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original scientific paper

## Assessment of Sorbate and Benzoate Content in Mustard, Ketchup and Tomato Sauce by Sub-Minute Capillary Electrophoresis

Preservatives in Sauces by Capillary Electrophoresis

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### SUMMARY

*Research background.* Sorbate and benzoate are important preservatives in food products, but these compounds can also have genotoxic effects, causing health risks to their consumers. In this regard, this study aimed to determine the concentrations of sorbate and benzoate in Brazilian samples of mustard, ketchup, and tomato sauce using a sub-minute capillary electrophoresis method adequately validated.

*Experimental approach.* In this study, sorbate and benzoate were evaluated in sauces samples by capillary electrophoresis using a simple sample preparation procedure. Previously, the method was validated according to Eurachem guidelines, and its greenness was assessed by Eco-scale.

*Results and conclusions.* The fitness for purpose of the method, as well as its suitability for the analysis of the studied matrixes and its agreement with the principles of green chemistry were checked and confirmed. Also, according to our findings, among the 30 commercial samples assessed, six of them presented some mislabeling or non-compliance with European

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or Brazilian legislation, reinforcing the constant need for quality assessment and surveillance of food products.

*Novelty and scientific contribution.* So far, few studies have been found related to investigating these preservatives in these types of foods, highlighting the significance and contribution of this manuscript to the improvement of knowledge in the field.

**Key words:** analytical validation, food control, food preservatives, food security, green analytical chemistry, quality assessment

## INTRODUCTION

Sorbic and benzoic acids and their salts play a significant role as preservatives in the food industry. Due to its capacity to inhibiting yeasts, molds, and bacteria, these additives are widely used in foods and beverages such as bread, sauces, soft drinks, cheeses, juice, and fermented products (1–4).

Due to the low solubility in water of sorbic and benzoic acids, potassium sorbate and sodium benzoate is preferred due to their high water solubility (4,5). Each preservative is used in a pH range, in which sorbates are effective at pH=6.5 or less, at which point its antimicrobial activity increase as the pH decreases. At the same time, the benzoates are most effective in the pH range between 2.5 and 4.0 (4,6).

Until recently, the use of sorbate and benzoate was considered safe, although studies in cells and animals have evidenced that besides allergic reactions, their consumption may be related to the generation of carcinogenic and mutagenic compounds (7). In this sense, the maximum acceptable daily intake was established as 5 mg/kg body mass/day for benzoic acid and its salts, while for sorbic acid and its salts, the maximum value was allowed 25 mg/kg body mass/day (8).

In this regard, regulatory agencies established maximum levels for their application in food products, including sauces and the like, since these preservatives are widely used in these types of products, and these foods are commonly consumed daily. The European Commission established the concentration of 1000 mg/kg as the maximum limits for the sum of sorbate and benzoate in non-emulsified sauces, for example (9). The Brazilian legislation also authorizes these preservatives in some food products such as mustard and tomato sauce at the maximum concentration of 1000 mg/kg of sorbic and benzoic acids or their salts, which can be used either individually or in combination. For ketchup, only sorbic acid and their salts are allowed at the maximum concentration of 1000 mg/kg (10).

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Considering the importance of monitoring sorbate and benzoate in food products to guarantee consumers' health, several analytical methods to determine these analytes in foodstuff have been reported in the literature, and most of them are based on liquid chromatography (2,4,11,12). Despite the high sensitivity, the liquid chromatography methods present some drawbacks, especially in regard to the acquisition and operational costs, and in general, they are not in agreement with the concept of green analytical chemistry. Also, other methods are reported in the literature, including gas chromatography (3,13–15) and capillary electrophoresis (CE) (16–19). Therefore, it is important and essential to developing methods that are simple, quick, and economical to determine sorbate and benzoate in food products. CE is recognized by its fast separations, and among the separation techniques is one that most fits on the principles of green analytical chemistry, because it mainly uses aqueous solvents, generally do not involves laborious and multi-step sample preparation, require reduced amounts of reagents and sample, resulting in an equally reduced amount of waste (20,21). Therefore, the use of green and validated CE methods is very promising for a fast and reliable evaluation of sorbate and benzoate in food products.

