

## Optimization Conditions For Determination of Cosmetic Preservatives using Ion-Exclusion Chromatography

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**ABSTRACT:** An ion chromatography (IC) method for simultaneous determination of benzoic, salicylic and sorbic acids was developed. In this method the samples were purified by solid-phase extraction. The analytes were then eluted with methanol from C<sub>18</sub> cartridge. The separation was performed on ion-exclusion column by an isocratic elution with 0.25 mmol /L sulfuric acid solution containing 20% (v/v) acetone as elute. The calibration curves showed good linearity with suppressed conductivity detection. Under this condition, separation of the three components was achieved in less than 23 min. The detection limit calculated as the concentration corresponding to three times the background noise, was 0.1, 0.15 and 0.2 mg/ L for benzoic acid, salicylic acid and sorbic acid respectively. The method was successfully applied to analysis of various cosmetic products.

**Key words:** Ion chromatography, Conductivity detector, Cosmetics, Preservatives

### INTRODUCTION

Of important preservatives used to prolong the useful life of food, pharmaceuticals and cosmetic products are benzoic, salicylic and sorbic acids. Non-volatility and good stability are general properties of these compounds. Benzoic acid has an inhibition effect in the bacterial growthment, while sorbic acid is effective against yeasts and moulds. salicylic acid has antiseptic and dandruff properties. combination of these compounds is increased the biochemical stability of food and cosmetic products for a long time (Raye *et al.*, 2006; Rieger *et al.*, 2001; Mitsui, 1997). In recent years, it has been increased interest in preservatives, due to the adverse side-effect of these preservatives, such as metabolic acidosis, convulsions, their tendencies to induce allergic contact dermatitis and even potentially inducing human breast tumors (Harveg *et al.*, 2003; Jensen *et al.*, 2005).

So far, numerous articles concerning preservative analysis have been published which mainly

focused on food products. But little attention has been paid to the analysis of active ingredients of cosmetics such as benzoic, sorbic and salicylic acids (Huang *et al.*, 2003; Marengo *et al.*, 2004; Prevot *et al.*, 2000; Mikami *et al.*, 2002). Analysis of these compounds are often difficult because of matrix complexity. In addition, depending on the nature of the cosmetics, conditions of analysis are varied widely and are usually time-consuming (Salvador *et al.*, 2007).

One of the important techniques in the separating and analyzing weakly acidic or basic compounds is ion-exclusion chromatography (IEC). This technique which based on a Donnan exclusion mechanism is a powerful technique in the separation of small organic acids (Novirc *et al.*, 2006). Separation of organic acids are achieved by changing the pH, because at low pH weak organic acids are undissociated or weakly dissociated and can diffuse into the resin pores on the stationary phase, While, anionic and or

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natural species are rejected. The aim of this study was to evaluate the ability and efficiency of ion exclusion column in combination with conductivity detector in the determination of benzoic, salicylic and sorbic acids in some of the samples consist of shampoos and a mouth wash.

## MATERIALS & METHODS

HPLC grade acetone was purchased from Fluka. Sorbic acid was obtained from Applichem. Methanol (HPLC grade) and other reagents (analytical grade) were purchased from Merck. A Metrohm IC system consisting of the following components was used, 733 IC separation center with a 20 µl injection loop; 762 IC interface; 732 IC conductivity detector; 709 IC pump and 753 suppressor module. Chromatographic separation was carried out on metrosep organic acid (10 µm, 250\*7.8 mm I.D., Metrohm Co.).

