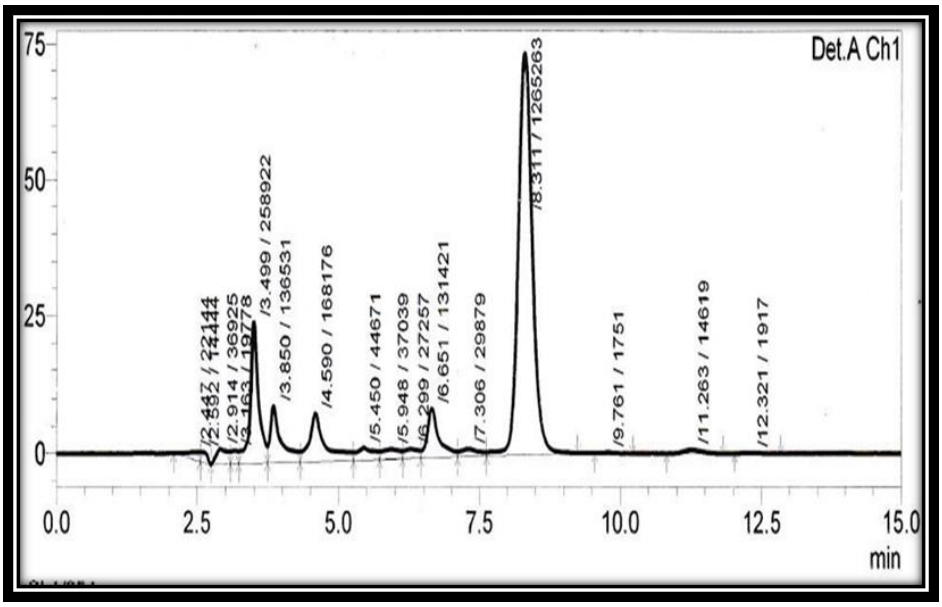

FIG. NO 4: HPLC CHROMATOGRAM OF LYOPHILIZED MIXTURE OF

MOUTHWASH SOLUTION

Run standard solution six times and test solution in duplicate. By using standard Average peak area and test sample peak area calculate the content of lyophilized mixture. Content analysis of the lyophilized mixture was found to be 20.49% within the specified limit as per I.P.

TABLE 11: CONTENT ANALYSIS OF LYOPHILIZED MIXTURE

Lyophilized mixture	Assay (%)
Mouthwash solution + Sorbitol	20.49 %

3.3 Formulation studies

3.3.1 Excipients

The prerequisite for selection of the excipients for preparation of a fast dissolving tablet was reconstituted as the ultimate goal was to develop a mouthwash with water as the final vehicle. So with a view to formulate a mouthwash, excipient chosen were glycine as disintegrant, menthol as a flavoring agent, Eco cool as cooling agent, ribitol and aspartame used as sweetening agent, brilliant blue as coloring agent and PEG 6000 as lubricant

3.3.2 Selection of process

Method of direct compression was selected for the preparation of fast dissolving tablet.

3.3.3 Evaluation of tablet blend

The prepared blend was subjected for the study of different micromeritics properties. The result for analysis of F1, F2 and F3 batches were summarized. The analysis result of tablet blend indicates that all the batches possess good flow ability and compressibility.

3.3.4 Tablet Evaluation parameter

3.3.4.1 Appearance and shape

All the tablets of design batches were having light blue color uniformly distributed, 8 mm in diameter with circular curved surface.

3.3.4.2 Thickness

Excessive variation in tablet thickness can result in problem with packaging as well as consumer acceptance. There was no marked variation in thickness of tablet within each formulation (5%) indicating uniform behavior of blend throughout the compression process. Thickness of design batches were found in range of 2.46 to 2.48 mm.

3.3.4.3 Friability

Friability of the tablet is measure of the tablet strength. Tablets with friability less than 1% of their weight are acceptable. The friability of the design batches were in the range of 0.34 to 0.79.

3.3.4.4 Disintegration test

Fast dissolving tablets are expected to disintegrate within 3 min. The disintegration time of optimized batch was found to be 160 seconds.

