



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.

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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 (Kuprovskyye, Pranaityte, & Padaruskas, 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 to 15 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 Millex 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 (¹³C 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 μm 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.

3.1. Detection limits, limits of quantification and linearity

3.1.1. HPLC–UV and LC–MS/MS methods

The limits of detections (LODs) were determined from the calculation of S/N ratios for both sodium benzoate and potassium sorbate. The LOD values of sodium benzoate and potassium sorbate were found to be 0.023 and 0.001 mg/L for both HPLC and LC–MS/MS methods, respectively. The determined LOD concentrations were reported using a signal to background noise (S/N) ratio of 3. The limits of quantification (LOQs) were estimated as 10 times the S/N for the above concentrations. The LOD and LOQ values of benzoate and sorbate for both HPLC and LC–MS/MS methods are given in Table 1. The sample chromatogram for LOD is given in S. Fig. 3 in supporting information.

Calibration curves for benzoate and sorbate were obtained by plotting peak areas versus the analyte concentrations in mg/L. Linearity was evaluated using linear regression analysis of a six-point linear plot consisting of three replicates per point and estimating the squared correlation coefficients, r^2 per analyte.

In the HPLC method, linear regression analyses for sodium benzoate and potassium sorbate were performed by plotting the peak area versus the concentrations in mg/L, giving the following equations for sodium benzoate and potassium sorbate respectively; $y = 38.581x + 2.22$ and $y = 97.266x + 2.41$. The equations were obtained as $y = 1.3732x - 0.031$ for benzoate and $y = 0.5803x - 0.0313$ for sorbate in the LC–MS/MS method.

For both analytes, the relationship between peak area and concentration was found to be linear from 0.1 to 100 mg/L (for HPLC) and 0.01 to 100 mg/L (for LC–MS/MS) with typical correlation coefficients of $r^2 = 0.99999$.

3.2. Recovery, repeatability and intermediate precision

3.2.1. HPLC–UV and LC–MS/MS methods

Recovery studies for the HPLC–UV method were performed with spiked ketchup samples at three fortification levels covering lower, intermediate and high concentration ranges, 1, 10 and 100 mg/L with 3 replicate, respectively. Recovery studies for the LC–MS/MS method were performed with spiked ketchup samples at 5 mg/L concentration level. Replicate recovery tests were conducted on the same day and on different days with different concentrations. Similar recovery studies were also performed for orange juices and soda. For the ketchup samples, the recoveries were found as 90% (1.0 mg/L) for sodium benzoate and 89% (1.0 mg/L) for potassium sorbate. For commercial orange juices and soda samples, higher recovery rates were attained. Sodium benzoate and potassium sorbate recoveries for orange juice and soda samples with 3 replicates were in the ranges of 95–96% and 93–94%, respectively. The recovery results for both benzoate and sorbate are given in S. Table 4 in supporting information.

For the precision study, six-ketchup samples fortified at three concentration levels (1, 10 and 100 mg/L) were tested on different days over five weeks. The repeatability (RSD) values for benzoate and sorbate, ranged between 0.1–1.7 and 0.1–0.6, respectively were obtained using the corresponding peak area of 6 replicate analyses performed for each sample.

Table 1

LOD and LOQ values of sodium benzoate and potassium sorbate in HPLC and LC–MS/MS systems.

	Benzoate (mg/L)		Sorbate (mg/L)	
	HPLC	LC–MS/MS	HPLC	LC–MS/MS
LOD	0.023	0.001	0.012	0.002
LOQ	0.077	0.003	0.040	0.008

For the reproducibility studies, 6 spiked ketchup samples were prepared and tested on different days over a period of 3 weeks, which displayed recoveries ranging between 89% and 91% for sodium benzoate and 88–90% for potassium sorbate. These results are comparable or even better than the methods reported in food and drinks (Berger & Berger, 2013).

