Int. J. Bio-Inorg. Hybd. Nanomat., Vol. 2, No. 3 (2013), 429-435

International Journal of Bio-Inorganic Hybrid Nanomaterials

Determination of Potassium Sorbate and Sodium Benzoate in "Doogh" by HPLC and Comparison with Spectrophotometry

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Received: 4 June 2013; Accepted: 17 August 2013

ABSTRACT

There are various methods for the analysis of Potassium Sorbate and Sodium Benzoate in food products, but a rapid and reliable method for identification of these preservatives in Doogh (an Iranian traditional dairy drink) is a procedure, in which high performance liquid chromatography (HPLC) utilized and followed by UV diode array detection of the two preservatives. The aim of this case study was determination of Potassium Sorbate and Sodium Benzoate in Doogh, Samples consumed in the city of Tehran, Iran by HPLC in compare of Spectrophotometry method. In this study, 27 samples were analyzed. The HPLC determination of the preservatives was performed reversed-phase; C_{18} column and UV detected at 225 nm for sodium benzoate and 255 nm for potassium sorbate. In Spectrophotometry method, Sodium benzoate and Potassium sorbate were detected in 228 nm and 250 nm, respectively. The results of spectrophotometry in low concentrations, showed high values in comparison to what had been mention by HPLC. In high concentration, spectrophotometry showed the low value in comparison to HPLC. In conclusion, spectrophotometry could not detect and determine the Potassium sorbate and Sodium benzoate in a sample at the same time with reliable and exact results.

Keyword: Preservatives; Sodium benzoate; Potassium sorbate; Doogh; Detection; Reversed-phase.

1. INTRODUCTION

Chemical preservation has become an increasingly important practice in modern food technology with the increase in the production of processed foods. These preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods and to extend shelf

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life and quality of foods; they also prevent hazards for consumers due to the presence of microbial toxin or pathogenic microorganisms and economic losses due to spoilage. The most commonly used preservatives in many types of foods are Benzoic and Sorbic acids, Nitrate and Nitrite (Kucukcetin et al., 2008; Santini et al., 2009). Benzoic and Sorbic acids and their respective sodium, Potassium and Calcium salts are the most commonly used preservatives in food stuffs. They are generally used to inhibit Yeast and Mould growth and being also effective against a wide range of bacteria. These compounds are most active in foods with low pH value and ineffective at neutral pH value (Santini et al., 2009; Tfouni and Toledo, 2002). At acidic pH, where Sorbic and Benzoic acids and their relative salts are so effective, the lipophilic undissociated molecule is freely permeable across the cell membrane. Subsequently upon encountering the higher pH inside the cell, the molecule dissociates resulting in the release of changed anions and protons, which cannot cross the plasma membrane (Cigic et al. 2000). The importance of food preservatives for consumers has always been a health safety issue (Kucukcetin et al., 2008). Although Benzoic and Sorbic acids and their salts are generally recognized as safe (GRAS) but the development of allergic reactions to Benzoate in humans, such as Uriticaria, non-immunological contact Urticaria, metabolic acidosis, convulsions, hyperpnoea, weak clastogenic activity and asthma has been reported in some studies (Tfouni and Toledo, 2002; Wen et al., 2007; Santini et al., 2009; Lino and Pena, 2010). Further studies showed that Sorbic acid has a relatively low toxicity to humans, explained by the fact that it is rapidly metabolized by path ways similar to those of other fatty acids. In humans a few cases of idiosyncratic intolerance to Sorbic acid and Sorbate salts have been reported (non-immunological contact Urticaria and pseudo-allergy) (Santini et al., 2009; Tfouni and Toledo, 2002). According to aforementioned reasons, Sorbic acid and Sorbate salts (especially Potassium sorbate) have become the leading preservatives for a wide variety of food products (Santini et al., 2009). For these reasons, the uses of food additives in different countries have been limited by specific regulations. These preservatives are allowed by legislation but their use demands special care. Iran follows regulations of Institute of Standard and Industrial Research of Iran (ISIRI) on the safe use of food additives (Kucukcetin et al., 2008). The acceptable daily intake (ADI) values, determined by the joint FAO/WHO expert committee on food additives (JECFA) is 25 mg/Kg of body mass for Sorbic acid and Sorbates salts. According to ISIRI, Potassium sorbate and Sodium benzoate usage in dairy products is prohibited.