TABLE 12: EVALUATION OF TABLET BLEND

Batch Code	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Carr's Index (%)	Hausner's Ratio	Angle of Repose (°)
F1	0.4873 ± 0.010	0.6137 ± 0.012	20.59 ± 3.354	1.25 ± 0.027	30.11 ± 1.12
F2	0.4529 ± 0.008	0.5406 ± 0.005	19.36 ± 1.215	1.19 ± 0.044	29.24 ± 1.40
F3	0.430 ± 0.012	0.493 ± 0.010	12.92 ± 2.231	1.14 ± 0.033	28.31 ± 1.23

all the reading taken in replicate represented as mean ± SD

TABLE 13: EVALUATION TABLETS PROPERTIES

Batch Code	Diameter (mm)	Thickness (mm)	Friability (%) (n=3)	Hardness (kg/cm^2)
F1	8	2.46 ± 0.029	0.34	3.1 ± 0.287
F2	8	2.48 ± 0.017	0.79	3 ± 0.268
F3	8	2.48 ± 0.023	0.44	3.1 ± 0.290

TABLE 14: FORMULATION CHARACTERISTICS OF TABLETS

Batch Code	Disintegration time (sec.)
F1	195
F2	180
F3	160

3.4 Reconstitution study

3.4.1 Content analysis (Assay %).

The analysis of content of the batch F1, F2 and F3 reconstituted solution of Mouthwash tablet was carried out utilizing IP assay of Mouthwash solution by High Performance Liquid Chromatography method (HPLC). Run standard sample six times and test sample duplicate.

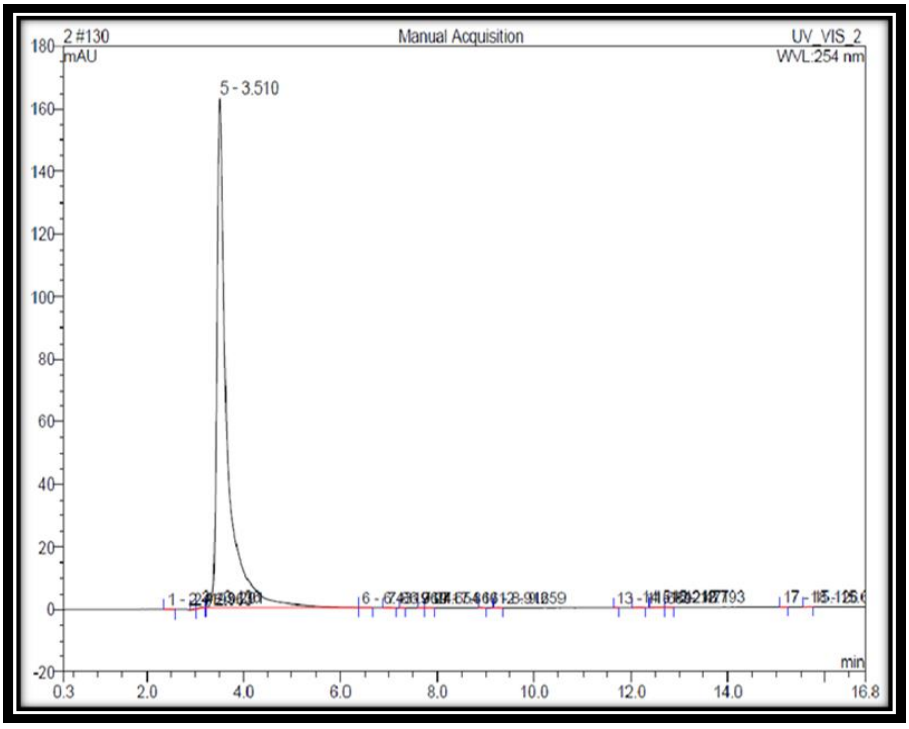

FIG. NO 5: HPLC CHROMATOGRAM OF STANDARD OF MOUTHWASH SOLUTION.