The same methodology was applied for the LC–MS/MS method, for which recovery test was performed with spiked ketchup samples at 5 mg/L concentration level. The nine replicate of sample were carried out on the same day. The recovery rates of sodium benzoate and potassium sorbate were found to be 92% and 90%, respectively.

For the repeatability test, the RSD% values for both benzoate and sorbate were calculated to be 3.58 and 4.56, respectively, from the response factors of the samples.

3.3. Estimation of uncertainty sources

For the calculation of the uncertainty associated with the final results, initially, sources of standard uncertainties require to be identified and then measured (Eurachem/CITAC guide, 2012). Thus, it was considered that the concentrations of benzoate and sorbate were mainly affected by the following sources of uncertainties for both HPLC and LC–MS/MS methods:

- Standard uncertainty associated with the sample weighing.
- Standard uncertainty associated with the volumetric glassware used for preparing sample solution.
- Standard uncertainty associated with the preparation of the calibration standard solutions.
- Standard uncertainty associated with the interpolation of the sample reading in the calibration curve.
- Standard uncertainty associated with the recovery rate repeatability.

The equation for calculating concentrations of the benzoate and sorbate is given as,

$$C_{cd} = \frac{1000 \times m \times P}{V} = \dots\dots\dots \text{mg/L}$$

where C_{cd} : concentration of analyte in stock solution (mg/L), m : weight of analyte (mg), P : purity of analyte, V : volume of stock solution (mL), 1000: conversion factor.

3.3.1. Uncertainty contribution from the sample weight (U_w)

Weighing was performed on an analytical balance having a sensitivity of 0.01 mg. The uncertainty was given in the calibration certificate of the balance as $\pm 0.1 + 1.59 \times 10^{-5} W$ at 95% confidence level. For 20 g sample, it was calculated as 1.003×10^{-4} g. Following the control measurements, the repeatability of the balance was found to be 0.02 mg (SD). As weighing process involves a difference (tare and sample weighing), the uncertainty from this source was considered twice.

3.3.2. Standard uncertainty associated with volumetric glassware used for preparing sample solution

The sample was dissolved in 100 ml flask and brought up to the volume, from which 1 ml of solution was transferred into a 10 ml volumetric flask and diluted to the volume for injection. Uncertainty values of volumetric glassware, 1 ml, 10 ml, and 100 ml, were given in their calibration certificates as ± 0.01 , ± 0.018 and ± 0.024 , respectively. Uncertainties for 1 and 10 ml flasks from weighing were not included as it was an experimental repeatability test. Temperature change in the laboratory was considered as $\pm 3^\circ\text{C}$, and the thermal expansion coefficient for water was $2.1 \times 10^{-4}^\circ\text{C}$.

The standard uncertainties coming from volume were calculated according to following formula:

$$U(V) = \sqrt{u_{k_{tv}}^2 + u_{s_{tv}}^2 + u_{t_{tv}}^2}$$

where, $u_{s_{tv}}$ = volume temperature change. thermal expansion coefficient/ $\sqrt{3}$, $u_{k_{tv}}$ = standard uncertainty from calibration of volumetric flask, $u_{t_{tv}}$ = standard uncertainty from repeatability of volumetric flask.

3.3.3. Standard uncertainty associated with the preparation of the calibration standard solutions,

Uncertainty from stock solution was found as $U_{\text{cstock}} = 2.78$, which was calculated as a combination of the uncertainties contributed by purity of standard, weighing process and volume measurement, using the following formula:

$$\left(\frac{u_{\text{Cstock}}}{C_{\text{stock}}}\right) = \sqrt{\left(\frac{u_p}{P}\right)^2 + \left(\frac{u_m}{m}\right)^2 + \left(\frac{u_v}{V}\right)^2}$$

where $\left(\frac{u_p}{P}\right)$: ratio of uncertainty from purity of standard material to its value, $\left(\frac{u_m}{m}\right)$: ratio of uncertainty from weighing of standard material to its value, $\left(\frac{u_v}{V}\right)$: ratio of uncertainty from volume of standard material to its value.

