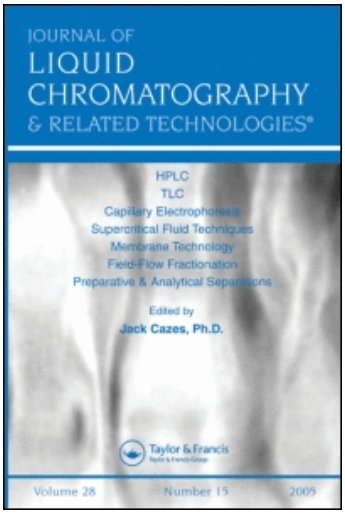

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DEVELOPMENT OF AN HPLC/DIODE-ARRAY DETECTOR METHOD FOR SIMULTANEOUS DETERMINATION OF SODIUM BENZOATE AND PHENOLIC COMPOUNDS IN QUINCE JAM

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ABSTRACT

A simple, rapid, sensitive, reproducible, and accurate reversed-phase HPLC procedure is proposed for the determination of sodium benzoate in quince jam samples. The sample preparation was simple, involving only extraction with methanol. The chromatographic separation was achieved using a reversed-phase column Spherisorb ODS2 (5µm; 25.0 x 0.46 cm). Gradient elution was carried out using water-formic acid (19:1) (A) and methanol (B). The effluent was monitored by a diode-array detector and chromatograms were recorded at 280 nm. The detection limit value of the method for sodium benzoate was 0.5 µg/mL and the method was precise (SD=0.003; CV%=2.76; n=6). Recovery values of sodium benzoate from spiked quince jam samples were between 94.7 and 100 %. This technique can also be useful for the simultaneous definition of the phenolic profile (phenolic acids, flavonoids, and glycosides of procyanidin polymers) of quince jam.

INTRODUCTION

Benzoic acid and its salts, namely sodium, potassium, and calcium benzoates can be used as preservatives, either alone or as a mixture with other additives such as sorbates and *p*-hydroxybenzoates in "marmelada". This is a jam that is industrially manufactured, or made at home, during the September/October months by boiling a mixture of sugar and quince puree (pulp of fruit of *Cydonia oblonga* Miller, var. *maliformis* or *piriformis*) until a convenient texture is obtained (usually to reach 65-72°Brix).

As quince is a seasonal fruit with strong acidity, the industry needs to preserve purees against yeasts and moulds, combining refrigeration with these permitted antimicrobial agents. Benzoates are the most popular preservative agent in quince puree that can be found until 1.5 g/Kg of final product.[1]

Typical quince jam should be prepared with genuine puree. Nevertheless, for agricultural reasons, in years in which quince production is low, quince jam is frequently adulterated by addition of apple (*Malus communis* Lamk) and pear (*Pirus communis* Lin.) purees.

By employing the phenolic analysis of genuine purees of these three fruits we were able to previously demonstrate the usefulness of its phenolic diagrams for the determination of genuineness of quince jam.[2]

After previous studies in commercial brands of quince jam to test the validity of our previous study and after having made several attempts to identify some visible unknown peaks in the chromatograms it was possible to observe a peak corresponding to sodium benzoate.

In spite of the several methods[3-7] that have been published to quantify benzoates we found it helpful to present this paper, where we describe a useful methodology for simultaneous quantification of sodium benzoate and evaluation of the authenticity of quince jam.

MATERIALS AND METHODS

Quince Jam Samples and Standards

Quince jams were purchased in the Portuguese market. In order to study the possibility of co-elution of sodium benzoate with other compounds, one quince jam sample was prepared by us with quince puree and sugar (50:50).

The standards were from Sigma (St. Louis, MO, USA) and from Extrasynthése (Genay, France). 3- and 4 -*O*-caffeoylquinic acids were not commercially available, so they were prepared by transesterification of 5-*O*-caffeoylquinic acid using tetramethylammonium hydroxide.[8,9] HPLC grade methanol and formic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Extraction of Sodium Benzoate and Phenolic Compounds from Quince Jam

Each jam sample (ca. 40 g) was thoroughly mixed with methanol (≈350 mL), until there was a negative reaction with NaOH. The extract was then filtered and evaporated to dryness under reduced pressure (40°C), redissolved in methanol (10 mL) and 20 µL were analyzed by HPLC.

HPLC Analysis of Sodium Benzoate and Phenolic Compounds

Separation of compounds was achieved as described previously[2] with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 x 0.46 cm; 5µm, particle size) column. The solvent system used was a gradient of water-formic acid (19:1) (A) and methanol (B). The best resolution was obtained at a solvent flow rate of 0.9 mL/min, starting with 5% methanol and installing a gradient to obtain 15%B at 3 min., 25%B at 13 min., 30%B at 25 min., 35%B at 35 min., 45%B at 39 min., 45%B at 42 min., 50%B at 44 min., 55%B at 47min., 70%B at 50 min., and 75%B at 56 min. Detection was achieved with a diode array detector, and chromatograms were recorded at 280 nm. The compounds in each sample were identified by comparing their retention times and UV-vis spectra in the 200-400 nm range with the library of spectra previously compiled by the authors.