In this context, this study aimed to determine sorbate and benzoate in Brazilian mustard, ketchup, and tomato sauce using a sub-minute CZE method; (ii) validate the method for these food matrixes; (iii) to determine the greenness of the method using the Eco-scale.

## MATERIALS AND METHODS

### *Reagents and solutions*

The reagents Tris(hydroxymethyl)aminomethane (TRIS), 2-hydroxyisobutyric acid (HIBA), and sorbic, benzoic, and salicylic acids were purchased from Sigma-Aldrich (St. Louis, CO, USA). The stock solutions of TRIS and HIBA (components of the background electrolyte) were prepared in ultrapure water (Milli-Q, Millipore, Bedford, MA, USA) at the concentration of 100 and 125 mmol/L, respectively. The stock solutions of sorbic, benzoic, and salicylic acids were prepared in methanol (Merck) at the concentration of 1000 mg/L and kept under refrigeration ( $5\pm 2$  °C) until the analysis when they were diluted to obtain the working concentration levels.

### *Instrumental*

The analyses were performed in a CE system (Agilent Technologies, model 7100, Palo Alto, CA, USA) equipped with a diode array detector. The data acquisition and treatment software were supplied by the manufacturer (HP ChemStation®).

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The electrophoretic separations were performed according to Costa *et al.* (16) in an uncoated fused-silica capillary of 32 cm (8.5 cm effective length × 50 µm inner diameter × 375 µm outer diameter). The short-end injection mode was applied in this study because it allowed a faster analysis with excellent separation of the compounds. The applied voltage was 30 kV, with positive polarity at the injection side, the temperature was maintained at 25 °C, and UV detection was conducted at 200 nm for benzoate and salicylate, and 254 nm for sorbate. The background electrolyte was composed of 25 mmol/L of TRIS and 12.5 mmol/L of HIBA, at pH=8.1. Salicylate was used as the internal standard (I.S.)

### *Samples and sample preparation*

A total of 30 samples of mustard ( $N=10$ ), ketchup ( $N=10$ ), and tomato sauce ( $N=10$ ) from different brands were purchased in a local supermarket of Florianopolis, Santa Catarina state, Brazil.

The sample preparation procedure was modified from that proposed by Javanmardi *et al.* (12), which used an aqueous dilution followed by dispersive liquid-liquid microextraction (DLLME). In the original protocol, 5 g of sauce sample was diluted in 50 mL of water and centrifugated at 4000 rpm for 5 min. An aliquot of 5 mL of the supernatant was transferred to a test tube in which 750 mg NaCl and 1.2 mL acetone (disperser solvent) containing 250 µL chloroform (extraction solvent) were added. The mixture was homogenized for 2 min and centrifugated at 4000 rpm for 10 min. The sediment phase was quantitatively placed into another test tube and evaporated with nitrogen gas. The residue was re-dissolved in 100 µL mobile phase for High Performance Liquid Chromatography (HPLC) analysis. In the present study, a simple sample preparation based only on aqueous dilution was employed as follows: samples ( $1.0 \pm 0.05$  g) were weighed into polypropylene tubes, and 10 mL of ultrapure water was added. The tubes were sealed, and the resulting solution was stirred with a vortex (Fisatom, model 772, São Paulo, Brazil) for 1 min and centrifuged for 5 min at 14000 rpm (Mini Spin plus, Eppendorf, Hamburg, Germany). The supernatant was filtered through syringe filters (0.45 µm pore size, Millipore, USA) and diluted in the proportion of 5:1 (v/v) with salicylate (internal standard, I.S.) at the concentration of 120 mg/L before being injected at the CE system. Subsequent dilutions were performed when necessary. For all experiments (quantification and validation) were considered the corrected peak area ( $\text{area}_{(\text{analyte})} / \text{area}_{(\text{I.S.})}$ ) and the corrected migration time ( $t_{m(\text{analyte})} / t_{m(\text{I.S.})}$ ).

All samples were prepared in three independent replicates and analyzed before the expiration date.

