

Estimation of the Components in Oral Care products Available in Indian Market – Part II: Toothpaste

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ABSTRACT

Commercial toothpaste samples, randomly collected from the open market were analysed for their constituents by using wet chemical as well as instrumental methods. The accuracy of the measurements of the level of constituents depends upon the method of solubilisation and extraction of the individual ingredients from the toothpaste. The best results were obtained with diluted hydrochloric acid extraction. Moisture contents of samples were calculated and varied from 8%w/w – 54%w/w, with 50% of the sample having water levels above 40 wt%. The pH of the toothpaste ranged from 6 - 10, with 25% of them having near-neutral pH. The concentrations of constituents quantitatively measured were: Fluoride: 800-1040 ppm, phosphates: 0.51-1.86 % by wt, potassium nitrate: 0.51-6.16 % by wt., methyl & Propyl Parabens: 0.10-0.20 % by wt., Silica: 1.5-24% wt, Calcium carbonate: 35-39% wt, Titanium dioxide: 0.02-0.30% wt, Sodium lauryl sulphate: 1.2-2.5% wt, Sorbitol (70% w/v): 14-59% wt and Glycerine: ~ 10% wt. The oral health implications of the products in terms of their claims and the claim ingredients in their respective formulations have been discussed.

Keywords: HPLC, GC, IC, Spectrophotometer, Toothpaste.

INTRODUCTION

Use of dentifrice in various forms for oral care application dates back to over 2000 years. Colonial America, used their own home-made mixes of powders and pastes, and the popularity of their usage increased with the re-invention of toothbrush by William Addis at around 1770. Dentifrices usually were a "family pot". The modern day toothpaste was initially invented in 1892 by Dr. Washington Wentworth Sheffield. Subsequently, the fluoridated toothpaste was developed by Bibby in 1942 and the first clinical trial of fluoride toothpaste was carried out.

Conventional toothpaste contained dicalcium phosphate, silica, calcium carbonate or alumina. In 1954, Muhler et al., introduced calcium pyrophosphate also as an abrasive system in the toothpaste formulation along with sodium fluoride as the anti-caries¹ active ingredient. This product showed about 10 % reduction in caries formation.

Discovery of new fluoridating molecule viz., sodium monofluoro phosphate in 1940, by Dr. Harold Hodge, at Ozark Mahoning Company, Oklahoma, followed the use of various new ingredients such as sodium N-lauroyl sarcosinate by Colgate and stannous fluoride in Crest by P&G².

In August 1958, a report entitled "Analysis of Competitive Toothpastes", TPDU/206/8, (1), was issued and since then this report has

served as a basis for toothpaste analysis. It was written, not a complete method for the analysis of any toothpaste since this is seldom necessary, but as a scheme which could be used by local laboratories to detect major changes in the composition of principle competitive toothpaste.

Since this time major changes occurring in toothpaste formulae and a large range of materials is now used. It is necessary to develop analytical methods³ by using sophisticated instruments to understand the composition of the constituents of competitive toothpaste. Here we developed and analyzed the competitive products.

Therefore, in the present study, the available toothpaste 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 toothpaste products.

MATERIALS AND METHODS

This series of experiments is designed to show how a variety of techniques and instruments are used to analyse toothpaste samples of unknown compositions. Analytical grade reagents and distilled water were used for solution preparations. The prepared solutions were stored in cleaned glass / plastic bottles,

depending on the nature of analyte. Toothpastes (20) from the Indian market were randomly sampled, labelled (A) to (T) and analysed. The qualitative and quantitative analyses protocols were designed and conducted using either single or complementary techniques⁴.

The following are the toothpaste products procured for the composition analysis:

1. Colgate Strong Teeth;
2. Colgate Total 12;
3. Colgate Fresh Energy Gel;
4. Colgate Cibaca Family Protection;
5. Colgate Active Salt;
6. Colgate Sensitive;
7. Colgate Max Fresh Spicy Fresh;
8. Colgate Max Fresh Citrus Blast;
9. Colgate Herbal;
10. Colgate Advanced Whitening;
11. Close-up Milk Calcium Nutrient;
12. Pepsodent Germ check+;
13. Pepsodent Whitening;
14. Close-up Red Hot;
15. Pepsodent G Gum Care;
16. Babool;
17. Meswak;
18. Promise Anti – Cavity;
19. Dabur Red, and
20. Sach Icy Mint Fresh.