The analytical determination of these preservatives is not only important for quality assurance purposes but also for consumer interest and protection. The most common analytical method for the determination of Benzoic acid (BA) , Sorbic acid (SA) or Sodium benzoate (E211) and Potassium sorbate (E202) has been reversed-phase HPLC (Saad et al., 2005; Theron and Rykerslues, 2011), although other analytical methods such as Capillary Electrophoresis (Tang and Wu, 2007), Spectrophotometry (Hofer and Jenewein, 2000), Gas Chromatography-Mass Spectrometry (Galli and Barabas, 2004), Thermal description Gas Chromatography (Wang et al., 2006), HPLC (Ferreira et al., 2000; Pylpiw and Grether, 2000; Chen and Wang, 2001; Cigic et al., 2001; Tfouni and Toldo, 2002; Saad et al., 2005; Chinnici et al., 2005; Kucukcetin et al., 2008) and SPME-HPLC (Wen et al., 2007) have also been reported. Such a method become so important as there seem to be an increasing trend in using combination of preservatives in food stuff. Here we report on a simplified procedure of separation Sodium benzoate (E211) and Potassium sorbate (E202) mixture followed by HPLC. The method was applied to the analysis of these preservatives in 27 samples.

Doogh as a traditional drink in Iran with a high value of nutrients (same as fermented milk and Yoghurt) and remedial property has a numerous effects on human's healthiness such as: Improving lactose digestion; lowering serum cholesterol levels and stimulating the immune system (Olson and Aryana, 2007).

The purpose of this study was to investigate the

existence of Sodium benzoate, Potassium sorbate, Nitrate and Nitrite in strained yoghurt, kasar cheese, tulum cheese and ayran which were commercially available on the local markets in Antalya, Turkey in order to compare their levels to allowable ones.

2. MATERIALS AND METHODS

2.1. Sampling

The samples of Doogh with different brands were purchased from vendors in Tehran, Iran. A total of 27 samples were chosen as a representative of what a consumer would find in market-basket. Samples' sizes ranged from 500 mL to 1 Liter. Each sample was tested for the two preservative, Sodium benzoate and Potassium sorbate.

2.2. Standards and chemicals

HPLC grade acetonitrile and other reagents such as Ammonium acetate, Glacial acetic acid, Chloridric acid and Petroleum benzene (analytical grade) were purchased from Merck (Darmstradt, Germany). Commercial standards of Sodium benzoate and Potassium sorbate were used (Sigma chemical). Deionised water used for chromatography processing was obtained from a Millipore Milli-Q water purification system (ELGA, UHQ-II-MK3 and UK). For the filtration of sample prior to injection, a Millex HV0.45µm filter (Millipore) was used.

2.3. Mobile phase preparation

The mobile phase consists of 90% ammonium acetate buffer with 10% HPLC-Grade acetonitrile was prepared in two steps (Pylypiw and Grether, 2000):

Step1: Acetate Buffer: exactly 0.30 gr of ammonium acetate were dissolved in approximately 900 mL of deionised water in a 1 L beaker. Then approximately 0.5 mL of Glacial acetic acid added to this solution acid and the pH adjusted to 4.2. After that, buffer solution was transferred to 1 L volumetric flask, brought to the volume and filtered through a 47 mm × 0.45 µm nylon filters.

Step2: Completion: Exactly 900 mL of the Acetate buffer solution was mixed with 100 mL of HPLC grade Acetonitrile. This was mixed, degassed in degasser (ultrasonic clear sweep system) and used for sample dilution, (standard dilution and HPLC mobile).

2.4. Analysis of sodium benzoate and potassium sorbate

2.4.1. Sample preparation

2.4.1.1. HPLC method

The Liquid Chromatography Technique was used to determine the concentrations of Sodium benzoate and Potassium sorbate in the samples, following the procedures described by Pylypiw and Grether, 2000. Each of Doogh samples degassed in an ultrasonic bath and 1.0 mL of sample was diluted (1:10) with mobile phase. After that, the obtained aqueous phase solution transferred into dry falcon and put in centrifuge (biofuge primco 6000 Heraeus) for 6000 rpm/15 min. The clear aqueous solution on top of samples in falcons were caught with pipettes and filtered through a 25 mm × 0.45 µm nylon Acrodisk filter in order to remove particulate matter from the samples and prevent these particles from damaging the pumping or injection system or clogging the column. After that, aqueous phase solution was transferred to dry vials of HPLC and put on Auto sampler of HPLC for determination and detection.

