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FULL SHORT COMMUNICATION

Simultaneous Determination of Benzoic Acid and Sorbic Acid in Food Products by CE after On-line Preconcentration by Dynamic pH Junction

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Abstract An online dynamic pH junction method has been proposed for sensitive determination of benzoic acid (BA) and sorbic acid (SA) by capillary electrophoresis (CE). It was based on the decreased mobility of the analytes when they migrated from sample zone with low pH to background electrolytes with high pH. Under optimized conditions, the limits of detection (S/N = 3) of BA and SA were 0.03 and 0.02 mg L^{-1}, respectively. According to the permitted limits of BA and SA in food (GB2760), the samples can be diluted about 2,000–20,000-folds to estimate whether the contents of BA and SA are in the safety range. Beverage, vinegar and fruit jam samples were analyzed after simple dilution, and the results were satisfactory with the recoveries in the range of 91–108%. The whole analysis process needs about only 10 min, which is much faster than the method of liquid–liquid extraction followed by CE determination.

Keywords Capillary electrophoresis · Dynamic pH junction · Benzoic acid · Sorbic acid

Introduction

Benzoic acid (BA), sorbic acid (SA) and their salts are the permitted chemical preservatives in food products to prevent transformation and degradation by microorganisms during storage. However, BA and SA may be harmful to consumers in case of excessive addition [1, 2]. Many countries have restricted the usage of preservatives in daily food. For example, the upper limits for BA and SA in foods are in the range of 0.2–2 g kg^{-1} in China (GB2760). In order to assess their level in foods, it is very important to develop convenient, inexpensive and fast analysis methods for these preservatives.

Several methods were described for the determination of BA and SA in foods, including spectrophotometry [3, 4], chemiluminescence [5, 6], gas chromatography (GC) [7–9] and high-performance liquid chromatography (HPLC) [10–13]. Spectrophotometric and chemiluminescent methods are generally unable to achieve simultaneous determination of BA and SA, and always suffer from matrix effects. HPLC and GC are valid separation methods and are capable of detecting BA and SA simultaneously, but they always require relatively long analysis time, large consumption of the mobile phase and extensive sample pretreatment such as liquid–liquid extraction (LLE) and solid phase extraction (SPE).

Owing to the advantages of high separation efficiency, low reagent consumption and short analysis time [14–19], capillary electrophoresis (CE) has been commonly used in food analysis [20, 21]. It provides another good choice for simultaneous determination of BA and SA in food products. However, the matrix in food products such as high contents of salts, proteins, carbohydrates, and organic acids can affect CE separation and determination, and thus a sample pretreatment step was always required. LLE method and auto-SPE system have been utilized for effective elimination of the matrix effects during analysis of BA and SA in food products, but they demand of either laborious procedures or complicated experimental set-up [22–24]. In CE, diluting the samples is a simple and effective approach for the elimination of matrix effects, but

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the dilution factor is always limited by the low concentration sensitivity caused by short optical path in the case of UV detection. If an online preconcentration technique is adopted, the dilution factor can be significantly improved and the matrix effects will be further alleviated correspondingly.

Online dynamic pH junction, one of the most efficient preconcentration techniques, provides a very simple and fast focusing scheme for CE. It has been applied for improved detection of peptides [25], purine metabolites [26], arsenic compounds [27], riboflavin [28] and cationic analytes [29]. BA and SA are weak electrolytes and their motilities can be easily changed by varying the pH of sample solution. Therefore, dynamic pH junction can also be applied for the focus of BA and SA. In this work, it was utilized for preconcentration of the BA and SA so that the common food samples such as vinegar, beverage and fruits jam can be analyzed directly with CE after only simple dilution. The method features the advantages of simplicity, rapidness and low reagent consumption for sample pretreatment.

Experimental

Reagents, Standards and Samples

All the chemical reagents were at least of analytical grade. The stock solutions of 1,000 μg mL^{-1} BA and 1,000 μg mL^{-1} SA were prepared by dissolving 0.0500 g BA and SA in 50 mL doubled distilled water (DDW), respectively. These stock solutions were kept in a refrigerator at 4 °C. Working standard solutions were prepared daily by appropriately diluting stock solution with 20 mmol L^{-1} pH 2.5 phosphate buffer solution. The 50 mmol L^{-1} pH 9.0 borate solution was used as running buffer. NaOH and HCl solutions (both 1 mol L^{-1}) were used to adjust pH to the desired value. The vinegar, beverage and fruit jam samples were obtained from the local market. Vinegar and beverage samples were diluted with 20 mmol L^{-1} pH 2.5 phosphate buffer solution for 1,000- and 20-folds, respectively, and then filtrated through 0.45 μm membrane before detection. For fruit jams, about 1 g sample was dissolved in 250 mL 20 mmol L^{-1} pH 2.5 phosphate buffer solution, and the obtained sample solution was filtered through 0.45 μm membrane before detection.