All the samples were conventional toothpastes except for samples (P), (Q) and (S) described as Herbal toothpastes by manufacturers.

The products were analysed for their physical properties viz., specific gravity, foam level, and viscosity. The pH of these toothpastes was also recorded. The qualitative identification of some of the component ingredients was performed to confirm their presence in the formulation prior to proceeding for their quantification. The quantitative composition of each component was analysed using instrumental techniques such as HPLC, IC and UV-Spectro-photometer.

The technique used for the analysis of each ingredient is tabulated in Table1.

Table 1: The technique used for the analysis of each ingredient

Ingredient	Technique
Fluoride	IC
Silica	Gravimetric & Spectroscopy
Calcium	IC
Potassium	IC
Phosphate	IC
Titanium dioxide	Spectroscopy
Sodium lauryl sulphate	Titrimetry
Parabens	HPLC-UV
Triclosan	HPLC-UV
Sodium saccharin	HPLC-UV
Sorbitol and Glycerine	HPLC-RI

Physical Analysis

pH

The pH of the toothpaste samples was measured using Orion 3-Star pH meter, having Automatic Temperature Compensation (ATC) probe. The pH meter was calibrated using standard buffer solutions of pH 4.01, 7.00 & 10.01, prior to the measurements.

Viscosity

Viscosity was measured by using Brookfield viscometer using T-spindles at 20 rpm using a helipath.

Foam level

Foam level was measured as per Indian standard test procedure IS 6356:2001.

No. of Layers of packing material

The number of layers of co-extruded laminate tubes was measured by treating the part (3 square centimetres) of the tube with carbon tetrachloride on heating.

Water content

Water content of toothpaste was measured by Karl Fisher titrator using about 0.2g of toothpaste for the analysis.

The results observed for the analysis of physical parameters for all the toothpaste samples are tabulated in Table 2.

Table 2: General appearance of the products and their physical characteristics

Sample	Appearance	pH	Viscosity (cps)	Foam level	Water (%w/w)	Layers of packing material
A	White and glossy colour	9.44	1,52,000	160	21.6	3
B	Two Coloured Paste. Green colour glossy stripes on white	7.80	85,500	140	42.6	3
C	Blue colour gel	6.05	2,00,000	140	34.7	3
D	Blue colour and glossy	9.80	1,55,000	140	30.1	3
E	Two Coloured paste (White & Blue). Four Blue stripes	9.50	1,56,000	110	35.2	3
F	Blue gel	7.92	1,85,000	140	39.6	3
G	Red colour gel, infused with dissolvable cooling crystal	6.70	1,22,000	180	30.0	3
H	Green colour gel and infused with dissolvable cooling crystals	6.83	2,25,000	160	43.7	3
I	Two Coloured (White & Green) with four streaks	9.50	2,10,000	140	8.3	3
J	White and Glossy colour	8.65	1,40,000	140	47.2	3
K	Center white colour, outer blue colour filled gel	7.44	2,00,000	140	22.5	3
L	Light pink colour	9.65	1,65,000	170	38.9	3
M	Light Blue stripe finely dispersed blue particles	9.64	80,500	140	35.5	3
N	Red colour gel	7.08	1,43,000	130	30.6	3
O	White colour and Glossy	6.52	1,12,000	130	40.3	3
P	Slight Off White colour	9.85	1,10,000	150	31.7	3
Q	light off white colour glossy	9.82	1,28,000	140	28.0	3
R	Glossy blue colour	7.65	1,30,000	130	48.5	3
S	Red colour	8.88	1,35,000	150	27.3	3
T	Blue colour gel with dissolvable white menthol cool beads	7.25	1,55,000	150	33.6	3

Chemical Composition Analysis

Qualitative Identification

Initially, the list of key ingredients from the pack labels of the individual products is noted. 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 many of 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.

Quantitative Estimation

The components present in the toothpaste products, as confirmed by their qualitative testing, were analysed for their quantitative

composition using HPLC with UV-Visible detector / Ion Chromatography / UV-Visible Spectrophotometer. Primarily, the components analysed are: (i) Humectants – Sorbitol and glycerol; (ii) Preservatives – Sodium benzoate

and parabens; (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.

