



Comparison of validation results of HPLC-UV/PDA and LC-MS/MS methods for the determination of sorbates and benzoates in food

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ABSTRACT

Sorbates are GRAS (Generally recognized as safe) additives, while benzoates are more of concern and can be converted to benzene through decarboxylation. Preservatives like sodium benzoate and potassium sorbate may contribute to intestinal dysbiosis and inflammatory bowel diseases by altering gut microbiota. Animal studies show teratogenic and neurotoxic effects on zebrafish embryos and chromosome aberrations in human lymphocytes. The health benefits of sorbates and benzoates have been explored in scientific literature, particularly in the context of their therapeutic potential beyond their antimicrobial properties. Sodium benzoate, a metabolite of cinnamon, upregulates neurotrophic factors, suggesting potential benefits in treating neurodegenerative disorders such as Alzheimer's and Parkinson's disease. The clinical administration of sodium benzoate is well-established for treating urea cycle disorders, where it helps manage hyperammonaemia by facilitating ammonia excretion. The aim of this study was comparing suitability of two methods, HPLC-UV/PDA and LC-MS/MS. High-Performance Liquid Chromatography (HPLC) is the most widely used method due to its high sensitivity, specificity and ability to separate and quantify sorbates (e.g., sorbic acid) and benzoates (e.g., benzoic acid) simultaneously. For trace-level detection, complex matrices, or multi-residue methods, LC-MS/MS is superior due to its unmatched sensitivity and selectivity, albeit at higher cost and complexity. Comparison, using paired sample t-test, using T-distribution (two-tailed), of the sorbate content of 36 foodstuffs analysed by the two methods, showed a non-significant small difference between the results of HPLC (mean = 358 mg/kg, SD = 378.5) and results of LC/MS-MS (mean = 335.6 mg/kg, SD = 336.6), $t(35) = 1.9$, $p = 0.071$.

1. Introduction

Sorbates (e.g., potassium sorbate) and benzoates (e.g., sodium benzoate) are food preservatives used to inhibit mould, yeast, and bacterial growth. Common foods that are preserved by adding sorbates are cheese, yogurt, dried fruits, baked goods, beverages, soft drinks, fruit juices, sauces, and pickled products (Choyam *et al.*, 2019). Sorbates are

generally recognized as safe by regulatory agencies like the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) when used within acceptable daily intake (ADI) limits (Chen *et al.*, 2020). Both sorbates and benzoates are rapidly metabolized and excreted, minimizing accumulation in the body. Adverse effects are rare at typical dietary levels but can occur in sensitive populations

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or with excessive consumption (Piper and Piper, 2017). Individuals with compromised liver function or specific allergies may be at higher risk. Regulatory bodies (FDA, EFSA) monitor usage levels to ensure safety. Safe daily intake (ADI) for sorbates is considered to be 11 mg/kg body weight (EFSA, 2019; FAO/WHO, 2021). Sensitive populations, people with allergies, asthma, or histamine intolerance may be more prone to adverse reactions (Abily-Donval *et al.*, 2015a). Cumulative exposure risk may increase with frequent consumption of multiple preserved foods (Udovicki *et al.*, 2024). The aim of this study was to compare suitability of two methods for determination of sorbates in food. Samples of different food were analysed by high performance liquid chromatography HPLC UV-PDA and liquid chromatography with tandem mass spectrometry (LC/MS-MS) methods.