3.3.3.1. Uncertainty derived from purity stock solution uncertainties (U_{cstock}). The purity assessment of potassium sorbate and benzoic acid were determined by Q NMR, using the NIST 350b (benzoic acid) and UME CRM 1301 (chloramphenicol), respectively. Purity of sodium benzoate and potassium sorbate were determined to be $99.7 \pm 0.2\%$ and $99.1 \pm 0.3\%$ by Q NMR. 4-Hydroxy benzoic acid (^{13}C labelled) was used as an internal standard (IS) for LC–MS/MS measurement.

The uncertainty calculation of the standards, sodium benzoate and potassium sorbate, coming from their purity was conducted using the values ± 0.2 and ± 0.3 for sodium benzoate and potassium sorbate, respectively.

3.3.3.2. Uncertainty derived from weighing of standards. A sensitivity of 0.01 mg was applied for weighing chemical standards. Calibration uncertainty of the balance was $\pm 0.02 + 1.54 \times 10^{-5} \text{ W mg}$. Repeatability value of balance was found and as 0.02 mg (SD). As weighing was derived from the difference of tare and the sample, the calibration uncertainty was included twice.

3.3.3.3. Standard volume uncertainty. Volumetric flask (25 ml) was used for preparation of standard solution, having uncertainties certificate value of $\pm 0.03 \text{ ml}$ at 95% range. The laboratory temperature difference was 3 °C. Volume coefficient for water was $2.1 \times 10^{-4} / ^\circ\text{C}$. As flask was not part of the experimental repeatability value, it was not included in the uncertainty calculation.

$$U(V) = \sqrt{(u_{\text{cal}})^2 + (u_t)^2 + (u_T)^2}$$

3.3.4. Standard uncertainty associated with the interpolation of the sample reading in the calibration curve

3.3.4.1. HPLC method. The correlation coefficient, for both sodium benzoate and potassium sorbate, calculated from calibration curves, was 0.99999 for the linear range of 0.5–100 mg/L. In the equation of slope, the a and b values were found to be $a = 2.22$ and $b = 38.581388$ (benzoate) and $a = 2.41$ and $b = 97.266701$ (sorbate).

The uncertainties, calculated from the calibration curve, using the following equation, were 0.019 and 0.035 for benzoate and sorbate, respectively.

$$U(c_0) = \frac{S}{B_1} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{S_{xx}}}$$

where S : residual standard deviation from calibration curve, B_1 : from slope of calibration curve, p : number of samples, n : number of measurements for calibration points, c_0 : concentration of solution used in the measurements, \bar{c} : mean concentration of the calibration standards, S_{xx} : $(c_i - c_{\text{mean}})^2$, i : indice for calibration standard, c_i : sample reading in the calibration curve.

3.3.4.2. LC–MS/MS method. The correlation coefficients, for both sodium benzoate and potassium sorbate, were calculated from the calibration curves (using curve.exe) as 0.9999 for the linear range of 0.01–5 mg/L. In the equation of slope, the values for a and b were found to be; $a = -0.02169$ and $b = 1.370875$ (benzoate) and $a = -0.021950$ and $b = 0.577939$ (sorbate).

Uncertainty from the calibration curve was calculated as 0.029 and 0.030 for benzoate and sorbate, respectively, using the equation in Section 3.3.4.1.

3.3.5. Standard uncertainty associated with the recovery rate repeatability

3.3.5.1. HPLC method. Uncertainty of experimental repeatability was calculated from the recovery test results as RSD%. Pooled RSDs% for both benzoate and sorbate were found to be 0.84 and 0.11, respectively. Dividing these results by 100 gave the repeatability uncertainties of 0.0084 for benzoate and 0.0011 for sorbate.