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### *Analytical validation and greenness assessment*

The analytical method was originally proposed and validated by Costa *et al.* (16) to assess sorbate and benzoate in soft drinks, juices, and teas. In regard to the application of this method to different matrixes (mustard and tomato-based samples) and a change performed in the linear range, it was necessary, in this study, to reassess the validation parameters such as linearity, matrix effect, selectivity, precision, and accuracy. The validation protocol followed the Eurachem guidelines (22). In addition, the greenness of the analytical procedures employed in this study was quantitatively determined using the Eco-scale proposed by Gałuszka *et al.* (23).

## RESULTS AND DISCUSSION

### *CZE method and sample preparation adaptations*

In this study, the CZE method proposed by Costa *et al.* (16) for non-alcoholic beverages was successfully applied to determine sorbate and benzoate in mustard, ketchup, and tomato sauce, as shown in Fig. 1.

This method allowed the separation of both ions and the I.S. in just 28 s. The total analysis time of the method, which includes flushing, vial permutation, injection, and separation, was experimentally determined and took about 1.3 min, achieving an analytical throughput of approximately 40 samples/hour. Moreover, it is noteworthy the high throughput of the CE method in comparison to chromatographic methods such as NMKL 124 (24), in which the target analytes separation takes over 15 minutes.

As shown in Table 1, different separation conditions and BGE composition can be used for sorbate and benzoate determination by capillary electrophoresis. However, the method proposed by Costa *et al.* (16) and employed in the present study allowed the separation of both analytes in less than 30 s. In contrast, separation times between 5 and 7 min were usually necessary when other separation conditions were employed. In addition, the limits of detection and quantification found by Costa *et al.* (16) were similar to those reported by the other studies indicating that the short separation time did not impact the sensibility of the method.

The successful application of the CZE method in this study was also dependent on the use of suitable sample preparation. The extraction of the analytes was based on modifications to the procedure proposed by Javanmardi *et al.* (12), which determined sorbate and benzoate in ketchup and other foods using the pre-preparation step followed by DLLM coupled with high performance liquid chromatography. Besides the non-utilization of DLLM, the modifications

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performed in the present study made the extraction procedure simpler and most appropriate for green chemistry by reducing the sample mass and the residue generation. In addition, the proposed method proved to be adequate to extract the analytes from the analyzed samples, eliminating the need to use one more step of cleaning and concentration of analytes, such as DLLM. Despite the use of minimum sample preparation steps, the electropherograms exhibited low interference on the separation zone, as shown in Fig. 1 (b) and (c).

### *Analytical validation*

To ensure the fitness for purpose and the *veracity of the data*, it was necessary to reassess the validation parameters of the CZE method for the new food matrixes investigated in this study.

#### Linearity and matrix effect

To assess the linearity, were prepared calibration curves of the analytes in standard solution and matrix. Each calibration curve was prepared in six equally spaced concentration levels (5 - 30 mg/L) and three independent replicates, being randomly injected in the CE system.

Two matrix calibration curves were prepared, one for mustard and another one for ketchup, both prepared by standard addition to blank samples. Due to the similarities in the composition of ketchup and tomato sauce, we assume that the interfering is comparable and, therefore, just one curve was built for ketchup to represent all styles of tomato-based sauces. In order to reproduce accurately the presence of possible interfering of the matrix, the blank samples used were obtained at the extraction and dilution conditions described in the sample preparation, section 2.3.

After the CE analysis and data acquisition, calibration curves were built by plotting the corrected peak area (sorbate/I.S. and benzoate/I.S.) *versus* concentration, and all curves presented  $R^2 > 0.99$ . Residual plots were assessed by visual inspection, and no departure of linearity or heteroscedasticity was verified. The assumptions of normality (25), homoscedasticity (26), and independence (27) were confirmed, and no lack of fit was observed, confirming the linearity at this range for standard solution and matrix calibration curves. The validation parameters are shown in Table 2.

The matrix effect was assessed by comparing the slopes obtained for the standard solution and matrix calibration curves (mustard and ketchup). Differences were considered statistically significant at the 5 % level ( $p < 0.05$ ) through the *t*-test bi-caudal. The presence of

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matrix effect was observed for the ions sorbate ( $p=0.0002$ ) and benzoate ( $p=0.01$ ) in ketchup and sorbate in mustard ( $p=0.006$ ). In this regard, all quantifications were performed using matrix calibration curves.

