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Analytical Methods

HPLC and LC–MS/MS methods for determination of sodium benzoate and potassium sorbate in food and beverages: Performances of local accredited laboratories *via* proficiency tests in Turkey

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ABSTRACT

High Performance Liquid Chromatography LC–UV and LC–MS/MS methods were developed and validated for quantitative analyses of sodium benzoate and potassium sorbate in foods and beverages. HPLC–UV and LC–MS/MS methods were compared for quantitative analyses of sodium benzoate and potassium sorbate in a representative ketchup sample. Optimisation of the methods enabled the chromatographic separation of the analytes in less than 4 min. A correlation coefficient of 0.999 was achieved over the measured calibration range for both compounds and methods (HPLC and LC–MS/MS). The uncertainty values of sodium benzoate and potassium sorbate were found as 0.199 and 0.150 mg/L by HPLC and 0.072 and 0.044 mg/L by LC–MS/MS, respectively.

Proficiency testing performance of Turkish accredited laboratories between the years 2005 and 2013 was evaluated and reported herein. The aim of the proficiency testing scheme was to evaluate the performance of the laboratories, analysing benzoate and sorbate in tomato ketchup.

1. Introduction

Food additives, having antimicrobial, antioxidant and buffer properties are used as preservatives in various foods to prevent their decay. Sodium benzoate and potassium sorbate are widely used to preserve processed foods such as fruit juice, soda, soy sauce, ketchup etc. These additives inhibit mould growth, prevent spoilage and preserve freshness of the products (Berger & Berger, 2013; Code of Federal Regulations., 1999; Mota, Ferreira, Cunha, Beatriz, & Oliveira, 2003; Pylypiw & Grether, 2000).

Although these preservatives are legally used in foods, they can be harmful if the uptake by a body is higher than the permitted limits (Kuprovskyte, Pranaityte, & Padarauskas, 2002; Mazdeh et al., 2013), which cause allergic effects, such as urticaria, non-immunological contact urticaria and asthma (Code of Federal Regulations, 1999; Tfouni & Toledo, 2002). Some cases of idiosyncratic intolerance to sorbic acid have been reported (Code of Federal Regulations, 1999; Hannuksela & Haahtela, 1987; Juhlin, Michaelsson, & Zetterstrom, 1972). Thus, several regulations limiting their use have been put into force. Concerning the use of benzoate and sorbate in processed food, the FDA allowed their maximum limit as 0.1% and 0.1–0.2%, respectively (Jones, 1992), as well as maximum levels of benzoate and sorbate as 200 mg/L to 2000 mg/L for different processed foods by European Regulation 92/2/EC.

As a result of regulations and symptoms of preservatives, analysis of food additives is an important issue for the food industry and regulatory authorities (Dzieciol, Wodnicka, & Huzar, 2010; Taylor et al., 2004). Measurement methods are essential for quality assurance efforts and are of assistance in the assessment of consumer intake levels for specific additives. Although, the AOAC 983.16 has been used for many years for the analysis of benzoate and sorbate, it is a time consuming method, in which the time required for the analysis of a single sample is more than 2.5 h (AOAC Official Method, 1983). Several studies have been reported

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on benzoate and sorbate determination, using spectrophotometry, gas chromatography, HPLC (Archer, 1980a, 1980b; Code of Federal Regulations, 1999; Gagliardi, DeOrsi, Manna, & Tonelli, 1997; Tfouni & Toledo, 2002), TLC (Vetter, 2000) and SPE (Lawn, Thompson, & Walker, 1997) systems. An HPLC method, comprising a C18 column (Nova-Pak 30 cm × 3.9 mm id, 4 μm particle size), PDA detector and a mixture of 81% water; 17% acetonitrile and 2% ammonium acetate buffer as eluent at pH 4.2 was reported to reduce the analysis time to15 min (Juhlin et al., 1972). An even more rapid HPLC method was reported by Pylypiw et al., which achieved a chromatographic separation in less than 11 min using an LC18 column (Supelcosil 25 cm × 4.6 mm id, 5 μm particle size) with PDA detector and a mixture of 90% ammonium acetate buffer and 10% acetonitrile as eluent at pH 4.2 (Code of Federal Regulations, 1999; Esfandiari et al., 2013; Kuprovskyte et al., 2002; Pylypiw & Grether, 2000; Mandrou & Bressolle, 1980; Mandrou et al., 1998; Pearce et al., 2004).

Food with nutritional benefits for human consumption can be stored for one or two years at ambient temperature through the addition of benzoic and/or sorbic acid or a combination of both (Rajchl et al., 2010). Ketchup is one these most commonly preserved foods. In addition, Ketchup possesses a more analytically challenging matrix than other non-alcoholic matrices. Thus, the method developed in this study was applied to the ketchup samples and we report herein a new and rapid LC–UV method for determination of sodium benzoate and potassium sorbate in ketchup and non alcoholic beverages. Moreover, in order to determine very low levels of these compounds in processed foods an LC–MS/MS method has been disclosed. Full method validation and uncertainty evaluation of the developed methods are reported. The results of preservative proficiency testing organised by TUBITAK UME over the last 10 years are reported herein.

