



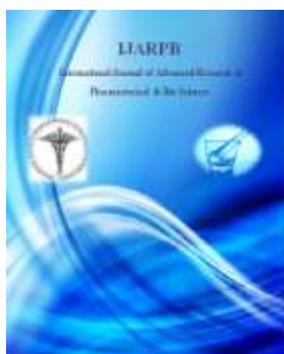
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Composition Analysis of the Oral Care products available in Indian market Part I: Mouthwashes

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ABSTRACT

Commercial mouthwash samples, randomly collected from the Indian market were analyzed for their constituents such as preservatives, sweetener, flavour ingredients, and actives, using High performance liquid chromatography and Gas chromatography methods. The quantitative composition of ingredients in any product is related to the final characteristics of the brand, marketing claims and consumer preferences. The pH of the mouthwash products are varying from 3.7 to 6.5, the mouthwashes containing ethyl alcohol in the range of 6 – 8 % w/v are having pH in the range of 6.0 - 6.5 whereas those containing alcohol higher than 20 % w/v are acidic in nature (pH below 4 also). The estimated quantities of constituents among the analyzed mouthwash products are found to be in the following range (% by wt): Methyl & Propyl parabens: 0.10-0.20; Sodium benzoate: ~ 0.10; Sodium saccharin: ~ 0.20; Thymol: 0.03-0.06; Eucalyptol: ~ 0.10. The alcohol (ethanol) content varied between 6.7 & 29 % by wt., and the fluoride varied between 0 & 250 ppm. The implications of these compositions in the mouthwash products for the oral care applications are discussed.

KEYWORDS: HPLC, GC, Mouthwash analysis, preservatives, actives, flavors;

(Research Article)**INTRODUCTION**

Oral cavity is the gateway to the body. Bacterial population in the oral cavity are the primary cause for all the problems associated with the oral cavity, viz., oral malodor¹, tooth cavity formation, hypersensitivity, gum bleeding, periodontitis and tooth loss, etc. To prevent the oral care problems and also to maintain good health, it is necessary to maintain good oral hygiene². Dentifrices and mouthwashes are the commonly used oral hygiene products by the consumers. These consumer products are manufactured by various industrial organizations and their compositions are proprietary information to those individual organizations. Hence, the only way to understand the market and technology trends in the product category is to analyze the products available in the market and arrive at plausible trend information.

Mouthwash is one of the formats of oral care Products that help in reducing the bacterial problems in the oral cavity and also providing the assurance of fresh breath, and also leading to the control of plaque, gingivitis and oral hygiene³. Mouthwashes are generally formulated⁴ using antibacterial agents (fluoride, alcohol, and cetylpyridinium chloride), flavours (thymol, eucalyptol, menthol, and mint oils), humectants (sorbitol, glycerol, propylene glycol) and colorants in an aqueous / alcoholic medium. The sweetener (sodium saccharin) may be used to reduce / eliminate the base taste of the product. Surfactants (sodium lauryl sulphate, cocoamidopropyl betaine) may also be used as foaming agents. Water is the medium. Most of the currently available mouthwash products in the market contain alcohol. Though alcohol acts as an antiseptic and antibacterial, it induces dryness in the mouth and makes the environment more susceptible to the growth of bad breath producing bacteria. Hence, the recent trend in the market is to move from the alcohol-containing products to alcohol-free mouthwashes.

Therefore, in the present study, the available mouthwash products from the Indian market were procured and analysed for their qualitative and quantitative compositions. The methods for the analysis of each component in the products were either taken directly from the reported standard methods or modified to suit the analysis of mouthwash products.

MATERIALS AND METHODS

The mouthwash products to be analysed were procured from the Indian Market. They were sampled randomly and analysed. Analytical grade reagents and distilled water were used for sample and solution preparations. The qualitative and quantitative analyses protocols were designed and conducted using either single or complementary techniques.

The following are the mouthwash products procured for the composition analysis: 1. Listerine coolmint-250mL; 2. Listerine coolmint-80mL; 3. Listerine; 4. AM PM Special; 5. AM PM Plus; 6. Colgate sensitive; 7. Colgate Plax peppermint, and 8. Colgate Plax Fresh mint.