Apparatus

All the electrophoretic experiments were performed on a commercial capillary electrophoresis instrument (CL-1030, Beijing Cailu Science Co., Beijing, China) with an UV–Vis detector. An uncoated 50 μm inner diameter (i.d.) fused-silica capillary (Hebei Reafine Chromatography Ltd., Hebei, China) with 55 cm total length and 47 cm effective length was used. The analytes, BA and SA, were both detected at 230 nm.

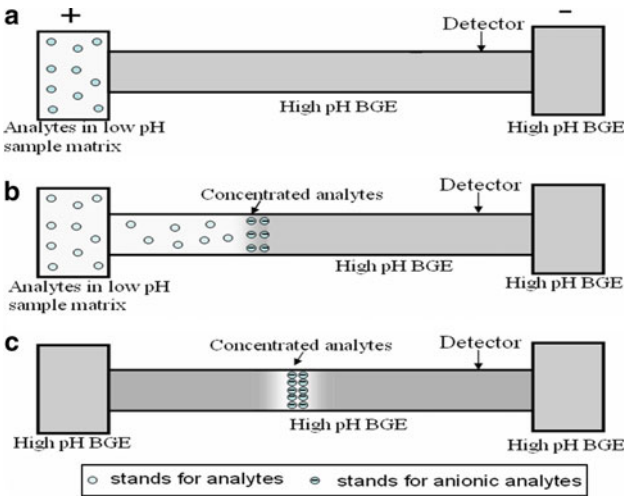

Fig. 1 Schematic diagram of accumulating BA and SA by dynamic pH junction. **a** capillary is conditioned with BGE, and ready for injection; **b** analytes in low pH sample matrix was electrokinetically injected and focused in the boundary between the two electrolytes; **c** focusing process continued before separation

CE Procedures

Before the first use, the capillary was rinsed with 0.1 mol L^{-1} NaOH for 30 min, and then with 0.1 mol L^{-1} HCl, double distilled water and running buffer solution for 10 min. Between two runs, the capillary was flushed with 0.1 mol L^{-1} NaOH for 2 min and running buffer solution for 2 min. During each run, the separation capillary was filled with running buffer solution. The separation voltage was kept at 25 kV. The sample solution was injected electrokinetically at 10 kV for 10–100 s when pH junction stacking mode was utilized. During common capillary zone electrophoresis (CZE) detection, the sample solution was injected by raising inlet end of the capillary 15 cm higher than the outlet end for 10 s. Quantification was achieved by measuring the CE peak heights.

Results and Discussion

Dynamic pH Junction Technique for BA and SA

The pK_a values of BA and SA were 4.20 and 4.76, respectively. Therefore, both of the two analytes were dissociated in pH > 4.76 buffer solutions and their effective mobility decreased correspondingly. In the experiments, when the analytes in low pH sample matrix was injected electrokinetically (Fig. 1a), the neutral BA

and SA molecules in the sample vial quickly moved into the capillary with electroosmotic flow. Subsequently, as the OH^- in higher pH background electrolyte (BGE) invaded the sample zone, they were negatively charged and decelerated rapidly. The deceleration processes led to the fast accumulation of the two analytes (Fig. 1b). After injection, the sample vial was replaced with a BGE vial, and the accumulation process went on under the applied separation voltage (Fig. 1c). Meanwhile, the OH^- in the higher pH BGE further invade the sample zone, and its pH increased persistently. The accumulation process was finished and separation process began when the pH values of sample zone were higher than analytes' pK_a.

Optimization of Preconcentration Conditions

Effects of the Sample Matrix pH on Focusing and Separation

The sample matrix pH is an important factor, influencing the focusing of weakly ionic analytes in dynamic pH junction. The effects of sample matrix pH on the separation and focusing of the analytes were investigated in the pH range 2.5–5.0 with 20 mmol L^{-1} phosphate buffer solution. As shown in Fig. 2, when the pH of the sample matrix was 4.0, 4.5 and 5.0, the SA was not focused very well. As the pH of the sample matrix decreased from 4.0 down to 2.5, the focusing effect was improved gradually and the absorbance signals were also found to be enhanced correspondingly. For BA, it always has sharp peaks at the investigated sample matrix pH range, but the absorbance signal decreased gradually as the pH increased. Thus, in this work, the optimum sample matrix pH was 2.5.