Uncertainty from the recovery step was calculated, using ketchup samples fortified with sorbate and benzoate. The uncertainties derived from the recovery were calculated using the following equation.

$$U(R_m) = R_m \sqrt{\left(\frac{u(C_{\text{obs.}})}{C_{\text{obs.}}}\right)^2 + \left(\frac{u(C_{\text{cert.}})}{C_{\text{cert.}}}\right)^2}$$

where $C_{\text{obs.}}$ and $C_{\text{cert.}}$ are the experimental and theoretical (certificate) concentrations, respectively. The recoveries for both sodium benzoate and potassium sorbate were calculated as 90% and 89%, respectively. The percentage recovery value will be included in the calculation to obtain the final corrected recovery results.

The combined and overall uncertainties for this method were calculated following the recommendation of Eurachem/CITAC (Eurachem/CITAC guide, 2012) and uncertainty results were given in S. Table 5 in supporting information.

3.3.5.2. LC–MS/MS method. Repeatability values for uncertainty calculations are given as RSD%. The uncertainty values from repeatability calculation were found to be 0.0020 and 0.0030 for benzoate and sorbate, respectively.

The combined and overall uncertainties for this method were calculated following the recommendation of Eurachem/CITAC and uncertainty results were given in S. Table 6 in supporting information.

3.4. Application of the method

The both HPLC and LC–MS/MS methods were applied successfully for routine analyses of sodium benzoate and potassium sorbate in foods and beverages. They were found to be practical, time saving methods and well suited for the Food Control laboratories to perform large amount of analyses in a limited time of period.

Typical chromatograms obtained from analysis of a ketchup sample using HPLC–UV and LC–MS/MS method are presented in

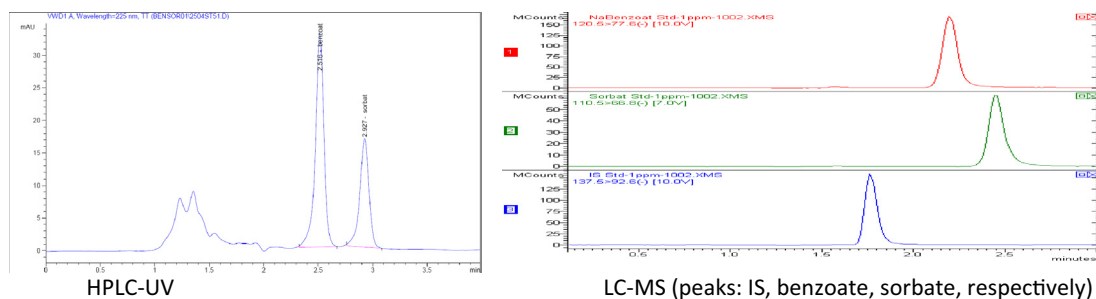


Fig. 1. Sample chromatograms for HPLC-UV and LC-MS/MS.

Table 2

Comparison of test results and z-scores of participants between the years 2005 and 2012.

Lab. No.	Benzoate (2005)			Sorbate (2005)		
	Result	s	z-Score	Result	s	z-Score
	Reference value = 317.16 mg/kg Target standard deviation = 21.33 mg/kg			Reference value = 119.38 mg/kg Target standard deviation = 9.30 mg/kg		
02	325.89	7.40	0.41	123.70	3.28	0.46
03	253.98	2.04	-2.96	100.48	5.35	-2.03
04	320.76		0.17	129.64		1.10
05	252.5		-3.03	81.3		-4.09
06	290.79	14.25	-1.24	126.70	3.66	0.79
07	340	4	1.07	130	2	1.14
08	262.6		-2.56	98.5		-2.25
09	279	2.68	-1.79	110.5	1.14	-0.95
	Benzoate (2012)			Sorbate (2012)		
	Reference value = 119.32 mg/kg Standard deviation = 9.28 mg/kg			Reference value = 65.51 mg/kg Standard deviation = 5.57 mg/kg		
	Result	s	z-Score	Result	s	z-Score
01	92.04	1.37	-2.94	56.76	0.150	-1.57
02	106	1.26	-1.44	65.4	0.45	-0.02
03	113.5	-	-0.63	65.5	-	0.00
04	160.8	-	4.47	21.1	-	-7.97
05	4.74	0.13	-12.35	2.71	0.032	-11.27
06	105.230	4.32	-1.52	71.397	1.77	1.06
07	110.0	0.511	-1.00	66.14	0.362	0.11
UME	119.32	0.38	0.00	65.51	0.683	0.00

Fig. 1, which indicate that benzoate and sorbate were eluted in less than 4 min with well-resolved chromatographic peaks.