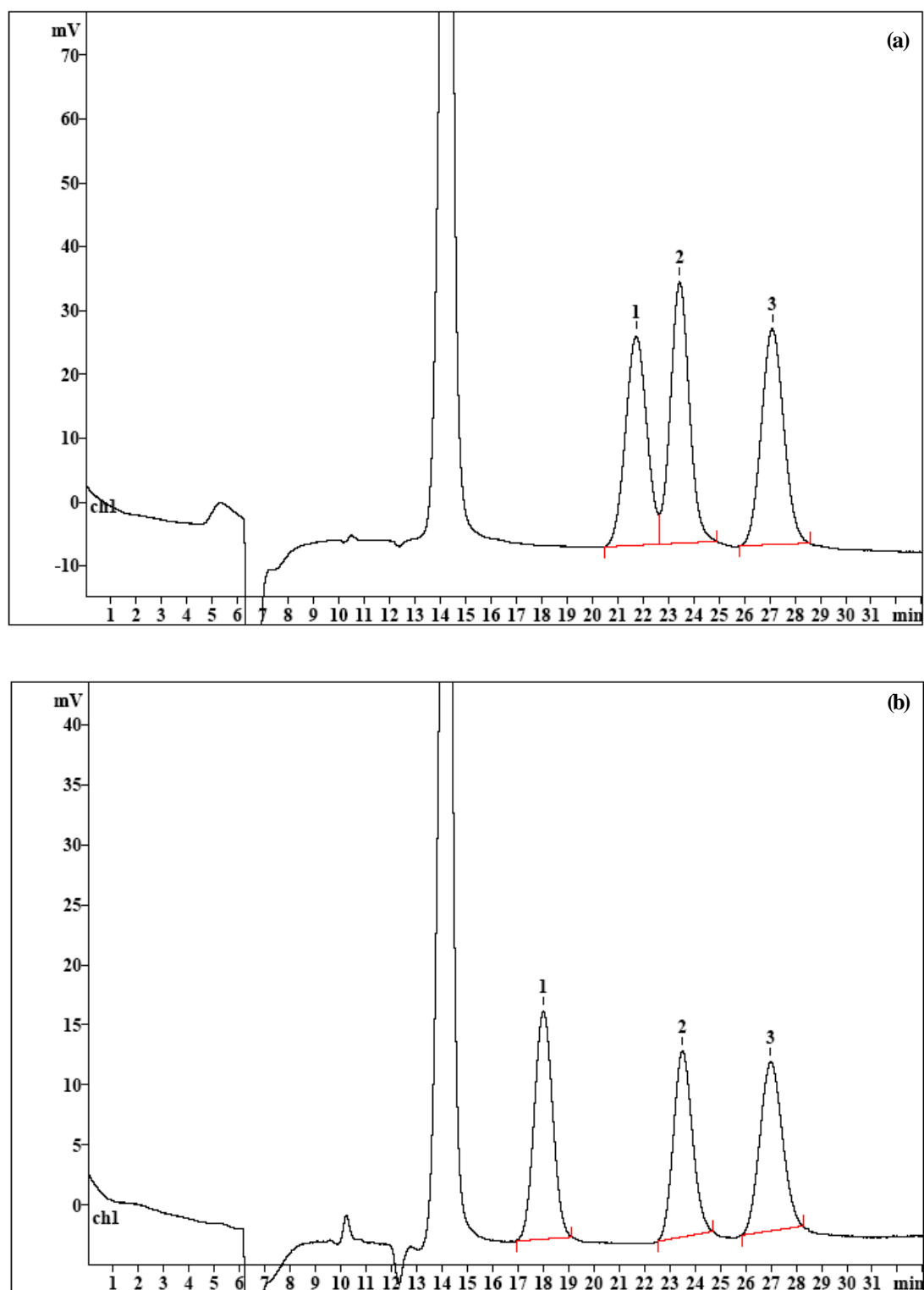
The chromatographic separation of benzoic, salicylic and sorbic acid was achieved with an Ion Exclusion column under isocratic condition at room temperature. A mixture of 0.25 mmol/L  $H_2SO_4$  and acetone (80:20) was performed as the mobile phase at a flow rate of 0.5 ml/min. the chromatogram was recorded for 33 min. The column effluent was passed into the suppressor column that has been washed with 50 mmol/L LiCl solution, and then three acids were detected with a conductivity detector. Standard stock solutions of benzoic, salicylic and sorbic acid (1000 mg/L) were prepared by dissolving of appropriate amounts of respective compounds in 150 ml of ultra pure water by mixing in ultrasonic bath at 40 °C. Then they were cooled to room temperature and diluted in a 200 ml volumetric flask. Seven standard solutions were prepared. An aliquot (20 µl) of each standard was injected. The peak areas for each standard solution were measured and calibration curve plotted by IC Net software. All measurements such as correlation coefficient and RSD were measured by IC Net software. The cosmetic product (1g) was accurately weighed in a 10 ml volumetric flask and diluted with methanol. The mixture was mixed well and the suspension solution was centrifuged at 5000 rpm for 30 min. 100 µl of clear part of centrifuged solution was diluted to 2 ml with ultra pure water.

## RESULTS & DISCUSSION

The preliminary experiments showed that the mixture of 0.5 mmol/L  $H_2SO_4$  with acetone (20%) has a weak efficiency in resolution of salicylic acid and sorbic acid (Fig. 1a). So, the mobile phase composition was varied continually. At first, the organic phase was changed to acetonitrile, but it showed no change in comparison with acetone. Methanol has been tested but it wasn't suitable to separate them. Finally, by modifying the acid concentration, two levels of acid, that is 1 mmol and 0.25 mmol/L  $H_2SO_4$  in 20% of acetone were selected. At higher concentration (Fig. 1b) resolution was reduced and retention time of salicylic acid was increased but at lower concentration (Fig. 1c) resolution was increased and retention times were decreased. Therefore, the last mobile phase mixture was selected and applied to all of analysis.