#### Selectivity, precision, and accuracy

The selectivity was evaluated experimentally by injecting the analytes (sorbate, benzoate, and I.S.) and the possible interferent (ascorbic acid) at the separation conditions. After the injection, the electropherogram was visually inspected, and the capacity of the method to accurately separate the analytes in the presence of the interferent was confirmed.

The parameter repeatability (intra-day) was assessed using three consecutive injections of a standard solution of the analytes (sorbate, benzoate, and I.S.). At the same time, the intermediate precision (inter-day) was determined by injecting three times this standard solution along three consecutive days. The method precision was confirmed since results for both intra- and inter-day precision were lower than 2.60 % relative standard deviation (Table 2).

The accuracy was established by recovering blank samples fortified at three concentration levels (10, 20, and 30 mg/L). The results for mustard ranged from 99.0 to 101.1 % and 98.8 to 101.6 % for sorbate and benzoate, respectively, while for ketchup, they ranged from 91.6 to 98.8 % for sorbate and 94.2 to 104.2 % for benzoate (Table 2). Therefore, these results indicated adequate method accuracy.

#### Analytical Eco-scale for greenness assessment

The scientific community has shown a growing interest in developing analytical methods environmentally sustainable. Several methods claim the status of “environmentally friendly”, but this claim often comes from an empirical denomination. Recently, some approaches have been proposed to quantitatively determine this appeal in analytical procedures. Some examples are the National Environmental Methods Index (NEMI) labelling, Analytical Greenness Calculator (AGREE), white analytical chemistry (WAC) and Eco-scale (28).

The Eco-scale is based on the assignment of penalty points to parameters of an analytical process that are not in agreement with the ideal green analysis. These penalty points are subtracted from a base of 100, the value considered for the ideal green analysis. The penalty points should contemplate all aspects of the analytical procedure, from the type

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and amount of reagents applied, energy consumption, waste generation, and until the occupational hazard to which the analyst is exposed (23,29).

As shown in Table 3, all the reagents and instruments used in the determination of sorbate and benzoate were considered to calculate the Eco-scale. To the proposed method were assigned 26 penalty points, resulting in an Eco-scale score of 74, which is characterized as acceptable in terms of greenness. The use of reagents as methanol and benzoic and sorbic acids resulted in important penalty points, which directly contributed to the score obtained for the method. However, parameters related to the occupational hazard and energy consumption were not significant, as well as the sample preparation steps also contributed to the greenness of the method since the procedure was based just on the sample dissolution, centrifugation, and filtration.

Recently, Nowak *et al.* (28) presented a new perspective for sustainable development in analytical chemistry named as WAC as an analogy to the 12 principles of green analytical chemistry. The concept of WAC was based in a tool known as Red-Green-Blue (RGB) model, being analytical efficiency represented by the color red; the environmental friendliness and safety represented by the color green; and the economic and practical aspects represented by the color blue. When the colors red, green and blue are on the higher saturation, the color obtained is white, which represents the ideal analytical method. The main idea of WAC assumes that an analytical method needs to balance between greenness and applicability.

The 12 principles of WAC were grouped as 4 red, 4 green and 4 blue principles:

- Red principles: scope and application; sensitivity (LOD and LOQ); precision and accuracy;
- Green principles: toxicity of reagents; number and amount of reagents and waste; energy consumption; impacts on health of humans and animals;
- Blue principles: cost-benefit; time-efficiency; requirement of personnel and infrastructure; simplicity of operation.

Alternatively assessing our study in terms of the principles of WAC, we can point out that the CE method follow most of the principles of WAC, since it can be applied to simultaneously determine three analytes; can be applied to several matrixes (soft drinks, juices, teas, sauces); has limits of detection and quantification consistent to the level of the analytes in the samples; good precision and accuracy. Most of the reagents used are non hazardous, and the hazardous one (methanol) was used at minimum amount; the sample preparation and the CE method were developed in order to obtain a minimum consumption of reagents and wast generation. The energy consumption of the CE method is low; by using



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correct disposal protocols the impacts for the environmental, humans and animals can be avoided. The method presents great cost-benefit; the time of analysis is considerable short allowing analyze several samples by hour; besides few laboratorial infrastructure is required. The only aspect of the WAC that the CE method may not fit properly is the last blue principle which recommends miniaturization and portability, a point of potential improvement in the future to make this method even more complete.