Effects of Sample Matrix Concentration on Focusing and Separation

Because the samples were prepared in phosphate buffer solution, the effects of sample matrix concentration was investigated by varying phosphate concentrations from 20 to 60 mmol L^{-1}. The sample matrix pH was kept at 2.5, and the injection time was 80 s. It was found that phosphate concentration has low effect on the focusing effect of BA and the peak heights were kept constant as the concentration varied. On the contrary, the focusing effect of SA was significantly influenced by phosphate concentration. As the phosphate concentration increased, the SA peak became broader and its height declined correspondingly. Finally, 20 mmol L^{-1} was considered as the best sample matrix concentration.

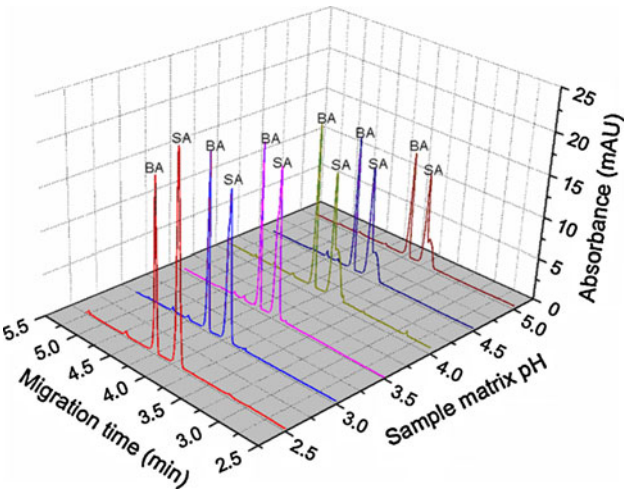

Fig. 2 Effects of sample matrix pH on separation and focusing. BGE, 50 mmol L^{-1} pH 9.0 borate buffer; sample matrix, 20 mmol L^{-1} phosphate buffer with varied pH; Applied voltage for electrokinetical injection 10 kV; Injection time, 60 s; Separation voltage, 25 kV; Effective capillary length 47 cm

Effects of Injection time on Focusing and Separation

The effect of injection time was investigated from 10–100 s, keeping the concentration of phosphate at 20 mmol L^{-1} and the pH of sample matrix at 2.5. As the injection time prolonged from 10 to 100 s, the peak heights of both BA and SA increased persistently, and no board effect was found for the two analytes. However, the separation resolution between BA and SA declined gradually as the injection time increased. This was due to the reduced effective length of separation capillary as the injection time increased. When the injection time was increased to 100 s, baseline separation was just maintained between the two peaks of BA and SA. Hence, we selected 100 s as the optimum injection time in terms of sensitivity and separation resolution.

Method Validation Under Optimized Conditions

The SA and BA with a concentration range from 0.1 to 25 mg L^{-1} were focused and separated well under optimized conditions. The calibration equations for BA and SA were $y = 2.137x + 0.2382$ and $y = 2.9849x - 0.4152$, and their coefficients of correlation (r) were 0.9997 and 0.9987, respectively.

The reproducibility of the peak heights and migration times was investigated by five consecutive injections of 5 mg L^{-1} BA and SA mixture solution. Relative standard deviations (RSDs) of peak heights for BA and SA were both less than 2.5%, and RSDs of migration time were <2%, indicating that this method has satisfactory reproducibility for both peak height and migration time.