2. Experimental

2.1. Chemicals

Sodium benzoate was obtained from Alfa Asar. Potassium sorbate, ammonium acetate and HPLC grade acetonitrile were purchased from Fluka Company, Germany. HPLC grade water was obtained using a Millipore Milli-Q water purification system. For the filtration of samples a Milex HV 0.45 μm filter (Millipore) was used. The purity assessment of potassium sorbate and sodium benzoate were also performed by Q NMR using the NIST 350b (benzoic acid) and UME CRM 1301 (chloramphenicol), respectively. Purity of sodium benzoate and potassium sorbate were determined as 99.7 ± 0.2% and 99.1 ± 0.3%, respectively, by Q NMR. 4-Hydroxy benzoic acid (^{13}C labelled) was used as internal standard (IS) for LC–MS/MS measurement.

2.2. Apparatus and chromatographic conditions

2.2.1. HPLC–UV method

Analyses were conducted, using an Agilent 1100 HPLC system, equipped with a quaternary pump, an auto-sampler with thermostat, a variable wavelength detector, a column and a sample thermostat and a Chem Station software.

Chromatographic separations were performed, using ACE-121-1504 C18 HPLC column (15 cm × 3.9 mm I.D., 5 μm). The mobile phase consisted of ammonium acetate buffer:acetonitrile (72:28, v/v). The acetate buffer was prepared with 0.30 g ammonium acetate in 900 mL HPLC grade water and glacial acetic acid to adjust the pH to 4.20. The ammonium acetate buffer solution was filtered through a 0.45 μm Millipore Millex-HV filter. The mobile phase was degassed for 10 min in an ultrasonic bath before use. The wavelengths selected for the determination of sodium benzoate and potassium sorbate were 225 and 255 nm, respectively. UV detection was carried out at two wavelengths simultaneously at a flow rate of 1 mL/min. The injection volume was 10 μL for all the samples. The column temperature was kept constant at 21 °C during the runs.

2.2.2. LC–MS/MS method

Analyses were carried out using a Zivak LC–MS/MS (Istanbul, Turkey) instrument equipped with a quaternary pump, an auto-sampler with thermostat, a mass detector, the column and sample thermostat systems and Zivak LC–MS/MS software.

Chromatographic separations were performed, using a phenomenex 150 × 2 mm, 4 μ HPLC column. The mobile phase consisted of ammonium acetate buffer:methanol (50:50, v/v). 1 L of 5 mM Ammonium acetate was prepared by dissolving ammonium acetate in deionised water and adjusting the pH to 4.2 using glacial acetic acid. Then, 50 mL of this buffer solution was diluted with deionised water to 1/4 (buffer:water, 1:4). The ammonium acetate buffer solution was filtered through a 0.45 μm Millipore Millex-HV filter. The mobile phase was degassed for 10 min in an ultrasonic bath before use. The samples were injected at a flow rate of 0.35 mL/min. The injection volume was 10 μL for all the samples. The column temperature was kept constant at 21 °C during the runs.

2.3. Preparation of standard solutions

Sodium benzoate (25 mg) and potassium sorbate (25 mg) were weighed and transferred to a 25 mL volumetric flask. The contents of the flask were initially dissolved in a portion of HPLC grade water then brought to final volume. Then, aliquots of primary solution were transferred into a 100 mL flask and diluted with mobile phase to prepare standard solutions with various concentrations, 0.1, 0.5, 1, 5, 10, 50 and 100 mg/L.

The standard solutions were prepared as 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 mg/L for the LC–MS/MS analysis.

2.4. Sample preparation

Different brands of orange juice, soda and ketchup were purchased. The juice and soda samples were centrifuged for 5 min and 1 mL aliquot of the supernatant was diluted to 10 mL with mobile phase, filtered through a 0.45 μm filter and injected to the column.

Ketchup (20 g) or beverage (pulpy) was weighed into a 100 mL capped jar using a calibrated balance. Then, 60 mL of the mobile phase added into the jar and vortexed in 5 min. The solution in the jar was poured into the 100 mL volumetric flask and the jar was rinsed twice with 10 mL of the mobile phase, after which the volumetric flask was filled to the mark with mobile phase. The content of the volumetric flask was shaken and mixed in an ultrasonic bath for 3 min. Then, 1 mL of the solution was transferred into a 10 mL volumetric flask and diluted to the mark with mobile phase. Finally, the sample solution was filtered through a 0.45 μm filter and injected into the HPLC or LC–MS/MS column for chromatographic separation.

3. Method validation

Of the matrices determined, the ketchup sample is composed of a more complex matrix. The ketchup sample was therefore selected as a representative product for the method validation of sodium benzoate and potassium sorbate determination in processed food.