#### *Determination of sorbate and benzoate in mustard, ketchup, and tomato sauce*

The validated method was applied to the analysis of sorbate and benzoate in 30 commercial samples of mustard, ketchup, and tomato sauce from different manufacturers. The results are shown in **Table 4**.

Among the analyzed samples, six of them declared “no preservatives” on the label, and this claim was confirmed experimentally. Also, of the thirty samples evaluated, seven presented some kind of non-compliance. The significant problems involving mislabeling and non-compliance with the Brazilian legislation were found in the ketchup samples, in which of the ten samples assessed, four presented some irregularity.

The Brazilian legislation only allows the addition of sorbic acid and its salts at the maximum concentration of 1000 mg/kg in ketchup (10). However, four analyzed samples presented sodium benzoate as a preservative. The ketchup A and G have reported the use of sorbate in their label, although benzoate was detected experimentally. In contrast, the ketchup D and I declared benzoate in the ingredient list, despite the regulatory recommendation. Besides these mislabeling, the ketchup G and I presented benzoate concentrations ( $1054.52 \pm 70.24$  and  $1479.12 \pm 36.34$  mg/kg, respectively) higher than the maximum limit established by the European Commission that is of 1000 mg/kg (9), which represents an infringement if these products are marketed in Europe. In the ketchup samples that sorbate was quantified, all of them were following the regulatory limit of both legislations.

Regarding tomato sauce, all samples were in accordance with the limits established by the European and Brazilian legislation that allows the use of sorbate and benzoate at concentrations of 1000 mg/kg (9,10). In tomato sauce, B and C were also verified mislabeling related to the presence of sorbate and benzoate in the ingredient list with the results obtained experimentally.

For mustard, European and Brazilian legislations allow the addition of sorbate and benzoate (9,10), but in nine of ten samples evaluated just benzoate was detected. For all

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samples, the concentrations of sorbate and benzoate were in accordance with the legislation (1000 mg/kg) and with their label.

Despite the adequacy of almost all investigated samples to the European and Brazilian legislations, the sorbate and benzoate levels found were, in general, higher than those reported by other studies. In Austrian mustards, the mean concentration was 118.9 mg/kg for sorbic acid and 195.5 mg/kg for benzoic acid, while in ketchup and tomato puree, the mean concentrations were 486.0 and 788.7 mg/kg for sorbic and benzoic acids, respectively (30). Low levels of sorbic and benzoic acids were also reported in Chinese ketchup at the concentration of 175.9 and 307.6 mg/kg, respectively (13). In Iranian ketchup, sorbate and benzoate are not allowed, but Javanmardi *et al.* (12) found benzoate levels of up to 38.7 mg/kg, but in tomato paste, none of them were detected (2). In contrast, 23.8 % of samples of Turkish ketchup showed a sum of sorbic and benzoic acids >1000 mg/kg (31).

Our findings reinforce the importance of the constant monitoring of sorbate and benzoate concentrations in these products, especially ketchup, associated with the labeling inspection since children and young people are frequent consumers of these products, and their excessive intake can represent health risks, especially for these consumers (7). In addition, the fact that the sorbate and benzoate levels found in this study were, in general, higher than those reported by other works lights up a warning signal about the possible excessive intake of these preservatives by Brazilian consumers since they are present in many different food products. Wherefore, the constant monitoring and the use of allowed sorbate and benzoate concentrations in mustard, ketchup, and tomato sauce are indispensable to ensure the lower risk to the health of their consumers.

## CONCLUSIONS

In this study, the proposed methodology employing a green CZE method was validated and showed to be suitable for monitoring sorbate and benzoate concentrations in mustard, ketchup, and tomato sauces, with the advantages of using a simple, fast, and solvent-free sample preparation procedure. Our findings indicated that of the 30 samples evaluated, six of them presented some kind of non-compliance with European or Brazilian legislation by using not allowed preservative or mislabeling, in which two of these samples (ketchup samples) presented benzoate, analyte not allowed in this product by Brazilian legislation, at a concentration higher than 1000 mg/kg. Therefore, these aspects highlight the need for this kind of assessment, especially by using fast and reliable methods.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## AUTHORS' CONTRIBUTION

ACOC conceived the idea of this manuscript, designed the experiments, supervised the work and revised the manuscript; LMP and FDB designed and performed the experiments, interpreted the data, wrote, edited, and revised the manuscript; SKTS, MS, and PN wrote, edited and revised the manuscript; LVG and RF supervised the work and revised the manuscript.