4. Proficiency testing in Turkey on benzoate and sorbate

The aim of local laboratory participation in this proficiency testing scheme is to allow laboratories to assess their ability to determine preservatives such as sorbate and benzoate in food matrices, and conservatively to improve their analytical performance for this type of analysis.

In this study, ketchup was selected as the food sample for the determination of sodium benzoate and potassium sorbate preservative content. About 1500 g of tomato ketchup sample (free from additives) was prepared by adding known amount of sodium benzoate and potassium sorbate, and homogenised. Then, 100 g of sample with capped jar stored at 4 °C before delivering was packed and delivered to participants by cargo to analyse benzoate and sorbate in the sample and participants sent back the reports to TÜBİTAK UME for the calculations of z score and mean values. Homogenisation and short term stability experiments were conducted according to ISO 17043 (ISO/IEC 17043, 2013). The homogeneity results and number of participant attending proficiency test due to the years were illustrated in Figs. 4 and 5, respectively in supporting information.

Participant used the following methods in this study:

AOAC 20.021, AOAC 983.16: 2002, AOAC Vol. 76 No.: 2: 1993, NMKL No.: 124.2:1997

The reference value of prepared sample was used in calculation of the z-score. Target standard deviation, calculated using Horwitz equation is given below.

Horwitz Equation:

$$\%RSD_R = 2^{(1-0.5 \log C)}$$

where %RSD_R: relative standard deviation in the same reproducibility conditions, [(s/X) × 100], C: concentration ratio (100 g/100 g = 1; 1 mg/kg (ppm) = 10⁻⁶).

The z scores was calculated to the following formula (Code of Federal Regulations, 1999):

$$z = \frac{x - X}{s}$$

where X: assigned value, x: participant's result, s: target standard deviation.

The comments on z-scores are as follows:

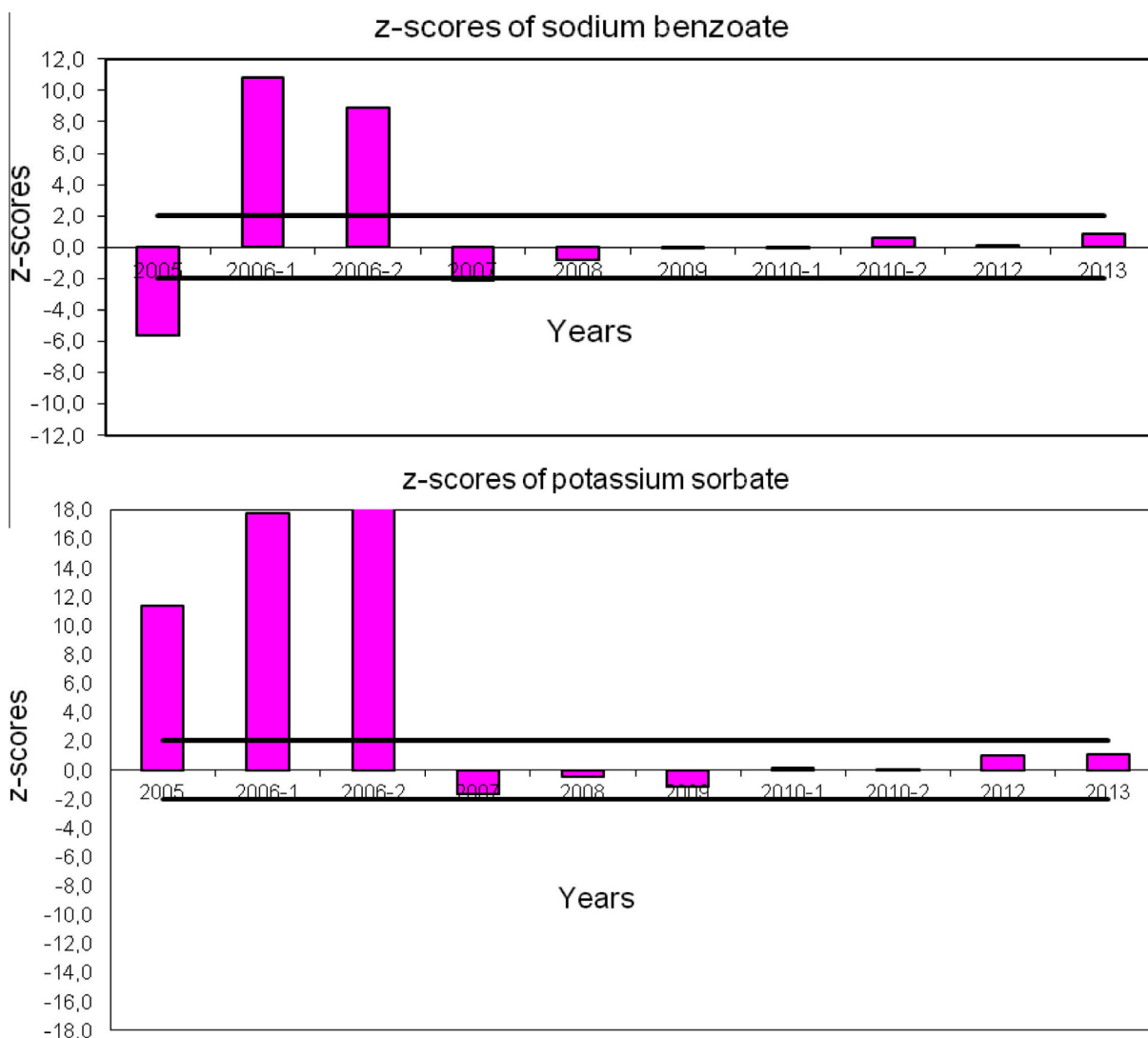
$|z| \leq 2$ "Satisfactory", $2 < |z| < 3$ "Questionable", $|z| \geq 3$ "Unsatisfactory"

Although proficiency tests are organised by TÜBİTAK UME every year, in this study, only the results of sodium benzoate and potassium sorbate in ketchup obtained between the years 2005 and 2013 were evaluated. The z-scores for both benzoate and sorbate (2005 and 2012) were given Table 2. All the z-scores for both benzoate and sorbate between the years 2005 and 2013 were summarised in Table 3 and illustrated with Fig. 2.

Table 3

Proficiency test results of benzoate and sorbate in ketchup sample obtained by Food Control Laboratory between the years 2005 and 2013.

Benzoate			Sorbate			Years
Ref. value (mg/kg)	Result (mg/kg)	z-Score	Ref. value (mg/kg)	Result (mg/kg)	z-Score	
344.99	216.00	-5.63	147.78	275.00	11.41	2005-1
167.00	296.64	10.80	96.00	202.50	17.75	2006-1
99.00	169.96	8.95	72.00	189.42	19.25	2006-2
112.00	89.50	-2.12	63.86	54.85	-1.65	2007
58.86	54.65	-0.83	41.74	41.08	-0.49	2008-1
76.31	76.12	-0.08	46.34	43.79	-1.19	2009-1
150.62	150.20	-0.04	67.59	68.63	0.18	2010-1
81.90	86.28	0.64	58.50	58.84	0.07	2010-2
122.75	123.32	0.10	66.07	66.81	0.99	2012-1
1063.80	1114.34	0.85	59.70	65.38	1.10	2013-1

**Fig. 2.** Distribution of z-scores of benzoate and sorbate obtained from Province Control Laboratory between the years 2005 and 2013.