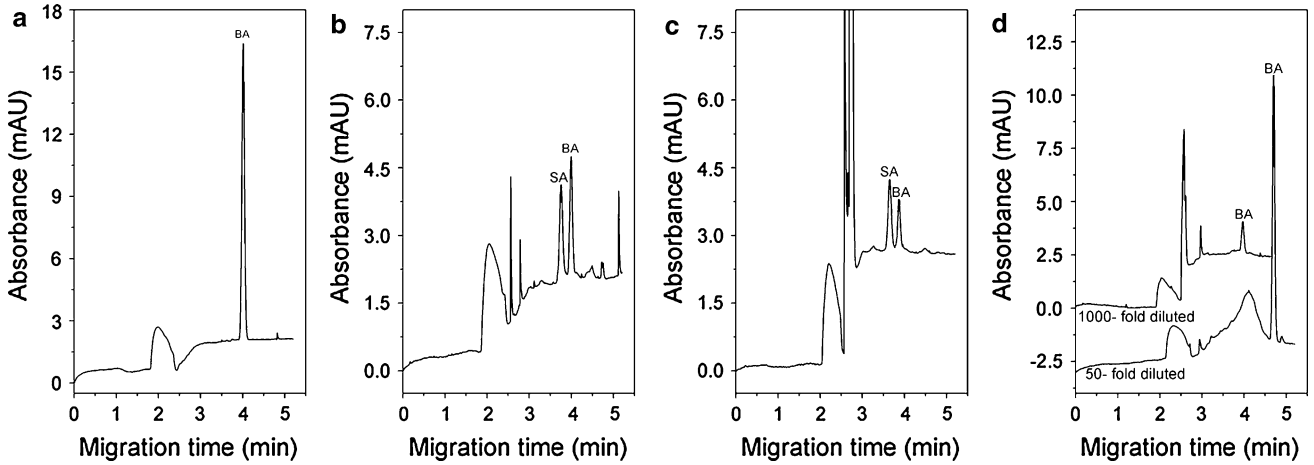

Fig. 3 Electropherograms of **a** beverage, **b** fruit jam I, **c** fruit jam II and **d** vinegar sample solution. Conditions: BGE, 50 mmol L^{-1} pH 9.0 borate buffer; sample matrix, 20 mmol L^{-1} pH 2.5 phosphate buffer; Applied voltage for electrokinetical injection 10 kV; Injection time 100 s; and other conditions were the same as Fig. 2

Table 1 Analytical results of BA and SA in food samples

Samples	Analyte	Found amount (mg kg^{-1})[a]	Added amount (mg kg^{-1})	Total found amount (mg kg^{-1})[a]	Recovery (%)[b]
Beverage	BA	80.1 ± 1.3	100.0	188.5 ± 4.5	108
	SA	nd	100.0	95.4 ± 3.2	95
Vinegar	BA	540.2 ± 31.9	500.0	1081.5 ± 26.7	108
	SA	nd	500.0	536.0 ± 23.3	107
Fruit jam I	BA	199.8 ± 7.5	250.0	427.3 ± 7.5	91
	SA	247.5 ± 15.3	250.0	482.5 ± 10.3	94
Fruit jam II	BA	97.2 ± 6.3	100.0	190.5 ± 10.5	93
	SA	103.4 ± 5.1	100.0	198.4 ± 8.9	95

nd not detected

[a] average ± one standard deviation of three trials

[b] Recovery = (Total found amount after spiked − found amount before spiked)/spiked value × 100%

The limits of detection (LODs) of this method for BA and SA (S/N = 3) were 0.03 and 0.02 mg L^{-1}, respectively, which were more than 100-folds lower than that obtained with common CZE method [23]. The limits of quantification (LOQs) of BA and SA (S/N = 10) were 0.1 and 0.07 mg L^{-1}, respectively. Because the permitted limits for benzoic acid and sorbic acid in foods are in the range of 0.2–2 g kg^{-1} (GB 2760), the food samples can be diluted 2,000–20,000-folds to estimate whether the contents of BA and SA are in safety range.

Sample Analysis

Four samples, beverage, fruit jam I, fruit jam II and vinegar were selected for the evaluation of the proposed method. After appropriate dilution and filtration through 0.45 μm membrane, the samples were analyzed directly using this method. The electropherograms of four investigated samples were exhibited in Fig. 3, no interference peak was found for the detection of BA and SA within the 5-min separation. Meanwhile, the migration times of BA and SA in the jam samples were the same as those of standard solution. However, when the two jams were analyzed directly by CE without dynamic pH junction, we found that that the migration times of the analytes in the two jams differed from those of their standard solutions, which was also found in Boyce's work [30]. The reason was probably that the sample was not diluted sufficiently, and the sample matrix affected CE separation. For the vinegar sample, the high amount of acetic acid can also significantly affect the migration time of BA when the sample was only 50-fold diluted (Fig. 3d). The alteration of migration time might influence not only identification of BA but also quantification of BA. Fortunately, the effect of acetic acid in the matrix was found to be little when the sample was diluted 1,000-fold, as exhibited in Fig. 3d.