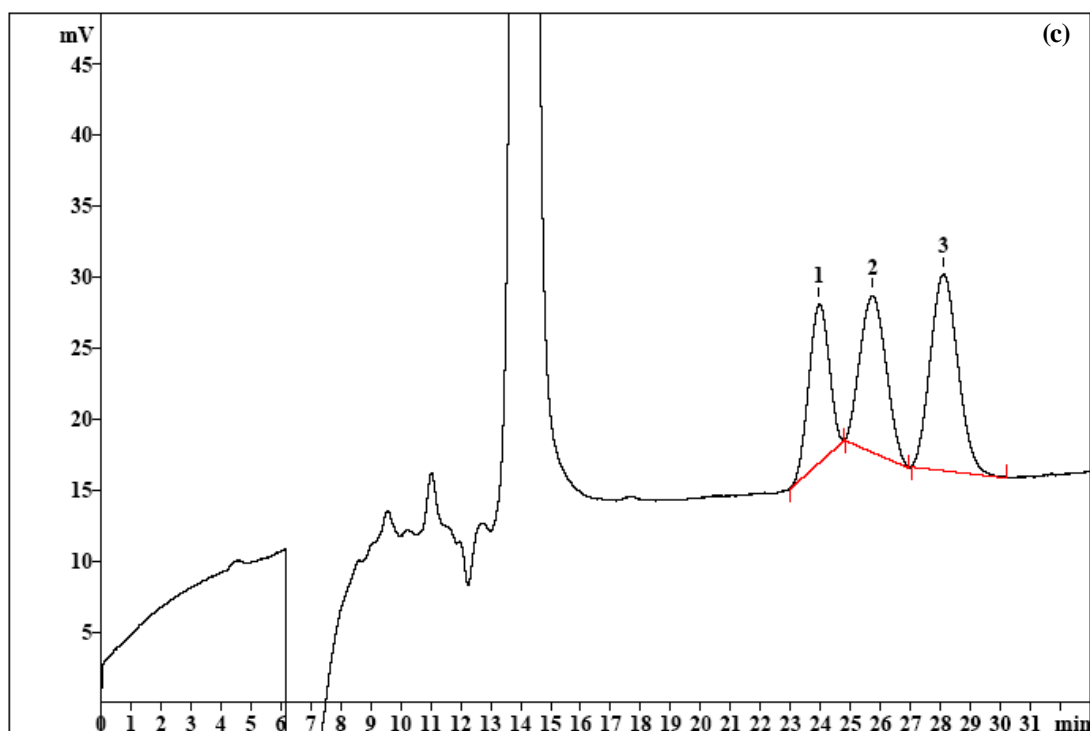
Quantification was based on the external standard method using calibration curves fitted by linear regression analysis. Linearity between the concentration of all three acids and the conductivity signals are in Table 1. The calibration curves and correlation coefficients for sorbic acid, benzoic acid and salicylic acid are shown in Fig. 2a, 2b and 2c respectively. The limit of detection (LOD) is defined as the smallest peak detected with a signal height three times of the baseline in our work, LODs were estimated by successively decreasing the concentration of the prepared standards, down to the smallest detectable peak. The detection limit for benzoic acid, salicylic acid and sorbic acid were 0.1, 0.15 and 0.2 mg/L respectively.

Peak identification of the preservatives in various samples was based on the comparison between the retention times of standard compounds and was confirmed by spiking known standard compounds to the sample. Two Foreign shampoos (Nivea and Panten), an Iranian shampoo (Firooz baby Shampoo) and an Iranian mouthwash (Irsha) were analyzed according to the experimental condition. None of three organic acids were detected in Iranian shampoo, but as shown in Fig 3 there was just benzoic acid as a preservative in both foreign shampoos as well as in mouthwash. Its quantity was approximately 1.2, 1.8, 3.6 mg/g in two shampoos and mouthwash respectively.



**Fig. 1a& b. chromatograms of standard solution**

a. The concentrations of benzoic acid, salicylic acid and sorbic acid are 10 mg/L and the eluent is 0.5 mmol/L  $\text{H}_2\text{SO}_4$  + 20% acetone (1. Salicylic acid, 2. Sorbic acid, 3. Benzoic acid). b. The concentrations of benzoic acid, salicylic acid and sorbic acid are 20 mg/L and the eluent is 0.25 mmol/L  $\text{H}_2\text{SO}_4$  + 20% acetone (1. Salicylic acid, 2. Sorbic acid, 3. Benzoic acid).