The products were analysed for their physical properties viz., specific gravity and turbidity. The pH of these mouthwashes were also measured and noted. The qualitative identification of the component ingredients was performed to confirm the presence of such ingredients in the formulation prior to proceeding for their quantification. The quantitative composition of each component was analysed using instrumental techniques such as HPLC and/or GC. The technique used for the analysis in each case is included in the tabulation along with the results obtained.

Physical Analysis

Turbidity: The turbidity / optical clarity of the mouthwashes⁵ were measured by using a HACH

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turbid meter (Model: 2100AN). The HACH turbid meter was calibrated using StablCal sealed vial standards of 0.1 NTU to 7500 NTU, prior to the measurements.

Specific gravity: The specific gravity (density) of these mouthwash samples were measured using a pycnometer as per the protocol reported in American Society for Testing and Materials (ASTM) D891.

pH: The pH of the mouthwash samples⁶ was measured using Digison pH meter, incorporating a glass cell and reference electrode. The pH meter was calibrated using standard buffer solutions of pH 1.68, 4.01, 7.00 & 10.01, prior to the measurements.

The analysis results observed for the various mouthwash products are tabulated as follows:

Table 1: General appearance of the products and their characteristics

Product name	Product Appearance	pH	Sp. Gravity	Turbidity (NTU)
AM PM Special	Sky blue liquid	6.15	1.04	5.46
AM PM Plus	Cherry red liquid	6.24	1.04	1.58
Colgate Sensitive	Light Pink liquid	6.51	1.06	5.21

Table 2: List of key ingredients in the mouthwash products as per the pack.

SI No	Brand name	Claim	List of key ingredients
1	AM PM Special	Triclosan-0.03 w/v Sodium fluoride-0.05w/v Alcohol-7.50w/v Thymol-0.02%w/v Eucalyptus oil-0.05%v/v	Ethyl Alcohol, Sodium fluoride, Triclosan, Thymol, Eucalyptus oil
2	AM PM Plus	Triclosan-0.03 w/v Sodium fluoride-0.05w/v	Ethyl Alcohol, Sodium fluoride, Triclosan, Thymol,

Colgate Plax Peppermint	Dark blue liquid	6.05	1.03	1.24
Colgate Plax Freshmint	Light green liquid	5.83	1.04	1.12
Listerine Coolmint, 250mL	Blue liquid	3.74	1.03	5.49
Listerine Coolmint, 80mL	Blue Liquid	3.80	1.05	4.28
Listerine 250mL	Light yellow	3.94	0.96	3.20

CHEMICAL COMPOSITION ANALYSIS**Qualitative Identification**

Initially, the list of key ingredients from the pack labels of the individual products is noted and the same information is summarized in Table 2. These are the key ingredients the marketing organization has claimed on the product pack that the product would contain. However, since there is no regulatory compulsion to declare the complete list of ingredients, only key ingredients are mentioned on the product packs. Hence, it is necessary to verify the presence of other ingredients that might have been used in these product formulations, prior to proceeding for their quantification.

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		Alcohol-7.50w/v	
3	Colgate Sensitive	Potassium nitrate-3%w/v Cetyl Pyridinium chloride-0.05%w/v	Potassium Nitrate, Sodium fluoride , Aqueous Base Q.S., Cetyl Pyridinium Chloride , Flavour
4	Colgate Plax Peppermint	Ethanol (95%)-7.2 v/v Available fluoride-225ppm	Glycerin, sorbitol, Propylene Glycol, Polysorbate 20, sodium Benzoate, Sodium fluoride, Cetylpyridinium Chloride, Sodium Saccharin, Ethyl Alcohol (95%), Flavour.
5	Colgate Plax Freshmint	Ethanol (95%)-7.2 v/v Available fluoride-225ppm	Glycerin, Sorbitol, Propylene Glycol, Polysorbate 20, sodium Benzoate, Sodium fluoride, Cetylpyridinium Chloride, Sodium Saccharin, Ethyl Alcohol (95%), Flavour.
6	Listerine Coolmint 250mL	Thymol I.P-0.06w/v Eucalyptol-0.09w/v Menthol-0.04w/v Ethanol (95%)-21.6 v/v	Thymol, Eucalyptol, Menthol, Ethanol
7	Listerine Coolmint 80mL	Thymol I.P-0.06w/v Eucalyptol-0.09w/v Menthol-0.04w/v Ethanol (95%)-21.6 v/v	Thymol, Eucalyptol, Menthol, Ethanol
8	Listerine	Thymol I.P-0.06w/v Eucalyptol-0.09w/v Menthol-0.04w/v Ethanol (95%)-26 v/v	Thymol, Eucalyptol, Menthol, Ethanol