The amounts of the preservative found in the investigated sample was listed in Table 1 ranging from 80.1 to 540.2 mg kg^{-1}, which are all lower than the corresponding permitted limits of vinegar, beverage and fruit jam in China (GB2760). The recoveries obtained by spiking standard solutions to the real samples were satisfactory, ranging

from 91 to 108%. The mark-label points out that there are many kinds of compounds such as minerals, carbohydrates, vitamins and organic acids. However, they did not affect the separation and detection of SA and BA after appropriate dilution and selective preconcentration of BA and SA by dynamic pH junction. The whole process for analyzing one sample, including dilution, filtration, preconcentration by dynamic pH junction and separation, just needs about 10 min, which is significantly faster than the LLE–CE method.

Conclusion

Online dynamic pH junction preconcentration technique was proposed for the determination of BA and SA in food samples by CE. The common BA- or SA- contained samples such as beverage, fruit jam, vinegar, etc. can be rapidly analyzed after simple dilution and filtration. Compared with the reported LLE- and SPE–CE methods, the proposed method has the advantages of simplicity, rapidness and low consumption of reagent for sample pretreatment. Therefore, hyphenated with this dynamic pH junction technique, CE will be a promising method for rapidly and conveniently estimating whether the contents of BA and SA are in safety range.

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References

1. Mota FJMI, Ferreira MPLVO, Cunha SC, Oliveira PP (2003) Food Chem 82(3):469–473
2. Tfouni SA, Toledo VMCF (2002) Food Control 13(2):117–123
3. Chen YQ, Ni YN (2009) Chinese Chem Lett 20(5):615–619
4. Marsili NR, Sobrero MS, Goicoechea HC (2003) Anal Bioanal Chem 376(1):126–133
5. Yu JH, Zhang CC, Ge L, Dai P, Ge SG (2009) Anal Sci 25(11):1351–1356
6. Wang ZJ, Song ZH (2003) Chinese Chem Lett 14(3):283–286
7. Dong CZ, Mei Y, Chen L (2006) J Chromatogr A 1117(1):109–117
8. Wang LL, Zhang XX, Wang P, Wang W (2006) Anal Chim Acta 577(1):62–67
9. Farahani H, Ganjali MR, Dinarand R, Norouzi P (2009) J Agric Food Chem 57(7):2633–2639
10. Lozano VA, Camina JM, Boeris MS, Marchevsky JJ (2007) Talanta 73(2):282–286
11. Saad B, Bari MdF, Saleh MI, Ahmad K, Talib MKM (2005) J Chromatogr A 1073(1–2):393–397
12. García I, Ortiz MC, Sarabia L, Vilches C, Redilla E (2003) J Chromatogr A 992(1–2):11–27
13. Mikami E, Goto T, Ohno T, Matsumoto H, Nishida M (2002) J Pharmaceut Biomed 28(2):261–267
14. Zhao SL, Song YR, Liu YM (2005) Talanta 67(1):212–216
15. Bi WW, Lei SR, Yang XP, Xu ZM, Yu HY, Xiao D, Choi MMF (2009) Talanta 78(3):1167–1172
16. Hu YY, Wu X, Su YY, Hou XD, Zhang JY (2009) Microchim Acta 166(3–4):289–294
17. Zhang XF, Xuan YL, Sun AM, Lv Y, Hou XD (2009) Luminescence 24(4):243–249
18. Zhang XF, Zhou Q, Wu L, Lv Y, Hou XD (2010) Microchem J 95(1):80–84
19. Woods L, Roddy ATP, Ewing A (2004) Electrophoresis 25(9):1181–1187
20. Zhang XF, Zhang JY, Wu X, Lv Y, Hou XD (2009) Electrophoresis 30(1):1937–1942
21. Kostal V, Katzenmeyer J, Arriaga EA (2008) Anal Chem 80(12):4350–4533
22. Tang YJ, Wu MJ (2007) Food Chem 103(1):243–248
23. Hu MZ, Wang WZ, Zhang YQ (2004) J Shanghai Norm Univ 33(3):63–66
24. Han F, He YZ, Li L, Fu GN, Xie HY, Gan WE (2008) Anal Chim Acta 618(1):79–85
25. Monton MR, Imami K, Nakanishi M, Kim J, Terabe S (2005) J Chromatogr A 1079(1–2):273
26. Kibbin PB, Chen DDY (2003) Chromatographia 57(1–2):87–93
27. Jaafar J, Irwan Z, Ahamad R, Trabe S, Ikegami T, Tanaka N (2007) J Sep Sci 30(3):391–398
28. Su AK, Chang YS, Lin CH (2004) Talanta 64(4):970–974
29. Kim J, Okamoto Y, Terabe S (2003) J Chromatogr A 1018(2):251–256
30. Boyce MC (1999) J Chromatogr A 847(1–2):369–375