**Fig.1. c. chromatograms of standard solution**

The concentrations of benzoic acid, salicylic acid and sorbic acid are 10 mg/L and the eluent is 1 mmol/L  $H_2SO_4$  + 20% acetone (1. Sorbic acid, 2. Salicylic acid, 3. Benzoic acid) Continues

**Table 1. Analytical characteristic of IC method**

Parameter	Sorbic acid	Benzoic acid	Salicylic acid
Linear range mg/L	1-400	0.5-500	1-200
Regression equation	$Y=0.9986X+0.7267$	$Y=0.9783X-1.2472$	$Y=1.0548X-0.7849$
RSD %	1.231	1.236	7.545
$R^2$	0.9998	0.9999	0.9995
LOD (mg/L)	0.2	0.1	0.15
LOQ (mg/L)	0.5	0.3	0.4

**Table 2. Recovery study**

sample	Benzoic acid			Salicylic acid			Sorbic acid		
	Added (mg/L)	Recovery (% , mean $\pm$ S.D.)	R.S.D (%)	Added (mg/L)	Recovery (% , mean $\pm$ S.D.)	R.S.D. (%)	Added (mg/L)	Recovery (% , mean $\pm$ S.D.)	R.S.D. (%)
Shampoo (Nivea)	1	97.5 $\pm$ 2.9	3.0	1	95.3 $\pm$ 3.7	3.9	1	93.4 $\pm$ 4.6	4.9
	10	98.0 $\pm$ 1.8	1.8	10	96.1 $\pm$ 3.2	3.3	10	94.0 $\pm$ 4.5	4.8
Shampoo (Panten)	1	96.3 $\pm$ 2.9	3.0	1	94.9 $\pm$ 4.1	4.3	1	93.7 $\pm$ 3.8	4.1
	10	97.2 $\pm$ 2.8	2.9	10	95.1 $\pm$ 4.3	4.5	10	92.2 $\pm$ 3.2	3.5
Mouthwash (Irsha)	1	98.5 $\pm$ 2.3	2.3	1	96.2 $\pm$ 2.6	2.7	1	95.2 $\pm$ 3.7	3.9
	10	98.7 $\pm$ 3.2	3.2	10	96.5 $\pm$ 2.5	2.6	10	95.3 $\pm$ 4.1	4.3