In this regard, the qualitative identification of various commonly used ingredients was carried out to confirm the presence or absence of (i) humectants, (ii) preservatives, (iii) sweetener, (iv)

flavour oil components relevant to oral care benefits and (v) other oral care active ingredients in them. The various techniques / methods used for the quantitative analysis are listed in Table 3.

Table 3: Technique used for the quantitative analysis of ingredients present in the various mouthwash products

S. No	Materials	Ingredients	Technique used for quantitative analysis
1	Humectants	Sorbitol	HPLC
		Glycerol	HPLC
		Propylene glycol	GC

2	Preservative	Sodium benzoate	HPLC
3	Sweetener	Sodium saccharin	HPLC
4	Flavour materials	Thymol	HPLC
		Menthol	GC
		Eucalyptol	GC
5	Other actives	Fluoride	IC
		Potassium nitrate	JV-visible Spectrophotometer
		Triclosan	HPLC

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		CPC	HPLC
6	Alcohol (ethanol)	Ethanol	GC

Quantitative Estimation

The components present in the mouthwash products, as confirmed by their qualitative testing, were analysed for their quantitative composition using Ion Chromatography / HPLC with UV-Visible Spectrophotometric detection / GC with Flame Ionization detection. Primarily, the components analysed are: (i) Humectants – Sorbitol, glycerol and propylene glycol; (ii) Preservatives – Sodium benzoate; (iii) Sweetener – Sodium saccharin; (iv) Flavour oil components relevant to oral care benefits – Thymol, Menthol & Eucalyptol; (v) other claimed oral care active ingredients – Fluoride, Potassium nitrate, Cetyl pyridinium chloride & Triclosan, and (vi) the alcohol (ethanol) content.

Selection of method and sample preparation

Selection of methods; preparation of standard and sample solutions were based on the ingredients list available on the pack. Sample solutions were prepared by taken directly from the mouthwash and diluted with respect diluent to obtain required concentration of components for the identification and estimation prior to injecting into the chromatographic column.

The suitability of the column and mobile phase was verified initially with a solvent run followed by a standard solution for all the chromatographic estimation methods.

A. Estimation of Ethanol content

The quantity of ethanol present in all the products except "Colgate Sensitive" was determined by using Gas chromatography (GC) technique with Flame Ionization Detection (FID)⁷.

The GC analysis was carried out using a stainless steel (SS) packed column (dimension: Length: 1.5m x diameter: 3.2mm), with 12% Sorbitol as the adsorbent material. The temperature of the injector port was set at 170°C whereas those of the column and detector were set at 150°C and 220°C, respectively. The carrier gas nitrogen was pumped at 40 mL/min with air and hydrogen used for the flame.

B. Estimation of Propylene glycol

The concentrations of propylene glycol in mouthwashes were determined by using Gas Chromatography method⁸. The gas chromatography method was performed by Gas chromatography with flame ionization detector on stainless steel column; 1.5m x 3mm, packed with 12% Sorbitol on untreated siliceous earth (such as Chromsorb W-NAW SINS). Temperature: Column at 165°C, Injector port and detector at 260°C. Carrier gas; nitrogen pumped at 30mL/min with air and hydrogen used for flame.