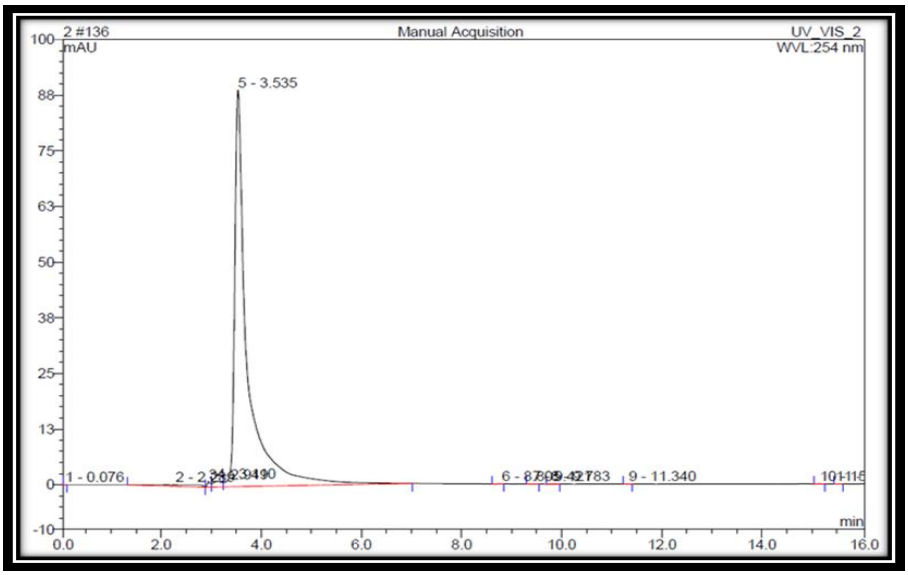

FIG.NO 6: HPLC CHROMATOGRAM OF BATCH F1 MOUTHWASH SOLUTION

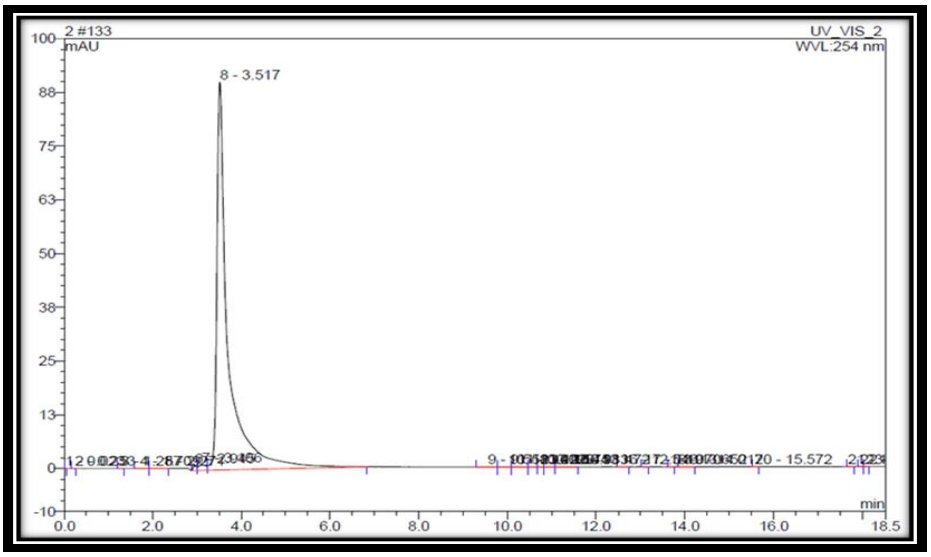

FIG.NO 7: HPLC CHROMATOGRAM OF BATCH F2 MOUTHWASH SOLUTION

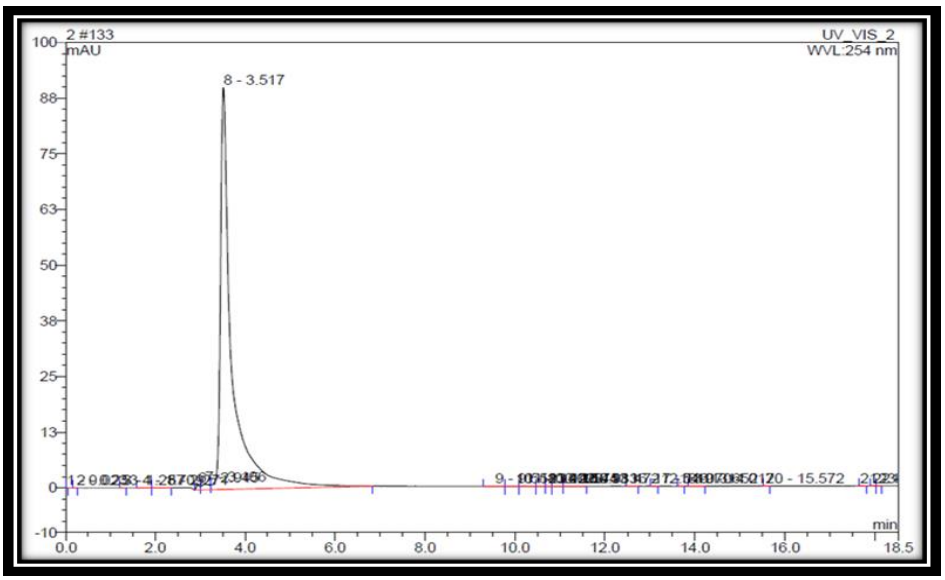

FIG.NO 8: HPLC CHROMATOGRAM OF BATCH F3 MOUTHWASH SOLUTION.

Mouthwash Solution WS standard solution average area was found to be 41.3316 mAU and test solution of mouthwash tablet formulation F1, F2 and F3 average area was found to be 34.64 mAU,34.47mAU and 35.17 mAU respectively. By using the average sample peaks of standard solution and test formulation calculate content in the formulation batch F1, F2 and F3 (Table 20).

TABLE 15: RECONSTITUTED FORMULATION BATCHES CONTENT ANALYSIS (ASSAY %)

Batch code	Average peaks of sample (mAU)	Average peaks of standard (mAU)	Assay (%)
F1	34.64	41.3316	98.20
F2	34.47	41.3316	97.70
F3	35.17	41.3316	99.68

3.5 Optimized batch

F3 batch was selected as optimized batch amongst design batches for the reconstitution of mouthwash tablet. Depending upon the evaluation of the tablet blend, tablet properties like hardness, friability, disintegration time. Also reconstitution study of the F3 batch assay within the specified limit as per I.P.