Selection of method & sample preparation

The dispersion, extraction, qualitative and quantitative analysis were carried out depending on the characteristics of the constituent being analysed as per the following flow chart.

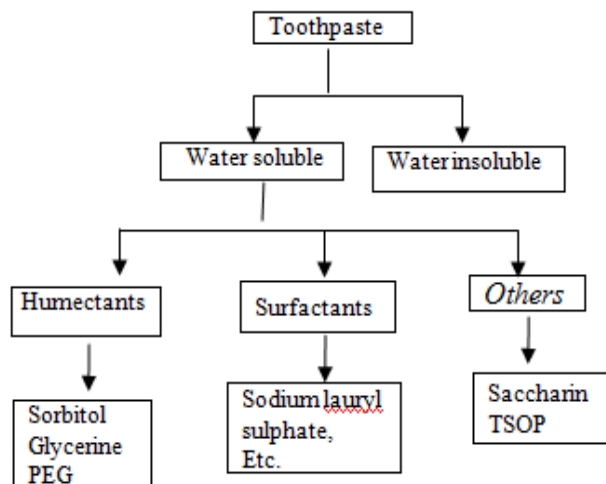


Fig. 1: Flow chart for the dispersion and extraction of ingredients of toothpaste in water

Selection of methods and the preparation of standard / sample solutions were based on the ingredients list available on the pack. Sample solutions for the analysis of individual

ingredients were prepared either by direct extraction or by isolation and dilution using respective diluent to obtain the concentration in the required range of analyte.

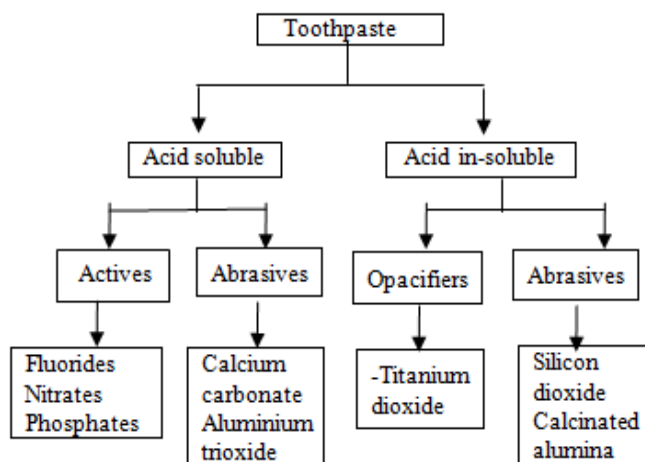


Fig. 2: Flow chart for the dispersion and extraction of acid-soluble ingredients of toothpaste using dilute hydrochloric acid.

A. Estimation of Fluoride content

The concentration of Fluoride⁵ was determined by using Dionex-2100 Ion Chromatography. A Dionex IonPac AS-18 cation separator column (250mm x 4mm), IonPac AG-18 guard column (50mm x 4mm), and EGC-KOH Cartridge were used. Suppressed conductivity ASRS ULTRA, 4 mm was used in the analysis. The column temperature was maintained at 30°C. Mobile phase was pumped at flow rate of 1.0mL/min with gradient elution as per the following profile.

Gradient profile

Time (min)	KOH (mM)
0-7	22
7-8	22-30
8-12	30
12-13	30-40
13-16	40
16-20	40-60
20-26	60-22
26-30	22

B. Estimation of Potassium and Calcium

The concentration of potassium was determined by using Dionex-2100 Ion Chromatography⁶. A Dionex IonPac CS12A cation separator column (250mm x 4mm), IonPac CG12A guard column (50mm x 4mm) and MSA cartridge were used. The eluent of 26 mM methanesulfonic acid (MSA) was generated on-line from reagent water with an EG40 eluent generator and EGC-MSA cartridge. The chromatographic experiment was performed at a flow rate of 1.0 mL/min. The column, loop injector, and the cell of the conductivity detector were maintained at 30°C with injection volume of 50µL.