C. Estimation of Menthol and Eucalyptol

The quantities of Menthol in mouthwash were estimated by using Gas chromatography with FI detector^{9,10}. The Gas chromatography method was performed on stainless steel column; 4m x 2mm, packed with diatomaceous support impregnated with 5 percent carbowax 20M, Sorbitol. The temperature of the injector port was set at 220°C whereas those of the column and detector were set at 240°C and 260°C, respectively. The carrier gas nitrogen was pumped at 30 mL/min with air and hydrogen used for the flame. The temperature ramp used during the analysis is as follows:

<u>Time</u> (Minute)	<u>Column temperature</u> (°C/min)	<u>Ramp rate</u> (°C/min)
0-2	80	--
2-22	240	8
22-30	240	--

(Research Article)**D. Estimation of Sodium benzoate and Sodium saccharin**

The concentrations of Sodium saccharin and sodium benzoate were determined by using HPLC method with UV detector^{11,12}. The HPLC method was performed on C18 column (150x4.6mm i.d., 5µm particle size) at ambient temperature; the mobile phase was mixture of acetate buffer and acetonitrile in the ratio of 80:20, v/v, pumped at flow rate of 1.0mL/min.and the UV detector was set at 230nm.

E. Estimation of Sorbitol and Glycerol

The concentrations of Sorbitol and glycerine were estimated by using HPLC method with RI detector^{13,14}. The HPLC method was performed on column with strong cation exchange resin sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form. (100x7.8mm i.d., 8µm particle size); Column oven temperature was set at 80 °C; the mobile phase was water, pumped at flow rate of 1.0mL/min.

F. Estimation of Thymol

The concentrations of Thymol were determined by using HPLC method with UV detector¹⁵. The HPLC method was performed on C18 column (150x4.6mm i.d., 5µm particle size) at ambient temperature; the mobile phase was mixture of water and acetonitrile in the ratio of 50:50, v/v, pumped at flow rate of 1.5mL/min.and the UV detector was set at 274nm.

G. Estimation of Triclosan

The concentrations of Triclosan were determined using HPLC method with UV detector¹⁶. The HPLC method was performed on C18 column (150x4.6mm i.d., 5µm particle size) at 40 °C temperature; the mobile phase was the mixture of

10mM monobasic hydrogen phosphate, 10mM dibasic hydrogen phosphate and acetonitrile in the ratio of 17.5:17.5:65.v/v/v, pumped at flow rate of 1.5mL/min.and the UV detector was set at 230nm.

H. Estimation of Cetyl pyridinium chloride

The concentrations of Cetylpyridinium chloride was determined by using HPLC method with UV detector¹⁷. The HPLC method was performed on Reprosil-pur basic C18 column (200x4.0mm i.d., 5µm particle size) at ambient temperature; the mobile phase was 0.1% trifluoroacetic acid and acetonitrile, pumped at flow rate of 1.5mL/min with gradient elution. The UV detector was set at 259nm.

Gradient elution:

<u>Time</u> (minute)	<u>Acetonitrile</u> (%)	<u>0.1% TFA</u> (%)
0-5	40	60
5-13	40 – 100	60 - 0
13-15	100	0
15-18	100 – 40	0 – 60

I. Estimation of Potassium nitrate

The amount of potassium nitrate present in the “Colgate Sensitive” mouthwash was analysed by spectrophotometer method¹⁸. Standard and sample were treated with hydrochloric acid and measure the absorbance at wavelength 220nm for Nitrate Ion reading and at 275nm for interference. Corrected abs = Abs at 220nm – 2 x Abs at 275nm. The correction value should not more than 10% of abs at220nm.

J. Estimation of Fluoride

Concentrations of available fluoride were determined by using Ion Chromatography method¹⁹. Ion Chromatography method was performed on Dionex Ionpac AS-18 column, (250x4.0) mm, with Guard column Dionex Ionpac AG-18 (4.0x5.0) mm. The mobile phase was

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potassium hydroxide solution, pumped at 1.0mL/min and set the detection on conductimetric with regenerating water suppression.

RESULTS**Table 4:** The composition of the mouthwash products as analysed by various techniques (Table 1).