RESULTS AND DISCUSSION

Analytical Curve and Detection Limit

Under the assay conditions described, a linear relationship between the concentration of sodium benzoate and the UV absorbance at 280 nm was obtained. This linearity was maintained over the concentration range 1.6-2000 µg/mL. The correlation coefficient for the standard curve exceeded 0.999. The calibration curve was obtained by triplicate determinations of each level, the peak area values (arbitrary units) were plotted as average values. The relative

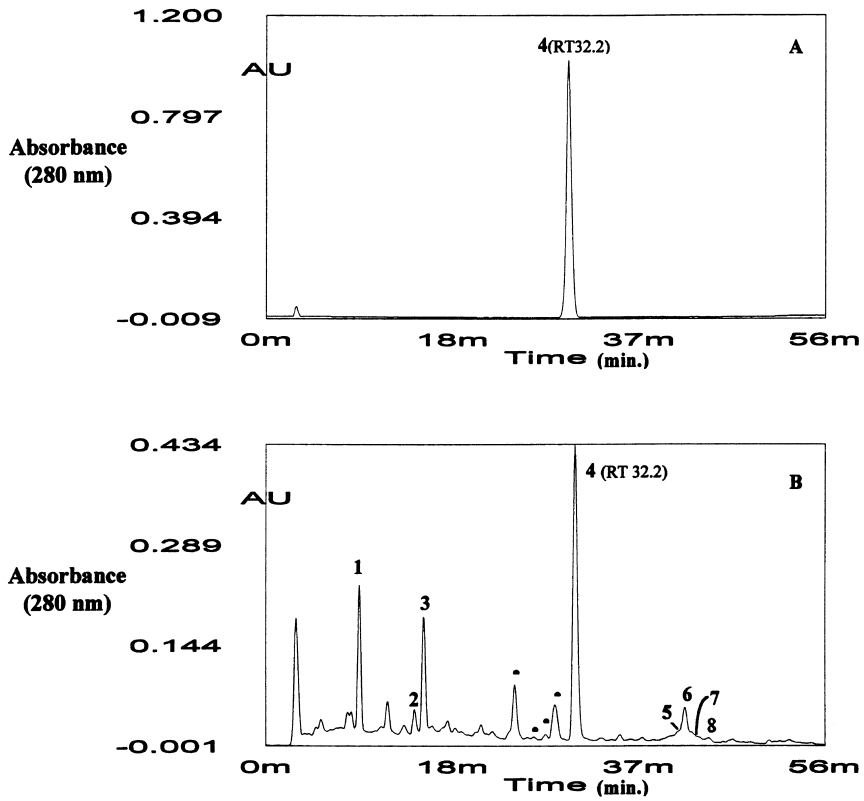

Figure 1. HPLC profiles of a standard solution of sodium benzoate (A) and of a quince jam extract (B). Detection at 280 nm. (1) 3-*O*-caffeoylquinic acid; (2) 4-*O*-caffeoylquinic acid; (3) 5-*O*-caffeoylquinic acid; (4) Sodium benzoate; (5) Rutin; (6) quercetin 3-galactoside; (7) quercetin 3-xyloside; (8) quercetin 3-rhamnoside. •unidentified characteristic glycosides of procyanidin polymers.

percent average deviations of triplicates were less than 2% in all cases. The average regression equation for sodium benzoate was, $y = 6.7 \times 10^6 x + 76569.56$. The detection limit value was calculated for sodium benzoate as the concentration corresponding to three times the standard deviation of the background noise and was 0.5 μg/mL.

Validation of the Method

The chromatogram obtained for standard sodium benzoate is shown in Figure 1-A. As an example, Figure 1-B shows the HPLC profile of a quince jam

Table 1

Sodium Benzoate Content in Quince Jam Samples[a]

Samples	Sodium Benzoate g/100g ± SD
A	0.074 ± 0.003
B	0.030 ± 0.004
C	0.026 ± 0.001
D	0.120 ± 0.003

[a] Values are expressed as mean ± SD of two determinations.

sample. The retention times (RT) obtained for sodium benzoate and each phenolic compound were: RT 9.6 min. for 3-*O*-caffeoylquinic acid; RT 15.3 min. for 4-*O*-caffeoylquinic acid; RT 16.2 min. for 5-*O*-caffeoylquinic acid; RT 32.2 min. for sodium benzoate; RT 42.0 min. for rutin; RT 42.3 min. for quercetin 3-galactoside; RT 44.3 min. for quercetin 3-xyloside and RT 45.5 min. for quercetin 3-rhamnoside. The unidentified compounds (•) had identical UV spectra when recorded with a diode-array detector (identical shape and maximum at 269.3 nm). The possibility of being glycosides of procyanidin polymers is not excluded in accordance with the Porter et al. *s*tudy,[10] their chromatographic behaviour, and their UV spectra.

The analysis of the chromatogram obtained with the quince jam sample prepared by us (without preservatives) showed no peaks at RT 32.2 min.. It was concluded that in commercial jams sodium benzoate did not co-elute with other compounds.

Results from the quantification of benzoate sodium applied to four quince jam samples are shown in Table 1. The precision of the analytical method was evaluated by measuring the peak chromatographic area of sodium benzoate of the same sample 6 times. The standard deviation was 0.003 and the coefficient of variation was 2.76 %.

In order to study the recovery of the procedure, one quince jam sample was added to known quantities of sodium benzoate. The sample was analyzed in triplicate before and after the addition of sodium benzoate. Thus, this procedure demonstrated the effectiveness of the extraction and the accuracy of the proposed method. The results are listed in Table 2. Recovery values were between 94.7 and 100.0 %.

Table 2

Recovery of Sodium Benzoate from a Spiked Quince Jam Sample

Present (g/100g)	Added (g/100g)	Found[a] (g/100g)	Standard Deviation	CV%	Recovery %
	0.010	0.084	0.003	3.1	100.0
0.074	0.040	0.108	0.004	3.2	94.7
	0.080	0.151	0.007	4.4	98.1

[a] Mean value found for 3 assays for each studied concentration.

In conclusion, this study suggests that the technique presented herein is quite useful for the simultaneous analysis of phenolic compounds and sodium benzoate in quince jam samples, allowing the separation and quantification of the main quince jam phenolic acids (cynnamic acids and their derivatives), flavonoids (flavonol glycosides), glycosides of procyanidin polymers and the preservative sodium benzoate.

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