2.4.1.2. Spectrophotometry method

Firstly, Doogh samples degassed in ultrasonic bath and then filtered with Watman paper No.42. , 5 mL of clear solution were caught and added 0.4 mL Chloridric acid 6 N and brought to 50 mL with petroleum Benzene and shake vigorously for 1 min. Sodium benzoate and Potassium sorbet detected in 228 nm and 250 nm, respectively.

2.4.2. Apparatus

2.4.2.1. Spectrophotometry conditions

Shimadzu, UV visible spectrophotometer Pharma Spec UV-17000, with UV detection at 228 nm for Sodium benzoate and 250 nm for Potassium sorbate were used. Correlation coefficient value was 0.994 for either preservative.

2.4.2.2. HPLC conditions

The chromatographic analysis was carried out in a high-performance liquid chromatography from Dionex, equipped as follows: ultimate 3000 pump, ASI-100 Automated sample injector, UVD 170U detector, thermostatted column compartment oven TCC-100. The HPLC operating mode was isocratic, the injection volume was 20 µL and the column temperature 20°C (room temperature). The chromatography column was a Supelcosil LC-18: 25 cm X 4.6 mm, 5 mm, Supelco, Bellefonte, PA, USA. Sample data collection was optimized to 30 min per sample with UV detection at wavelength of maximum absorption of the compounds, 225 nm for Sodium benzoate and 255 nm for Potassium sorbate, with the detector wavelength switched between analyses during each run. The optimal flow rate was determined 0.8 mL/min. Correlation coefficients value was 0.996 for either preservative.

2.4.3. Preparation of the standard curve

The External Standard Plot method was used. Duplicate injections of 20 µL Sodium benzoate and Potassium sorbate standard solutions were done to make linear regression lines (peak area versus concentration). The peaks were identified based on the retention time. The standard curves were obtained from five points for both of Sodium benzoate and Potassium sorbate. Concentrations values were 5, 10, 20 and 40 mg/L.

2.4.4. Recovery study

In order to verify the accuracy and precision of the analytical procedure, the recovery studies were carried out. The recovery of Sodium benzoate and Potassium sorbate added to the samples free of the two preservatives. Samples of Doogh were analyzed before and after addition of 100 and 200 mg of sodium benzoate and potassium sorbate to 100 mL of the samples.

3. RESULTS AND DISCUSSION

The Recent research which has been done in accordance to the legal obligations of preservatives usage in Doogh, legislated by authorized organizations, shows that HPLC method is more applicable in compression to the other ones. In this case study, HPLC method compared with spectrophotometry method's results. The analytical method used for extraction of Sodium Benzoate and Potassium Sorbate in samples was based on (Pylypiw and Greyher, 2000). Spiked samples were chosen as a prototype to validate this procedure. Recovery for sodium benzoate was 83-96% and 82-93% for potassium sorbate. The correlation coefficients of Sodium Benzoate and Potassium Sorbate were 0.9968 for either preservative.

In HPLC method, values found in the separation and the resolution of the peak, indicate that the analytical method proposed in this work completely separates the analytes. The approximate retention time was 9.80 min for Sodium benzoate and 26.50 min for Potassium sorbate. The limit of detection (LOD) for Sodium benzoate and Potassium sorbate were 0.15 mg/kg and 0.24 mg/kg in the samples, respectively. The limit of quantitation (LOQ) for Sodium benzoate and Potassium sorbate were 0.5 mg/kg and 0.8 mg/kg in the samples, respectively. Recoveries ranged from 93.1-96.3% and 92.9-99.7% respectively. The mean regression equations for concentrations of Sodium benzoate and Potassium sorbate versus arbitrary units of peak area were $Y= 48.92 X + 34.51$ (Y represents peak area, X represents concentration in mg/L) and $Y= 134.04 X + 11.30$, respectively. The correlation coefficients for standard curves of Sodium benzoate and Potassium sorbate were 0.9993 and 0.9988, respectively. Table 1 shows mean concentrations (mg/kg) of Sodium benzoate and Potassium sorbate in Doogh samples, whereas the typical chromatogram of standard of Sodium benzoate and Potassium sorbate are shown in Figure 1.