1.1 Sorbates as food additives

Potassium sorbate ($C_6H_7KO_2$) and sorbic acid ($C_6H_8O_2$) are two forms of this preservative in food. The metabolism pathway of sorbic acid starts in the gastrointestinal tract and ends metabolized primarily in the liver. It undergoes β -oxidation, similar to fatty acids, breaking down into carbon dioxide and water. The process involves conjugation with coenzyme A, followed by oxidation to smaller molecules excreted via urine or exhaled as CO_2 (Chen *et al.*, 2020). Minor amounts may be conjugated with glycine to form urocanic acid derivatives, excreted in urine. Potential adverse effects of sorbates if used in high doses can cause several issues. Potassium sorbate can contribute to intestinal dysbiosis and inflammatory bowel diseases (IBD) by altering gut microbiota (Jarmakiewicz-Czaja *et al.*, 2022). Allergic reaction, including skin irritation, urticaria, or contact dermatitis in sensitive individuals and gastrointestinal issues, i.e., nausea, diarrhoea, or stomach discomfort in rare cases (Trasande *et al.*, 2018). High doses can cause glycine depletion, impair memory, or increase attention deficit hyperactivity disorder (ADHD) symptoms in sensitive individuals (Abily-Donval *et al.*, 2015b). Animal studies show teratogenic and neurotoxic effects on zebrafish embryos and chromosome aberrations in human lymphocytes.

1.2 Benzoates as food additives

Benzoates are food additives in form of acid and salts with chemical formula sodium benzo-

ate ($C_7H_5NaO_2$) and benzoic acid ($C_7H_6O_2$). Sodium benzoate can also alter the gut microbiota and contribute to health issues (IBD), much like sorbates (Jarmakiewicz-Czaja *et al.*, 2022). The benzoate metabolism pathway starts with absorption in the stomach and small intestine. In the liver, benzoate is conjugated with glycine to form hippuric acid (benzoyl glycine), catalyzed by acyl-CoA synthetase and glycine N-acyltransferase. Hippuric acid is excreted in urine, typically within 6–12 hours (Ogbadu, 2014).

1.3 Analytical methods for preservatives in food

Determination of sorbates (and benzoates) involves various analytical methods to ensure accurate detection and quantification. HPLC is the most widely used method due to its high sensitivity, specificity, and ability to separate and quantify sorbates (e.g., sorbic acid) and benzoates (e.g., benzoic acid) simultaneously (Pylypiw and Grether, 2000). Gas chromatography with flame ionization detection (GC-FID) is used for quantification of volatile derivatives of sorbates and benzoates, often after derivatization (e.g., esterification to increase volatility). Gas chromatography-mass spectrometry (GC-MS) is used for confirmation and trace-level detection (Tungkijanansin *et al.*, 2020). Capillary electrophoresis (CE) separates charged molecules like benzoates and sorbates based on their electrophoretic mobility in a capillary tube. Detectors used are UV or diode array detector (DAD), typically at 200–230 nm (Mesquita *et al.*, 2018). Due to its lower sensitivity, CE is less common than HPLC, but since organic solvents are not used in CE, it is a green chemistry method. Spectrophotometric UV-Vis methods measure absorbance of sorbates and benzoates at specific wavelengths (e.g., 250 nm for sorbic acid, 225 nm for benzoic acid). These methods are cost effective, much like CE, but have weak specificity. LC-MS/MS combines HPLC with mass spectrometry for highly sensitive and selective detection, especially in complex matrices like dairy or meat products (Gören *et al.*, 2015). This method has advantage for trace level detection, but the disadvantage of being expensive because of the required equipment and maintenance. A reversed-phase C18 column is commonly used due to its ability to separate polar compounds like sorbates and benzoates. The hydrophobic nature of the C18 stationary phase effectively retains and separates these acidic preservatives in food matrices. Thin-layer chromatography (TLC) is sometimes

used for qualitative or semi-quantitative analysis of sorbates and benzoates (Khan et al., 1994). Ion chromatography (IC) separates sorbates and benzoates based on ionic interactions. It is a practical method for aqueous matrices like beverages (Iammarino and Taranto, 2013). Regulatory standards set by organizations like the FDA, EFSA, or Codex Alimentarius often specify HPLC or LC-MS/MS, while for official testing, HPLC with UV or MS detection is the gold standard for routine analysis of sorbates and benzoates in food due to its versatility and reliability. GC and CE are alternatives for specific applications, while spectrophotometry and TLC serve as cost-effective screening tools.