5. Discussion

Unlike previous reports on HPLC methods, in this study, shorter column for separation was used. A 15 cm C18 HPLC column with a diameter of 3.9 mm provided faster chromatographic separation with sodium benzoate and potassium sorbate, using much less mobile phase solvent. Optimisation of mobile phase (72:28% ammonium acetate: acetonitrile) and flow rate (1 mL/min) allowed

faster elution of analytes with a good chromatographic resolution, ($R_s = 0.28$). The representative chromatograms for standard mixture and ketchup sample displayed a very good base line separation and symmetrical peaks with the present method (Fig. 1).

Although there are slight differences between HPLC–UV and LC–MS/MS methods in terms of sample preparation, much lower LOD and LOQ values (using neat calibration standards) can be

reached by LC–MS/MS compare with HPLC method. As indicated by the LOD values, the content of benzoate and sorbate in much less concentrations (approx. 20 times) can be detected through LC–MS/MS method than that HPLC method.

When the proficiency testing results in 2005 are considered, a laboratory having the high $|z|$ scores could be observed, which are 3.03 for sodium benzoate and 4.09 for potassium sorbate in ketchup sample. This result corresponds 12.5% of all participants. Although the percentage of the participants, having $|z|$ scores higher than 3, looks high, it could be reasonable due to the small number of participants. Differences in the test results could also be due to the different methods applied. In 2012, the results of $|z|$ scores of the participants having higher than 3 were increased twice (25%) compared to the year, 2005.

Participants of the proficiency testing improved their ability of measurements by attending such tests. The performance pattern of a selected laboratory could be found in Table 3, which are the results and the z-scores of the laboratory participated to proficiency tests between the years 2005–2013. The years between 2005 and 2007, the z scores of the laboratories for both benzoate and sorbate tests were higher and out of the range. But, after 2007, the z-score values released from the same laboratory were in the limits. Fig. 2 shows z-scores of a laboratory by years, which indicate that proficiency tests have very good external quality control tools for the accreditation body and improves the quality of measurements in the accredited laboratories. The study of proficiency testing was assessed free from method development and uncertainty calculations. There are no uncertainty calculations of the proficiency testing results.

6. Conclusions

HPLC and LC–MS/MS methods were further developed and validated for the simultaneous determination of sodium benzoate and potassium sorbate in foods and beverages. While their detection was achieved at 225 (sodium benzoate) and 255 (potassium sorbate), using ACEC18 column in HPLC method, in LC–MS/MS, a mass detector was used and the run time of a sample was reduced less than 3 min. As expected, the LC–MS/MS method is faster, simple, sensitive, precise and accurate than HPLC method. Moreover, it is an economic method which consumes much less solvent for each chromatographic run. Therefore, it is convenient for the laboratories performing routine determination of sodium benzoate and potassium sorbate in foods and beverages. The most important difference between LC–MS/MS and HPLC methods is lower LOD and LOQ values. Detection of lower concentrations of benzoate and sorbate in foods and beverages by LC–MS/MS method could be possible compare with HPLC method. To the best of our knowledge, it is the fastest method reported so far for the determination of sodium benzoate and potassium sorbate in foods. Total analysis time, including sample preparation and chromatographic run, is only 15 min for ketchup and 10 min for the beverage samples.

Proficiency testing showed the ability of the measurements of laboratories attended the comparison. On the other hand, the number of participants is important for evaluating the results in a proper statistical logical. Laboratories participated more to the proficiency testing improved their measurement abilities. This idea can be supported by the results of z-scores of one laboratory between the years 2005–2013. Although the z-scores of the laboratory were very high and out of limits for both benzoate and sorbate analyses in the first three years, the z-scores have been in the limits in the following years. This demonstrates that there is a significant improvement in z-scores for both benzoate and sorbate analyses by years in Turkey.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.11.094>.

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