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**Table 1.** Separation conditions of benzoate and sorbate by capillary electrophoresis methods

Method	BGE and separation parameters	Separation time, LOD and LOQ
Our study / Costa <i>et al.</i> (16)	BGE: 25 mmol/L tris(hydroxymethyl)aminomethane and 12.5 mmol/L 2-hydroxyisobutyric acid, at pH=8.1 Short-end injection: 32 cm total length x 8.5 cm effective length x 50 µm inner diameter Voltage: +30 kV Temperature: 25 °C Detection: UV direct at 200 nm for benzoate and 254 nm for sorbate	30 s LOD: 0.3 mg/L sorbate; 0.9 mg/L benzoate LOQ: 1.1 mg/L sorbate; 1.3 mg/L benzoate
Petrucci <i>et al.</i> (17)	BGE: 20 mmol/L disodium hydrogen phosphate and 0.2 mmol/L sodium phosphate monobasic, at pH=8.5 Injection: 48.5 cm total length x 40 cm effective length x 75 µm inner diameter Voltage: +15 kV Temperature: 29 °C Detection: UV direct at 200 nm for benzoate and sorbate	6 min LOD: 0.7 mg/kg sorbate; 1.0 mg/kg benzoate LOQ: 2.2 mg/kg sorbate; 3.1 mg/kg benzoate
Coelho <i>et al.</i> (18)	BGE: 10 mmol/L histine, 100 mmol/L propanesulfonic acid, 0.2 mmol/L cetyltrimethylammonium bromide, and 10 % methanol, at pH=5.8 Injection: 80 cm total length x 71.5 cm effective length x 75 µm inner diameter Voltage: -27 kV Temperature: - Detection: conductivity detection at 633 kHz	7 min LOD: 1.2 mg/L sorbate LOQ: 4.1 mg/L sorbate
de Jesus <i>et al.</i> (19)	BGE: 10 mmol/L benzoic acid, 15 mmol/L histidine and 0.2 mmol/L cetyltrimethylammonium bromide, at pH=5.8 Injection: 75 cm total length x 67 cm effective length x 75 µm inner diameter Voltage: -25 kV	5 min LOD: 0.009 mg/L sorbate LOQ: 0.03 mg/L sorbate



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	Temperature: -	
	Detection: UV direct at 250 nm for sorbate	
Mesquita <i>et al.</i> (32)	BGE: 20 mmol/L sodium tetraborate, at pH=9.3 Injection: 48.5 cm total length x 40 cm effective length x 75 µm inner diameter Voltage: -20 kV Temperature: 25 °C Detection: UV direct at 255 nm for sorbate	7 min LOD: 0.4 mg/L sorbate LOQ: 1.0 mg/L sorbate
Nowak (33)	BGE: 50 mmol/L borax, at pH=10.5 Injection: 30 cm total length x 20 cm effective length x 50 µm inner diameter Voltage: -15 kV Temperature: 25 °C Detection: UV direct at 200 nm for sorbate and benzoate	3 min LOD: 0.4 mg/L sorbate and benzoate LOQ: 1.2 mg/L benzoate; 1.3 mg/L sorbate

BGE: background electrolyte; LOD: limit of detection; LOQ: limit of quantification; (-) not informed

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**Table 2.** Analytical performance of the capillary zone electrophoresis method for the matrixes assessed