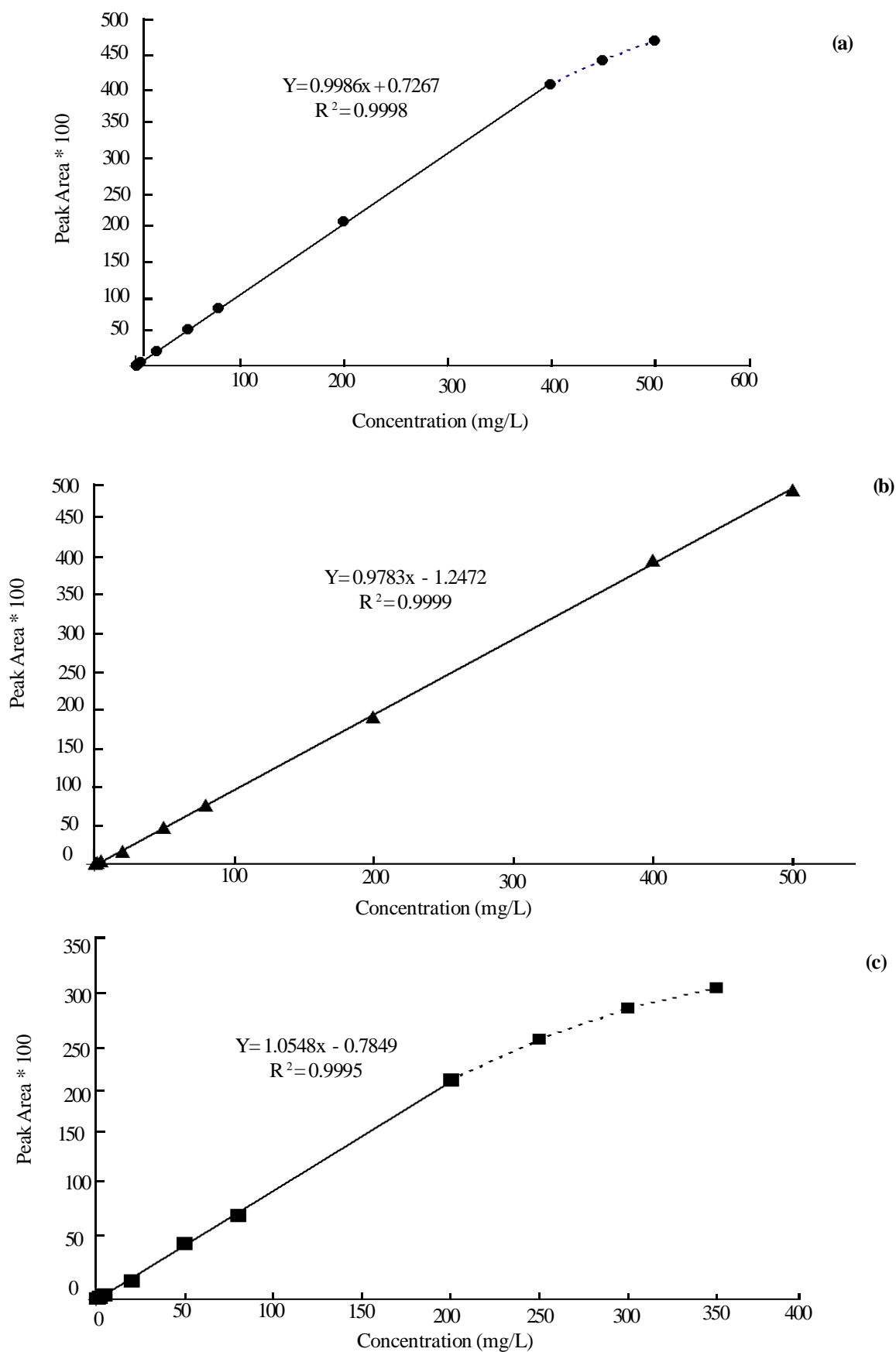


Fig. 2a, b & c. Calibration curves. a. sorbic acid b. benzoic acid c. salicylic acid

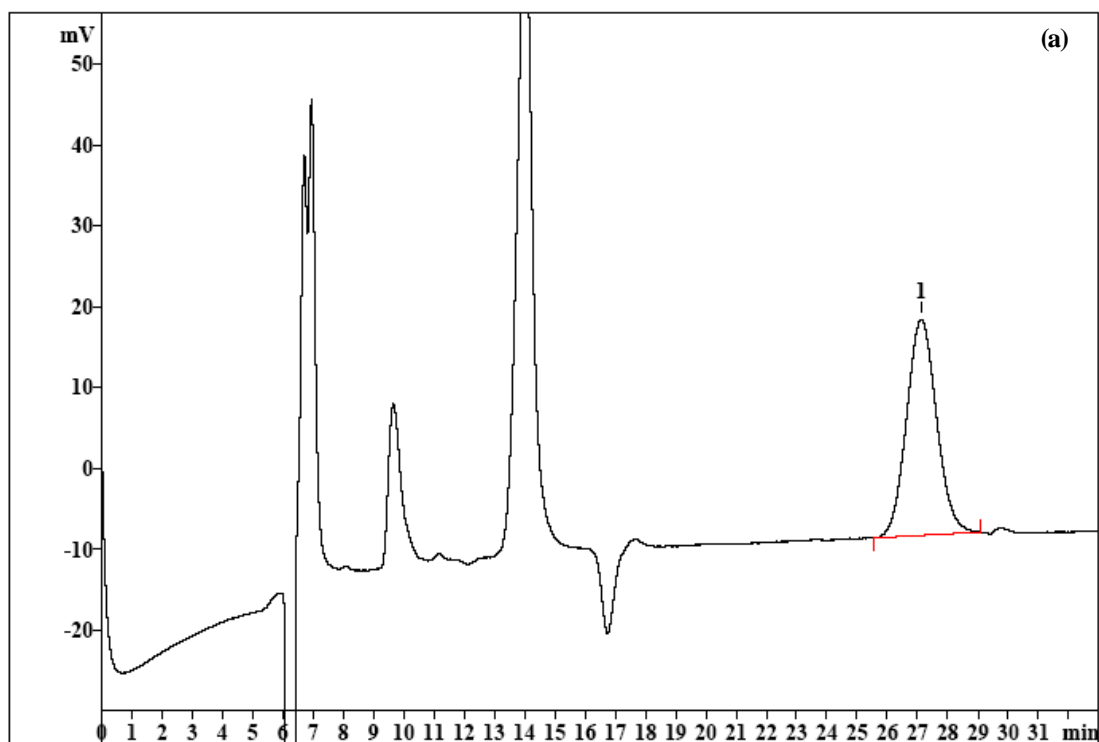


Fig. 3.a.