C. Estimation of Phosphates

The concentrations of phosphates were determined by using Dionex-2100 Ion Chromatography⁷. A Dionex IonPac AS18 anion separator column (250mm x 4mm) and IonPac AG18 guard column (50mm x 4mm) were used. KOH produced by an EG equipped with a EGC II KOH cartridge and CR-ATC. Suppressed conductivity ASRS 300, 4 mm, external water mode was used in the analysis. The column temperature was maintained at 35°C. Mobile phase was pumped at flow rate of 1.2mL/min with gradient elution as per the following profile.

Gradient profile

Time (min)	KOH (mM)
0-8	15
8-20	15-45
20-25	45-15
25-30	15

D. Estimation of Silica

The silica content was determined using gravimetric method and spectrophotometer method^{8,9}. The residual / dissolved silica was estimated by the colour development of the sample by treating with ammonium molybdate and 1-amino-2-naphthol-4-sulfonic acid. Finally, the absorbance was measured at 832nm and the total content calculated by combining the gravimetric and spectrophotometric results.

E. Estimation of Titanium dioxide

Concentration of titanium dioxide⁹ in toothpaste was determined by treating Ignition residue with hydrogen peroxide for colour development and measuring the absorbance at 400nm with Shimadzu UV-

Vis.Spectrophotometer using 1cm quartz cuvette.

F. Estimation of Sodium lauryl sulphate

Concentration of sodium lauryl sulphate in toothpaste was determined by using complexometric titration with benzithonium chloride, using dimethyl yellow-oracet blue B solution as indicator.

G. Estimation of Methyl and Propyl Parabens

The concentrations of methyl and propyl parabens were determined by using HPLC with UV detector¹⁰. The HPLC method was performed on C8 column (250 mm x 4.6 mm i.d., 5 µm particle size). The mobile phase composition was a mixture of acetonitrile and water in the ratio of 65:35. The mobile phase was pumped at a flow rate of 2.0mL/min and the eluents were detected at a wavelength of 254 nm.

H. Estimation of Triclosan

The concentration of Triclosan was determined by using HPLC with UV detector^{11, 12 and 13}. The HPLC method was performed using C18 column (150 mm x 4.6 mm i.d., 5 µm particle size). The mobile phase was 10 mM monobasic hydrogen phosphate – 10 mM dibasic hydrogen phosphate - acetonitrile (17.5:17.5:65 v/v/v), pumped at a flow rate of 1.5 mL/min and the UV detector was set at 230 nm.

I. Estimation of Sodium Saccharin

The concentration of Sodium saccharin was determined by using HPLC with UV detector^{14, 15}. The HPLC method was performed using C18 column (150 mm x 4.6 mm i.d., 5µm particle size). The mobile phase was Acetate buffer - acetonitrile (80:20, v/v), pumped at a flow rate of 1.0 mL/min and the UV detector was set at 230 nm.

J. Estimation of Sorbitol and glycerol

The concentrations of Sorbitol and glycerol were determined by using HPLC with RI detector¹⁶. The HPLC method was performed using a column with strong cation exchange resin - sulfonated cross-linked styrene-divinyl benzene copolymer, in the Lead (Pb) form (100 x 7.8 mm id, 8 µm particle size). The mobile phase was water, pumped at a flow rate of 1.0 mL/min.

RESULTS

Table 3: The composition of the toothpaste products as analysed by using various techniques