Sample	Sodium Benzoate (%w/v)	Propylene Glycol (%v/v)	Sorbitol (%w/w)	Glycerol (%v/v)
Colgate Sensitive	---	5.29	3.05	7.85
Colgate Plax Peppermint	0.18	4.72	3.32	7.54
Colgate Plax Freshmint	0.19	4.52	3.22	7.93

Table 5: The composition of the actives in mouthwash products as analysed by various techniques (Table 1).

Sample	Fluoride (ppm)	KNO ₃ (%w/v)	CPC (%w/v)	Ethyl Alcohol (%v/v)	Triclosan (%w/v)	Sodium Saccharin (%w/v)	Thymol (%w/v)	Eucalyptol (%v/v)	Menthol (w/v)
AM PM Special	240	---	---	7.8	0.03	---	0.03	0.05	---
AM PM Plus	234	---	---	7.6	0.03	---	---	---	---
Colgate Sensitive	234	2.99	0.05	---	---	---	---	---	---
Colgate Plax Peppermint	235	---	0.05	6.9	---	0.10	---	---	---
Colgate Plax Freshmint	228	---	0.05	7.4	---	0.12	---	---	---
Listerine Coolmint 250mL	3	---	---	21.7	---	---	0.05	0.09	0.04
Listerine Coolmint 80mL	2	---	---	22.1	---	---	0.06	0.09	0.04
Listerine	2	---	---	27.0	---	---	0.06	0.09	0.04

DISCUSSION

Commercially available mouthwash products from the Indian market were procured and were analysed for their constituents using various analytical techniques. The observed data for their

physical properties are tabulated in Table 3 and the analysed concentrations of constituents of these mouthwashes are compiled in Tables 4 & 5.

The pH of the mouthwashes are in the range of 3.74 – 6.51, with half of them having acidic pH and

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the rest of them are all of neutral pH. Most of the mouthwashes contained high levels of ethyl alcohol. These products contained thymol and menthol also. These are having acidic pH values (3.74 – 3.94). The water-based (aqueous) products and products with low levels of alcohol are of near-neutral pH. Hence, from the pH values of mouthwashes, we could get an initial indication of the level of alcohol present in them.

Most of mouthwashes available in the market are alcohol-based formulations. The alcohol content in them varies from 6.3%v/v to 27%v/v. Whenever the alcohol content is high, those products did not contain preservatives whereas the products with low levels of alcohol contained sodium benzoate as preservative.

The products with low or no alcohol contained humectants such as sorbitol, glycerol and propylene glycol. These would help in reducing the dryness of the oral cavity after product usage and also, they help in preserving the product.

All Colgate mouthwashes analysed in this study are formulated with 0.05%w/v cetylpyridinium chloride and about 5%v/v of propylene glycol. From the composition analysis of various mouthwash products and the information available about the history of the brands in the market, it may be said that the products with long history are with higher levels of alcohol whereas the recent products are with lower levels of alcohol and the most recent introductions are alcohol-free.

CONCLUSIONS

The various analytical methods utilized during this investigation are either already published and used with minor modifications or developed in-house to suit the requirements. Practically, there were no major issues in implementing these methods for the analysis of products with unknown compositions.

The various approaches were used for the separation and estimation of the constituents of the mouthwash products. Using these instrumental techniques, a very close estimation of the constituents can be achieved.

From the observed results, the following conclusions could be drawn:

1. The composition analysis of the mouthwash products using various instrumental techniques could be done with ease.
2. Products with high alcohol contents are preservative-free.
3. The humectants are used for better safety to the consumers and also, they help in reducing the dryness of the soft tissues in the oral cavity.
4. The market is moving towards alcohol-free products.
5. With the introduction of new products in the market by the category leader, it may be said that the market is expected to grow rapidly. Hence, it is expected that more products would be available in the market in the future to come.

In light of this, the technological trend of product category could be understood with the regular evaluation of the products in the market. The qualitative identification of the ingredients can throw some light on the physical quality and toxicological safety of the product whereas the quantitative estimation of the composition could lead us to predict the expected efficacy for the intended benefits.

In addition, this information could lead us to understand the currently available technological information about how to develop and deliver consumer-friendly products with improved aesthetics and efficacy.

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