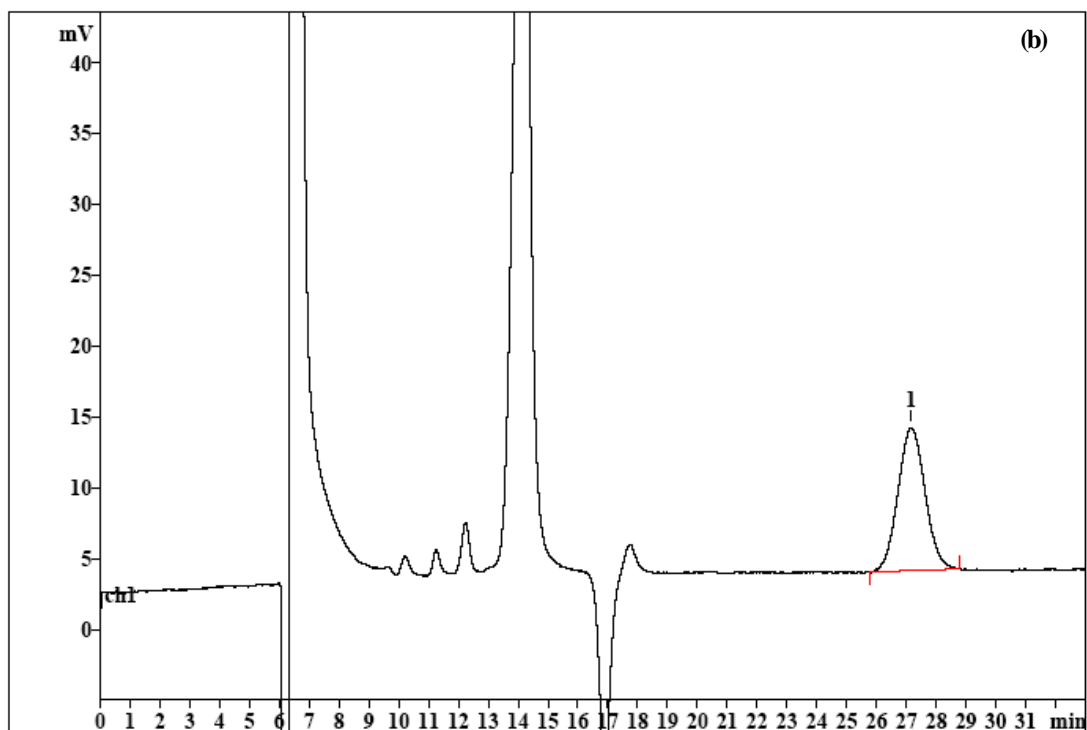
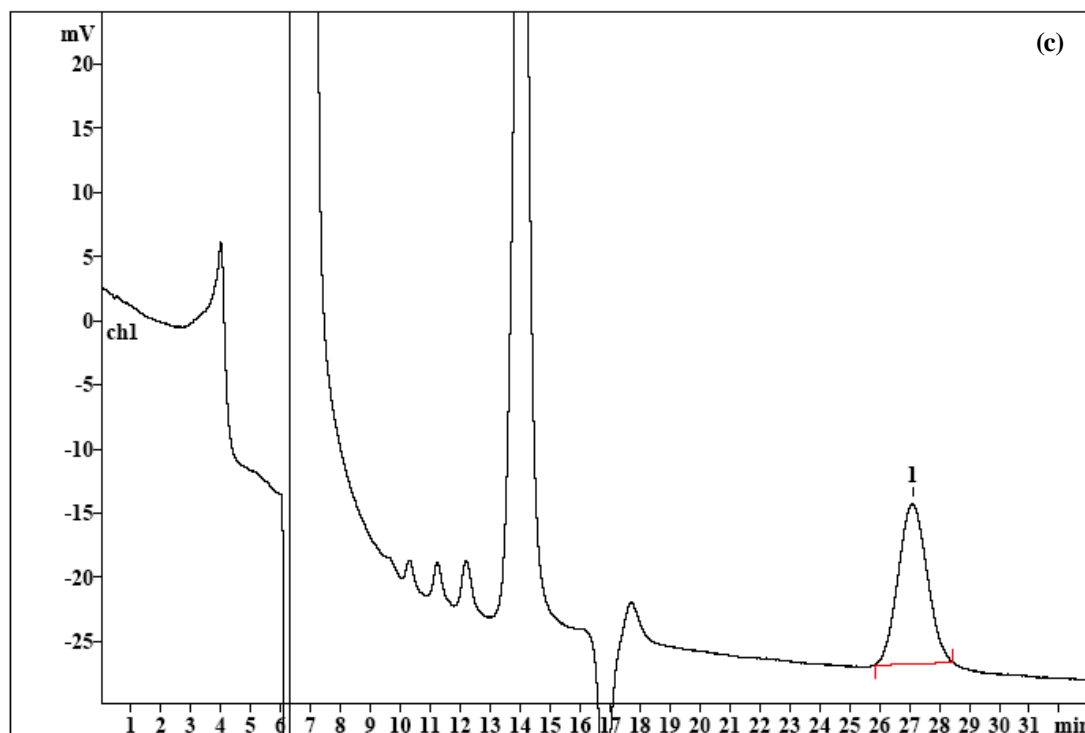