100% of Doogh samples contained Sodium benzoate in the range of 18.3-2345.16 mg/kg, which are not acceptable according to Institute of Standard and Industrial Research of Iran (ISIRI). 25.92% of samples contained concentrations of Potassium sorbate between 0~ 4961.3 mg/kg, which was not in compliance with the ISIRI

Table 1: *Concentrations (mg/kg) of sodium benzoate and potassium sorbate in Doogh.*

Method	Sodium benzoate		Potassium sorbate	
	Mean	Range	Mean	Range
HPLC	12.008	53.71	13.07	54.07
Spectrophotometry	195.893	1270.52	198.8	4961.3

legislations. Only 25.9% of Doogh samples contained both of Sodium benzoate and Potassium sorbate.

Our results were complying with previous studies. Results of HPLC were more exact in compression to spectrophotometry's results. Results of some samples in HPLC and spectrophotometry were so different. Achieved Results of spectrophotometry were varying, especially in samples which were spiked with two preservatives at same time.

Statistical analysis and spearman post hoc test was used to evaluate the correlation. A significant correlation was observed ($P<0.05$). Statistical calculation showed that in medium concentrations, there were overlap between HPLC and spectrophotometry's results and determined values became closer to each other. There were huge difference in high value and low value. The results of spectrophotometry in low concentration, showed high values in comparison to what had been mention by HPLC and it means that in low concentration of potassium sorbate, there were lots of differences in results of spectrophotometry. In high concentration, spectrophotometry showed low value in comparison to HPLC. In conclusion, spectrophotometry could not detect and determined the Potassium sorbate and Sodium benzoate in a sample at the same time and also does not have

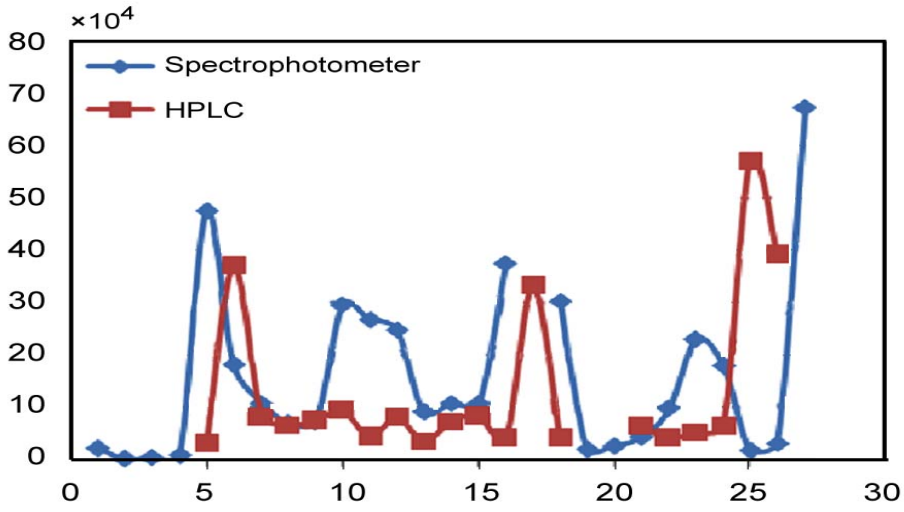
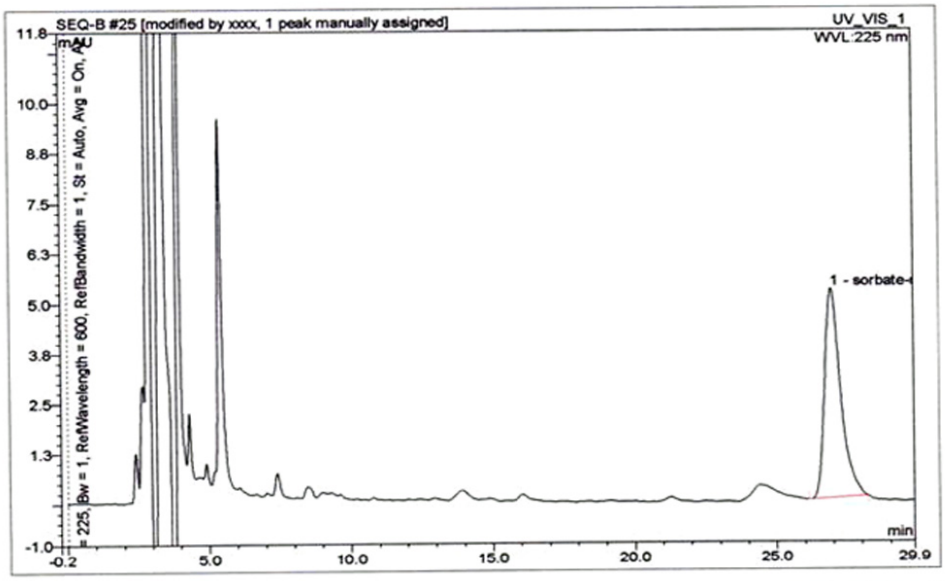
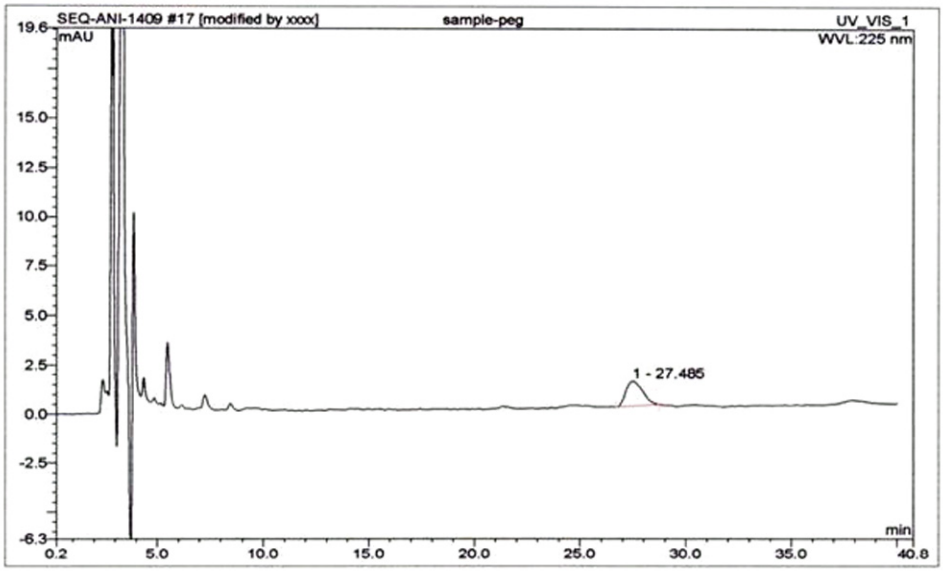