3.6 Antibacterial activity

The result for antibacterial activity of optimized EG6 was 85% inhibition that confirmed its antibacterial effectiveness to skin against microbes. The optimized formulations have strong antibacterial and antimicrobial activities, so considered safe oral use. The similar findings have been reported in previous studies of formulations. Mouthwash Solution are decrease inflammation. Also good analgesic and anti-inflammatory activity due to the presence of vitamin. cetylpyridinium chloride is expected to elicit a mechanism of action that decreases new dental plaque growth, decreases or removes existing dental plaque, diminishes the growth of pathogenic bacteria, and inhibits the production of virulence factors

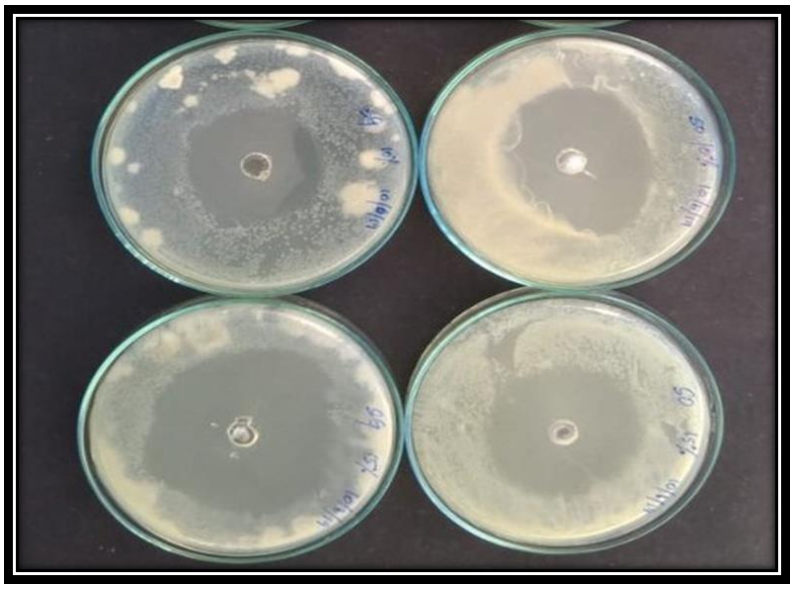

FIG. NO 9 ANTIBACTERIAL ACTIVITY

4. SUMMARY AND CONCLUSION

Recent trends of patient oriented practice demands design of patient oriented dosage form to achieve patient compliance and better therapeutic profile. The number of formulation related factors contributes to non-compliance and inadequate drug release profile. Hence, there is a need to design patient oriented drug delivery system.

Present work lead to the optimization of process for preparation of lyophilize Mouthwas solution and development of effervescent tablet comprising a solid water soluble excipient i.e. glycine, sorbitol, aspartame which dissolve in 160 seconds. Formulation being a solid dosage form, the predicted stability of Cetylpyridium Chloride and Azadirachta Indica is more, as Mouthwas solution is available in liquid form and possesses stability problems if not stored at low temperature. Further a fast dissolving tablet strip will always be preferred by an end user over a liquid mouthwash bottle. The said formulation will be widely useful for a traveler, tourist or a camper, as it is difficult to carry liquid mouthwash bottle with him because of weight, fragility or bulk hence offering patient compliance and also solving the problem of shelf space at the retail outlet and home.

Formulation had a pleasant mouth feel with long lasting cooling effect. Formulation passed test within specified limits

Thus, attempt to develop a fast dissolving tablet for mouthwash was feasible with added advantage mentioned above.

The better performance of the formulation necessitates further studies like excipient compatibility study, official tests for tablet evaluation and evaluation of the stability.

5. ACKNOWLEDGEMENT
The authors are thankful to the Principal, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda Maharashtra, India. Necessary facilities for research work.

6. CONFLICTS OF INTEREST
Authors have no conflicts of interest to declare.

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12. Ilango K, Chitra V(2009). Hepatoprotective and antioxidant activities of fruit pulp of Limonia acidissima Linn. Int J Health Res; 2(4):361-7. S. No. Phytochemicals Fruit pulp Leaf Stem 1 Amino acids - + + 2 Alkaloids ++ - - 3 Carbohydrates +++ - + 4 Flavonoids +++ +++ ++ 5 Tannins + + - 6 Cardiac glycosides ++ + + 7 Triterpenes - - + 8 Steroids + + - 9 Saponins - + ++ Table 5. Phytochemical analysis of wood apple 54. 6 Int. Res. J. Plant. Sci.
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