2. Materials and methods

Thirty-six samples of milk products (soft cheese products, milk spreads, seasoned milk fermented products), bakery products (soft cookies, fruit coated pies), fruit and vegetable products (spreads, sauces, brined fruit), fish products and pasteurized egg products were analysed for sorbates using both HPLC and LC-MS methods. Student’s t-test was used for comparison of results for two different methods (XLSTAT 2025).

2.1. LC-MS/MS method for sorbate and benzoate quantification

Sample preparation was matrix dependent, so for samples with certain protein amounts, simple protein precipitation with Carezz I and II solutions was used, or liquid-liquid extraction with methanol or acetonitrile to extract sorbates and benzoates, respectively, from food matrices like beverages, jams, and yogurt (Hatton and Warr, 2024). The temperature range 30–40°C ensured consistent retention times and peak shapes because it minimizes viscosity of the mobile phase and improves reproducibility. The column used was C18 100 Å, 50 × 2.1 mm; 2.6 µm. The mobile phase consisted of: A: 0.1% formic acid in water (to enhance ionization in MS) and B: Methanol with 0.1% formic acid. The gradient started with 10% of organic phase, reaching 90% at 10–12 minutes, and re-equilibrating to 15 minutes of total run. Formic acid enhanced negative ion formation for MS detection, and the gradient ensured separation of sorbates, benzoates, and potential matrix interferences (Gören et al., 2015). Electrospray Ionization (ESI) was in negative ion mode. Multiple reaction monitoring (MRM) transitions were with a collision energy of 10eV.

Table 1. Transitions of sodium benzoate and potassium sorbate for Shimadzu LCMS-8045

Component	Precursor ion: m/z	Product ion: m/z	Product ion: m/z
Sodium Benzoate	121.0	77.0	93.0
Potassium Sorbate	111.10	67.0	83.0

These transitions are specific and sensitive for quantification and confirmation. Thirty-six samples of different food with added preservatives were analysed and content of sorbates was quantified, using both HPLC-UV/PDA and LC-MS/MS.

1.2. HPLC- UV/PDA method for sorbate and benzoate quantification

The Waters Alliance HPLC 2695 model is a robust, modular system designed for reliable and reproducible separation, while the 2996 PDA detector provides UV-Vis spectral data across a wide wavelength range (190–800 nm). The method is in the scope of accreditation according to ISO 17025. Mobile phase was 20 min gradient of acetonitrile and potassium dihydrogen phosphate buffer, pH 2.5 (Sigma Aldrich). The column used for separation was C18, 150 × 4.6 mm, with particle size 5 µm. Sorbates

and benzoates, which have strong chromophores, show maximum absorbance of λ_{max} ~260 nm and λ_{max} ~230 nm for sorbic and benzoic acid, respectively. The main parameters of validation for the HPLC method were: reproducibility RSD_r=10.1; trueness: 96.6% and 99.7%; limit of detection/quantification LOD/LOQ=1.63/2.25 mg/kg and 0.66/1.23 mg/kg; linearity=0.9999, for benzoates and sorbates respectively. Paired sample T-test, using T distribution (two-tailed) from Microsoft Excel was used to assess data obtained from the two different techniques used for sorbate analysis of foods.

3. Results

According to the HPLC UV PDA method, LODs were 0.66–1.63 mg/l for sorbates and benzoates, but using LC-MS/MS, LODs were significantly lower 0.132–0.175 mg/l. Nonetheless, recovery

was between 97% and 104%, for both methods and analytes. The measurement uncertainty for HPLC/UV-PDA method was 10% for both analytes, sorbates and benzoates. During the validation period of the LC-MS/MS method, measurement uncertainty—including combined uncertainty from proficiency test data—resulted in $U_c=14\%$. Figure 1 shows results of sorbate quantitative analysis of the 36 food samples by the two methods, HPLC-UV/PDA and LC-MS/MS.