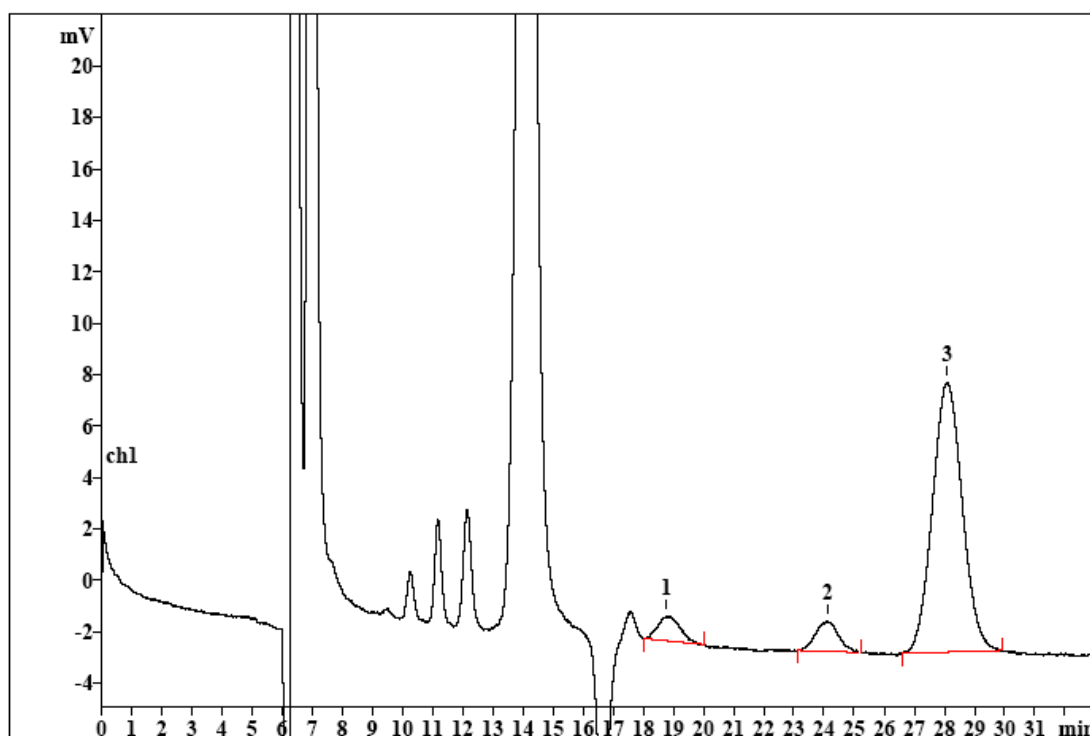