Sample	Total fluoride (ppm)	Free fluoride (ppm)	Silica (%wt)	CaCO ₃ (%wt)	TiO ₂ (%wt)	SLS (%wt)	KNO ₃ (%wt)	TSPP (%wt)	70% Sorbitol (%wt)	Glycerol (%wt)
A	912	750	5.0	35.1	0.20	2.51	0.00	0.00	34.0	0.0
B	1010	1000	19.5	0.0	0.28	2.10	0.00	0.00	35.5	0.0
C	900	852	14.2	0.0	0.00	2.52	0.00	0.50	58.0	0.0
D	905	702	1.5	35.0	0.00	2.31	0.00	0.00	24.7	0.0
E	945	780	6.5	38.5	0.00	2.20	0.00	0.00	20.3	0.0
F	935	888	20.2	0.00	0.00	1.22	6.16	1.20	20.5	10.25
G	972	872	14.0	0.00	0.05	2.23	0.00	0.00	58.5	0.0
H	971	875	14.0	0.00	0.04	2.25	0.00	0.00	59.0	0.0
I	955	720	4.5	38.8	0.05	2.35	0.00	0.00	21.0	0.0
J	1000	982	24.1	0.00	0.10	2.50	0.00	0.00	21.8	10.15
K	904	880	16.1	0.29	0.05	2.52	0.00	0.00	64.5	0.0
L	940	750	7.0	35.2	0.00	2.51	0.50	1.90	14.1	0.0
M	920	685	5.0	34.9	0.05	2.27	0.00	0.00	20.1	0.0
N	938	918	15.1	0.00	0.04	2.52	0.00	0.00	60.9	0.0
O	935	898	15.5	0.00	0.02	2.25	0.00	0.00	41.8	0.0
P	0	0	3.2	35.3	0.00	1.70	0.00	0.00	22.0	0.0
Q	0	0	3.6	39.2	0.00	1.73	0.00	0.00	20.3	0.0
R	0	0	15.4	0.00	0.04	1.85	0.00	0.00	27.8	0.0
S	0	0	0.00	0.00	0.00	1.85	0.00	0.00	25.0	0.0
T	1012	895	15.4	0.00	0.03	2.52	0.00	0.00	58.9	0.0

DISCUSSION

The commercial toothpastes were procured from the Indian retail market. The ingredients of toothpaste samples were extracted in water or dilute hydrochloric acid and analysed for their quantification. The pH of the toothpastes was ranged from 6.1 – 9.9, with 25 % of them having near neutral pH in water while the remaining 75 % are on the alkaline range. The toothpastes containing calcium carbonate and phosphates in them are having higher pH values (9 – 10), whereas silica based toothpastes are having near neutral pH. The pH values of toothpaste are giving an indication of the inorganic ingredients present, such as calcium carbonate and phosphate, as expected.

The water content of toothpaste was measured by the KF Titrator. It varied between 8.3% w/w and 48.5% w/w. Among them, 25% of the products are having water content more than 40% w/w and 45% of the products are having water content between 30% w/w and 40% w/w.

The toothpaste samples analysed were both fluoridated as well as non-fluoridated products. Among the fluoridated products analysed, the total fluoride content were in the range of 900 – 1000 ppm whereas the available / soluble fluoride content varied between 680 and 1000 ppm. This wide variation may be attributed to the time gap for the analysis from their respective date of manufacture. The toothpastes containing calcium carbonate as abrasive are having lower level of available

fluoride as compared to the products without calcium carbonate in them.

Another active ingredient, potassium nitrate was observed in 2 out of 20 toothpastes and having values of 0.51%wt and 5.12% by wt. Since, the toothpaste containing potassium nitrate come under the hypersensitivity product category, the number of products available in this category is low in Indian market, as of today. However, this segment is growing and can expect more products in this category in future.

All toothpastes analysed in this study contain sodium lauryl sulphate (SLS) as surfactant. The SLS content in toothpastes varies from 1.2% wt to 2.6% wt, in which about 80% of them are having more than 2% by wt, whereas it can be, used up to 3% by wt. by the regulatory standards.

The main abrasives found in the toothpaste of Indian market are either silica or calcium carbonate. Since the products contain either or both grades of silica viz., thickening grade and abrasive grade, the analytical results for silica would be the total contribution from the all the grades of silica and also the presence of silicate, in some cases.

The silica content in various toothpaste samples analysed varied from 1.3%wt up to 23.9%wt and the calcium carbonate is varying from 34.1% by wt. to 39.4% by wt. The abrasive content in the product depends on which is the abrasive – silica or calcium carbonate. Calcium carbonate based toothpastes contain silica to the extent of up to 7% by wt. and is contributed by the thickening

grade of silica and silicate, if present, in them. Among the toothpastes analysed, about 40 % of them are calcium carbonate based whereas about 60% of them are based on silica as abrasive. The later products contain silica to the extent of 14 to 24 % by wt. and are contributed by the thickening as well as the abrasive grade of silica.

All the toothpastes in the Indian market contain sorbitol as the humectant. However, some of the toothpastes contain glycerol and even PEG as additional humectants, though those products containing combination of humectants are only about 40% in the market. The Sorbitol content varies from 14.1% to 60.9% by wt. The toothpastes formulated as transparent gel contain high levels of Sorbitol, whereas in non-gel formulations and in combination of humectants such as glycerol and PEG, the sorbitol content is comparatively low.