Paired sample T-test, using T distribution (two-tailed) results indicated that there was a non-significant small difference between the results from

HPLC ($M = 358.3 \text{ mg/kg}$, $SD = 378.5$) and LC/MS-MS ($M = 335.6 \text{ mg/kg}$, $SD = 336.6$), $t(35) = 1.9$, $p = 0.071$. Since the $p\text{-value} > \alpha$, H_0 can not be rejected. The test statistic T was -1.864 , which was in the 95% region of acceptance: $[-2.0301, 2.0301]$. The subtraction difference (-22.64) was also in the 95% region of acceptance: $[-24.6575, 24.6575]$. The 95% confidence interval of subtraction difference was $[-47.2978, 2.0172]$. The observed effect size (d) was small, 0.31 . Results of sorbate analysis by the HPLC UV PDA and LC/MS-MS methods, according to foodstuff groups, are presented in Tables 2-6.

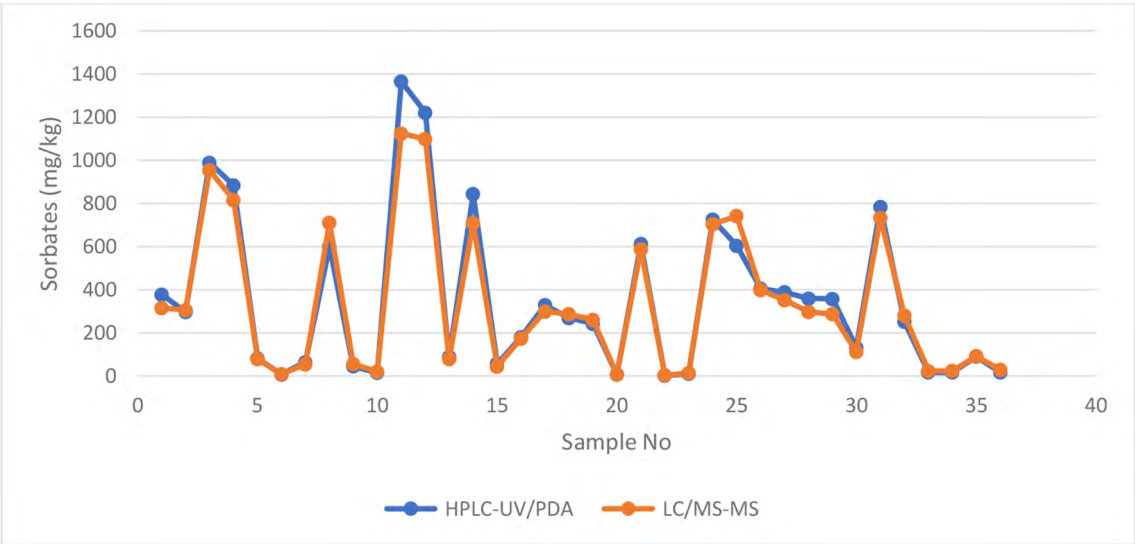


Figure 1. Sorbate (mg/kg) content in 36 foods, analysed by HPLC-UV/PDA and LC-MS/MS

Table 2. Sorbates (mg/kg) in milk products

MILK PRODUCT GROUP		Result HPLC	Result LC/MS-MS
S1	BURGER CHEDDAR	989.37	953.42
S2	TRAPIST CHEESE SLICE	82.32	79.22
S3	SOFT CHEESE	8.19	9.12
S4	FULL FAT CHEESE	64.64	54.95
S5	COTTAGE CHEESE	601.59	711.22
S6	GOUDA CHEESE SLICE	45.55	56.55
S7	TRAPIST CHEESE PIECE	15.46	21.25
S8	MIXED CHEESE	784.31	734.22
S9	SOFT CHEESE	252.32	280.21
S10	YOGHURT SALAD PICKLES	612.58	587.23
S11	YOGHURT SALAD HERBS	2.81	3.03