Fig. 3.b.

**Fig. 3. chromatograms of real samples**  
(1. Benzoic acid). a. Irsha mouthwash, b. Nivea shampoo (Continues)



**Fig. 3. chromatograms of real samples**  
(1. Benzoic acid). a. Irsha mouthwash, b. Nivea shampoo



**Fig. 4. Recovery study of real samples (1. Salicylic acid, 2. Sorbic acid, 3. Benzoic acid).** a. Panten Shampoo was spiked with 1 mg /L of benzoic acid, salicylic acid and sorbic acid b. Panten Shampoo was spiked with 10 mg/L of benzoic acid, salicylic acid and sorbic acid

In order to verify the precision and accuracy of the analytical method, all samples were spiked with two levels of standard of preservatives and analyses were carried out before and after the addition of 1 and 10 mg/L of benzoic acid, salicylic acid and sorbic acid. The results were summarized in Table 2. The Panten shampoos that were spiked with three organic acids are shown in Fig. 4.

## CONCLUSION

A method has been developed for the determination of Benzoic, Salicylic and sorbic acids by a common ion exclusion column and a suppressed system with conductivity detector. This method is conveniently applicable for quantitative analysis of Shampoo samples and related Cosmetics. Despite the weak acidic nature of the mentioned acids, which cause of some difficulties in determination with conductivity detector in ordinary conditions, the results of experiments have been show that the usage of both C<sub>18</sub> non-polar bonded silica phase cartridge and suppressor can be compensate the deficiency of their weak signals. In addition, application of cartridge and suppressor have been improved the determination limits for each of the three acids considerably compared with the other reported methods ( Chinnici *et al.*, 2005; Helaleh *et al.*, 2002; Yang *et al.*, 2000; Ohto *et al.*, 1991). In the separation of molecular or ionic species, it has been found that ion- exclusion mechanism to be affected by a large number of parameters. This behavior is considered as an great advantage for which, because degree of freedom in the selection of those parameters, can be increased the ability of the method for the analysis of other real cosmetic samples (Novirc *et al.*, 2006).

## REFERENCES

- Chinnici, F., Spinabell, U., Riponi, C. and Amati, A. (2005). Optimization of the Determination of Organic acids and sugars in Fruit Juices by Ion-Exclusion Liquid Chromatography. *J. Food Composition and Analysis*, **18**, 121-131.
- Jensen, C. D., Johansen, J. D., Menn'e, T. and Anderson, K. E. (2005). Methyl dibromo glutaronitrile contact allergy: effect of single versus repeated daily exposure. *Contact Dermatitis*, **52** (2), 88-92.
- Harvey, P. W. (2003). Parabens, Oestrogenicity, Underarm Cosmetics and Breast Cancer: A perspective on a Hypothesis. *J. Appl. Toxicol.*, **23** (5), 285-288.
- Helaleh, M. I. H., Tanaka, K., Taoda, H., Hu, W., Hasebe, K. and Haddad, P. R. (2002). Quantitative analysis of some carboxylic acids by Ion-Exclusion Chromatography with Atmospheric Pressure Chemical Ionization Mass Spectrometric Detection. *J. Chromatogr., A* **956**, 201-208.
- Huang, H. Y., Lai, Y. C., Chiu, C. W. and Yeh, J. M. (2003). Comparing Micellar electrokinetic Chromatography and microemulsion electrokinetic chromatography for the Analysis of Preservatives in pharmaceutical and Cosmetic Products, *J. Chromatogr., A* **993**, 153-164.
- Marengo, E., Gianotti, V., Angioi, S., Gennaro, M. C. (2004). Optimization by Experimental Design and Artificial Neural Networks of the ion-interaction Reversed-Phase Liquid Chromatographic Separation of Twenty Cosmetic Preservatives, *J. chromatogr. A* **1029**, 57-65.
- Mikami, E., Goto, T., Ohno, T., Matsumoto, H. and Nishida, M. (2002). Simultaneous Analysis of dehydroacetic acid, Benzoic acid, Sorbic acid and salicylic acid in Cosmetic products by Solid-Phase Extraction and High-Performance Liquid chromatography. *J. Pharm. Biomed. Anal.*, **28**, 261-267.
- Mitsui, T. (1997). *New Cosmetic Science*, Elsevier, 523.
- Novirc, M. and Haddad, P. R. (2006). Analyte-Stationary phase interactions in Ion-Exclusion Chromatography, *J. Chromatogr., A* **1118**, 19-28.
- Ohto, K. and Tanaka, K. (1991). Ion - Exclusion Chromatography of Carboxylic acids on Silica gel modified with Aluminum, *J. Chromatogr., A* **850**, 177-185.
- Paye, M., Barel, A. O. and Maibach, H. I. (2006). *Hand book of Cosmetic science and Technology*, 2<sup>nd</sup>. ed., CRC Press: Taylor & Francis Group, 1034.
- Prevot, A. B., Pramauro, E., Gallarate, M., Carlotti, M. E. and Orio, G. (2000). Determination of Micelle/water partition coefficients of cosmetic preservatives, Optimization of the Capillary Electrophoretic method. *Analytical chimica Acta*, **412** (1-2), 141-148.
- Rieger, M. M. (2001). Cosmetics, in Kirk-Othmer Encyclopedia of Chemical Technology 5<sup>th</sup> ed, Vol. 7, Page 820-865.
- Salvador, A. and Chisvert, A. (2007). Analysis of Cosmetic Products, Elsevier Sci. pub., 491.
- Yang, L., Liu, L., Olsen, B. A. and Nussbaum, M. A. (2002). The Determination of Oxalic acid, and Oxamide in a Drug substance by Ion - Exclusion Chromatography. *J. pharm. Biomed. Anal.*, **22**, 487-493.