Figure 1: *Potassium sorbate's results comparison in samples. The x axis represents the number of sample; y axis represents the measure (maybe ppm or mg/dl).*



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	26.95	sorbate-sample	5.228	3.437	100.00	n.a.	BMB*^
Total:			5.228	3.437	100.00	0.000	

Figure 2: *eak of sodium benzoate achieved from HPLC. The x axis represents Time (min); The y axis represents Reflect Wavelength (nm).*



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	27.49	n.a.	1.247	1.157	100.00	n.a.	BMB*
Total:			1.247	1.157	100.00	0.000	

Figure 3: *Peak of sodium benzoate achieved from HPLC. The x axis represents Time (min); The y axis represents Reflect Wavelength (nm).*

accurate results because potassium sorbate and sodium benzoate peaks over lapped. For this reason, in a Doogh sample with mixed of these two preservatives, HPLC must be used. In flavored Doogh, essence and flavors caused difficulties in detection and determination.

4. CONCLUSIONS

Many report's methods use complicated and labor-intensive pre-treatment procedures such as Steam Distillation Multiple Steps and Solid-Phase Extractions. Comparing to the previous methods (Tfouni and Toledo, 2002), the present analytical method simplifies the analysis considerably, reduces its cost and time also encompasses higher level of sensitivity. HPLC method is preferred one in using to quantitative determination of Sodium Benzoate and Potassium Sorbate in Doogh.

The extracted information about general detections of Sodium Benzoate and Potassium Sorbate in most samples shows that they are commonly used as a preservative in Doogh. The Sodium Benzoate and Potassium Sorbate usage in Doogh is prohibited by the Institute of Standard and Industrial Research of Iran (ISIRI). Therefore, using of Sorbate and Benzoate should be regulated and on the other hand more cooperation between producers, processors and the regional administration seems to be essential.

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