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 toothpaste 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 toothpaste products using various instrumental techniques could be done with ease.
2. Though it is difficult to exactly predict the composition of all the ingredients from the analysis of the product of unknown composition, we could get a good estimate of the specific ingredient with a reliable quantification.
3. The product compositions keep changing with time as the organizations keep doing research to improve the product quality. Hence, the product analysis should be done as and when we need to know the composition of the competitor's products in the market place. This regular analysis would also give us an idea of the technical trend in which the organization is doing the research.
4. In light of this, to derive the technological trend of product category in the market place, it is necessary to analyse regularly

the products of various players in the market.

5. The qualitative identification of the list of ingredients of the product could provide an idea about the toxicological safety of the product whereas the quantitative estimation of the composition could lead us to predict the expected efficacy for the intended benefits.
6. In addition, these studies could lead us to generate new ideas for the development of new and improved products that could deliver more consumer-friendly products with improved aesthetics and efficacy.
7. With this study, we could conclude that the modern analytical techniques could easily be utilized to understand the competition and the market. Also, it could give us clues to develop better products for consumers.

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REFERENCES

1. Journal of Dentistry, Oral Medicine and Dental education. 2009;3(1).
2. Bonnie Grider and Michele Johnson. "Anticavity Toothpaste design", Department of chemical, biological, and materials engineering, march 14, 2008.
3. Tonzelich J. Production and Origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontal. 1977;48(1):13-20.
4. Amparo Salvador and Alberto Chisvert. Analysis of Cosmetic products. Elsevier, ISBN: 978-0-444-52260-3.
5. Smee.BW, Hall GEM and Koop DJ. "Analysis of fluoride, chloride, nitrate and sulphate in natural waters using ion chromatography", Journal of Geochemical Exploration - Association of Exploration Geochemists. 1978;10:245-258.
6. Tauno Jauhiainen, John Moorea, Paavo Pera, Ema Ekib, John Deromec and Kirsti Derome. Simple procedure for ion chromatographic determination of anions and cations at trace levels in ice core samples. Analytica Chimica Acta. 1999;389:21±2
7. Herman J and Weiser JR. The Determination of Pyrophosphate in commercial triphosphate. The Journal of American Oil Chemist society, Vol.34.
8. Perfecto G Lim. Total Silica Analysis Using a Double Beam Atomic Absorption Spectrophotometer", Proceedings World Geothermal Congress 2005, Antalya, Turkey, 2005.
9. U.S.Pharmacopia, USP29-NF24, Page 880, 3432
10. Ting Fei, Haifang Li, Mingyu Ding, Masahito Ito and Jin-Ming Lin. Determination of parabens in cosmetic products by solid-phase microextraction of poly(ethylene glycol) diacrylate thin film on fibers and ultra high-speed liquid chromatography with diode array detector. J Sep Sci. 2011;34:1599-1606
11. Lida Fotouhi, Hamid Reza Shahbaazi, Azadeh Fatehi and Majid M Heravi, Voltammetric Determination of Triclosan in Waste Water and Personal Care Products. Int J Electrochem Sci. 2010;5:1390-1398.
12. Santo Scalia, Mario Guarneri and Enea Menegatti. Assayo f triclosani n deodoranst ticksa nd soaps by supercritical fluid extraction and HPLC. J SocC. OsmeCt h em.4,5, 35-42
13. Ana Sanches-Silva, Raquel Sendon-Garcia, Julia Lopez-Hernandez and Perfecto Paseiro-Losad., Determination of triclosan in foodstuffs. Journal of Separation Science. 2005; 28(1):65-72.
14. Cavrini AMV, Bonazzi D and Benfenati L. HPLC analysis of aspartame and saccharin in pharmaceutical and dietary formulations. Chromatographia. 30(3-4): 215-219
15. Mira Akar and Gordana Popovi. Determination of saccharin in pharmaceuticals by high performance thin layer chromatography. J Serb Chem Soc. 2006;71(6): 669-676.
16. Virginia Gordy, Baust JG and Hendrix DL. A High-Pressure Liquid Chromatographic Method for Analysis of Carbohydrates and Polyols from Lichens. *The Bryologist* Winter. 1978; 81(4):532-538