Parameter	<i>N</i>	Sorbate	Benzoate
Linear range (mg/L)	-	5 – 30	
Linearity – standard solution			
Linearity – slope	3	0.0210	0.0173
Linearity – intercept	3	0.0381	0.0334
Linearity – coefficient of regression, R <sup>2</sup>	3	0.999	0.998
Linearity – mustard			
Linearity – slope	3	0.0226	0.0165
Linearity – intercept	3	0.0492	0.0832
Linearity – coefficient of regression, R <sup>2</sup>	3	0.998	0.995
Linearity – ketchup			
Linearity – slope	3	0.0175	0.0153
Linearity – intercept	3	0.1007	0.1164
Linearity – coefficient of regression, R <sup>2</sup>	3	0.996	0.997
Precision (% RSD)			
Intra-day precision – corrected peak area	3	1.43	1.51
Intra-day precision – corrected migration time	3	0.18	0.04
Inter-day precision – corrected peak area	9	2.06	2.56
Inter-day precision – corrected migration time	9	0.50	0.21
Recovery (%)			
Mustard – concentration level (10 mg/L)	3	99.0±1.0	99.9±2.1
Mustard – concentration level (20 mg/L)	3	101.1±1.8	101.6±1.4
Mustard – concentration level (30 mg/L)	3	99.3±0.5	98.8±1.9
Ketchup – concentration level (10 mg/L)	3	98.8±1.7	104.2±1.2
Ketchup – concentration level (20 mg/L)	3	97.8±2.7	103.4±1.9
Ketchup – concentration level (30 mg/L)	3	91.6±1.8	94.2±0.9

*N*: number of observations; RSD: relative standard deviation

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**Table 3.** Green evaluation of the capillary zone electrophoresis method according to the Eco-scale

Aspects of the analytical procedure	Penalty points
Reagents	20
Energy consumption	0
Occupational hazard	1
Waste	5
Total	26
Eco-scale	74

**Table 4.** Concentrations of potassium sorbate and sodium benzoate in mustard, ketchup and tomato sauce samples

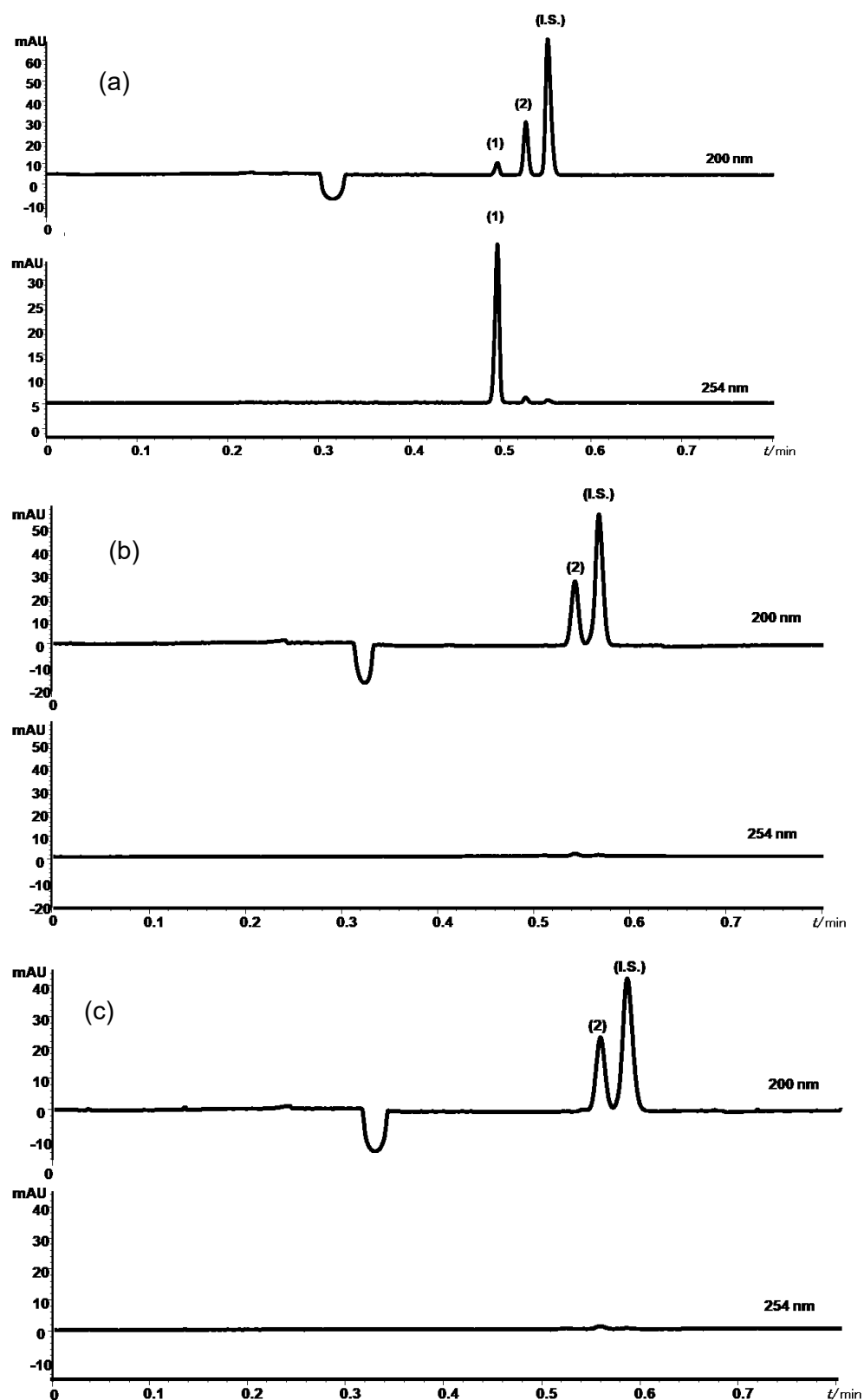
Sample	Preservative declared in the label	w(sorbate)/ (mg/kg)	w(benzoate)/ (mg/kg)
Mustard A	-	<LOD	<LOD
Mustard B	Sodium benzoate	<LOD	180.34±15.05
Mustard C	Sodium benzoate	<LOD	946.19±78.99
Mustard D	Sodium benzoate	<LOD	559.08±85.26
Mustard E	Sodium benzoate	<LOD	813.17±92.88
Mustard F	Sodium benzoate	<LOD	620.56±48.18
Mustard G	Potassium sorbate	606.71±10.35	917.29±14.18
Mustard H	Sodium benzoate	<LOD	952.35±44.76
Mustard I	Sodium benzoate	<LOD	991.53±0.77