Table 3. Sorbates (mg/kg) in fruit and vegetable products

	FRUIT AND VEGETABLE PRODUCT GROUP	Result HPLC	Result LC/MS-MS
S12	BLACK OLIVE IN BRINE	88.79	80.25
S13	GREEN OLIVE IN BRINE	843.41	709.11
S14	CANDIED DATES	180.46	174.545
S15	SAUERKRAUT	329.35	300.11
S16	KETCHUP HOT	604.62	742.11
S17	KETCHUP MILD	405.53	399.12
S18	KETCHUP PIZZA	388.79	352.44
S19	KETCHUP SPICY	359.85	298.41
S20	TOMATO SAUCE	357.98	287.45
S21	BBQ SAUCE	132.98	112.21
S22	BBQ COLESLAW SALAD	7.6	7.75
S23	SAUERKRAUT LEAF	725.17	704.00

Table 4. Sorbates (mg/kg) in bakery products

	BAKERY PRODUCTS	Result HPLC	Result LC/MS-MS
S24	APPLE PIE	378.31	316.51
S25	CHERRY PIE	297.33	305.54
S26	COOKIES	57.64	45.265
S27	CHERRY CAKE	269.61	269.8273
S28	APPLE CAKE	243.55	261.25

Table 4. Sorbates (mg/kg) in vegetable products

	VEGETABLE PRODUCT	Result HPLC	Result LC/MS-MS
S29	VEGETABLE SWEETENED CREAM	1365	1124.55
S30	FRUIT AND VEGETABLE SPREAD	1365	1098.74
S31	VEGETABLE SPRAY CREAM	10.84	14.21

Table 5. Sorbates (mg/kg) in fishery products

	FISHERY PRODUCTS	Result HPLC	Result LC/MS-MS
S32	BREADED FISHBURGERS	17.39	25.23
S33	SURIMI	17.39	24.18

Table 6. Sorbates (mean mg/kg) in egg products

	EGG PRODUCTS	Result HPLC	Result LC/MS-MS
S34	MAYONAISE	884.23	816.47
S35	PASTEURIZED LIQUID CHILLED EGG WHITE	90.8	92.54
S36	PASTEURIZED LIQUID CHILLED EGG YOLK	17.1	29.77

3. Discussion

Paired sample t-test, using the T distribution (two-tailed) indicated there was a non-significant small difference between results (sorbate content, mg/kg) obtained with the different methods, LC-MS/MS and HPLC-UV/PDA, for 36 foods. Validation parameters of both methods meet requirements of Serbian (Serbia, 2018) and European regulations (European Commission, 2011). The sorbate contents of 36 foodstuffs were within the permitted values stipulated for these groups of food. The observed effect size (d) was small, 0.31. This indicates that the magnitude of the difference between the average of the differences and the expected average of the differences is small. Validation measurements, such as LOD, LOQ, and measurement uncertainty, as well as repeatability, reproducibility and recovery were satisfactory for both methods, HPLC and LC-

MS, as other authors also found in their comparative research (Gören *et al.*, 2015; Horiyama *et al.*, 2010; Tungkijanansin *et al.*, 2020). Generally, LC-MS/MS is preferred for trace analysis and regulatory compliance (Horiyama *et al.*, 2010), while HPLC UV/PDA is suitable for high throughput of samples with limits up to 2000 mg/kg of sorbates.

4. Conclusion

Comparing the Waters Alliance HPLC with UV/PDA 2996 and the Shimadzu LCMS-8045 LC-MS/MS for analysing sorbates and benzoates involved evaluating their performance based on sensitivity, specificity, sample preparation, cost, and suitability for the specific method. The choice of method depends on the food matrix, required sensitivity, and available instrumentation.

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