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Mustard J	Sodium benzoate	<LOD	404.20±5.99
Ketchup A	Potassium sorbate	<LOD	585.92±33.30
Ketchup B	Potassium sorbate	429.96±6.67	<LOD
Ketchup C	-	<LOD	<LOD
Ketchup D	Sodium benzoate	<LOD	163.46±1.25
Ketchup E	Potassium sorbate	694.64±38.22	<LOD
Ketchup F	Potassium sorbate	444.35±21.48	<LOD
Ketchup G	Potassium sorbate	741.20±38.55	1054.52±70.24
Ketchup H	Potassium sorbate	604.84±10.10	<LOD
Ketchup I	Sodium benzoate	<LOD	1479.12±36.34
Ketchup J	-	<LOD	<LOD
Tomato sauce A	Sodium benzoate	<LOD	362.38±79.42
Tomato sauce B	Potassium sorbate	<LOD	351.79±33.95
Tomato sauce C	Sodium benzoate	413.84±44.39	<LOD
Tomato sauce D	Sodium benzoate	<LOD	325.65±17.96
Tomato sauce E	-	<LOD	<LOD
Tomato sauce F	Potassium sorbate	589.13±65.54	<LOD
Tomato sauce G	Potassium sorbate and sodium benzoate	430.59±18.98	446.78±20.84
Tomato sauce H	-	<LOD	<LOD
Tomato sauce I	-	<LOD	<LOD
Tomato sauce J	Potassium sorbate	849.12±24.30	<LOD

LOD: limit of detection; LOD sorbate: 0.3 mg/L; LOD benzoate: 0.9 mg/L; (-) label informed "no preservatives"

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**Fig. 1.** Electropherograms of (a) standard solution; (b) mustard sample G; and (c) ketchup sample I. Separation conditions: background electrolyte composed of 25 mmol/L of

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tris(hydroxymethyl)aminomethane and 12.5 mmol/L of 2-hydroxyisobutyric acid, at pH=8.1; hydrodynamic injection of +50 mbar for 3 s; voltage of +30 kV; and capillary of 32 cm (8.5 cm effective length × 50 µm inner diameter × 375 µm outer diameter). Peaks: (1) sorbate; (2) benzoate; and (I.S. – internal standard) salicylate.