SPECTRAL IDENTIFICATION AND HPLC QUANTIFICATION OF BENZOIC ACID FROM NATURAL JUICES

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Abstract. Benzoic acid from four natural juices was investigated in this paper. The identification of benzoic acid was achieved with FTIR using ATR technique. A high-performance liquid chromatography (HPLC) with photodiode array detection (PDA) method was developed for determination of benzoic acid. The highest concentration of benzoic acid was found in orange juice in the range of 71 mg·L^{-1}. Precision, linearity, sensitivity (limit of detection and limit of quantitation) and uncertainty were established. The obtained results suggested that ATR and HPLC are suitable methods for the identification and quantification of benzoic acid in natural juices.

Keywords: Benzoic acid, FTIR, HPLC, natural juices

1. INTRODUCTION

Phenolics are an important constituent of fruit quality because of their contribution to the taste, colour and nutritional properties of fruit [1].

Plant phenolics include phenolics acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids, tannins and the less common stilbenes and lignans [2].

Benzoic acid (C_6H_5–COOH), the simplest aromatic carboxylic acid, is present naturally either free or bound as benzoic acid esters and can be found in many natural products [3].

Benzoic acid is extensively used as preserving agent most suitable for foods, fruit juices, and soft drinks. It is generally effective in controlling mould and inhibiting yeast growth and also in preventing a wide range of bacterial aggressions. Although this prevents or delays nutritional losses due to microbiological, enzymatic, or chemical changes of foods during its shelf life, it is harmful at higher than permitted safety levels [4].

In humans, the acute toxicity of benzoic acid is low, this substance is known to cause non-immunological contact reactions (pseudo allergy). Cases of urticaria, asthma, rhinitis, or anaphylactic shock have been reported following oral, dermal, or inhalation exposure to benzoic acid and sodium benzoate. The symptoms appear shortly after exposure and disappear within a few hours, even at low doses [5].

Analytical methods for the determination of benzoic acid include spectrophotometric methods [6], which need extensive extraction procedures and are not very specific; gas chromatographic (GC) methods [7], which are more sensitive and specific but need lengthy sample preparation and derivatization prior to determination; and high-performance liquid chromatography (HPLC)[8], which has a high specificity and minimum sample preparation and does not require derivatization.

2. MATERIALS AND METHODS

Chemicals and materials

All reagents: acetic acid (CH_3COOH) glacial, methanol (CH_3OH), for HPLC, ammonium acetate (CH_3COONH_4), 0.01 mol/L solution, potassium hexacyanoferrate (II) trihydrate, $K_4[Fe(CN)_6]\cdot 3H_2O$, zinc sulfate heptahydrate, ($ZnSO_4\cdot 7H_2O$), 300 g/L solution used were of analytical grade. Benzoic acid was purchased from Sigma-Aldrich.

Four commercial juices (two orange juices, one multivitamin juice and one berry juice) were purchased from retail stores.

ATR analysis

Spectra were recorded on a Perkin Elmer Spectrum GX device type, the field 4000-600 cm^{-1} with 32 scans and a resolution of 4 cm^{-1}. Each standard and each sample were recorded three times and was a very good reproducibility of the spectra. Standard spectrum for benzoic acid was compared with spectra library of the instrument and a very good fitting was obtained.

Attenuated total reflectance, ATR, is an infrared spectroscopy analytical technique most used in recent years, according to the literature [9]. ATR allows analyze samples without any previous preparation and high speed of analysis. The lack of sample preparation for analysis and penetration depths to reach an all too small incidence of the sample, a few μm, placed on a diamond crystal, which has a high refractive index, are the main advantages of this analytical technique. Penetration depth is proportional to the incident beam wavelength and therefore increase the penetration depth is observed at wavelengths greater, so the smaller wave numbers.

The intensity of radiation absorbed depends on the quantity of sample comes in contact with the diamond surface and the number of contact points that radiation has the sample. By increasing the number of reflections by pressing the sample, IR absorption intensity increases and thus the spectrum intensity. Liquid or fine powder

samples have a perfect contact with diamond surface and that is these types of samples show no obstacle to the analysis. By this analytical method was analyzed both benzoic acid as reference material and four kinds of fruit juices, namely two oranges, one berry and one multivitamin.

HPLC analysis

Sample preparation

Extraction conditions were nearly similar to those presented in reference [11]. A sample volume of 10 ml was mixed with 75 ml of extraction solution (60 volume parts of ammonium acetate/acetic acid buffer solution with 40 volume parts of methanol) in a 100 ml volumetric flask. The content was introduced in a ultrasonic bath for at least 10 min and then dilute to the mark with extraction solution at 20°C. Then were added 1.0 ml of Carrez solution I and 1.0 ml of Carrez solution II for clarification. The solution was passed through a paper filter. Prior to injection, the clear extracts were filtered through 0.45-µm.

Chromatographic system

The chromatographic system consisted of an Agilent 1100 Series HPLC instrument equipped with a quaternary pump, a degasser, an autosampler, a diode-array detector and a Agilent Chemstation for data acquisition and analysis. Twenty microliters of the sample were injected with the autosampler. A Kromasil 100-5C18 column (250 mm×4.6 mm, 5µm) was used for the chromatographic analysis and the column temperature was set at 25 ^{0}C.

The benzoic acid analysis was performed with a binary solvent system ammonium acetate/acetic acid (A) and methanol (B): 50:40 (pH = 4.5 - 4.6) with a flow rate of 1 ml/min. Chromatograms were recorded at 235 nm.

3. RESULTS AND DISCUSSIONS

ATR analysis

By ATR FTIR analytical method was analyzed benzoic acid as reference material. Also by this method were analyzed four kinds of fruit juices, namely two oranges, one berry and one multivitamin. Both standards and fruit juices have been deposited on the diamond window and dried in air stream at ambient temperature, where appropriate.

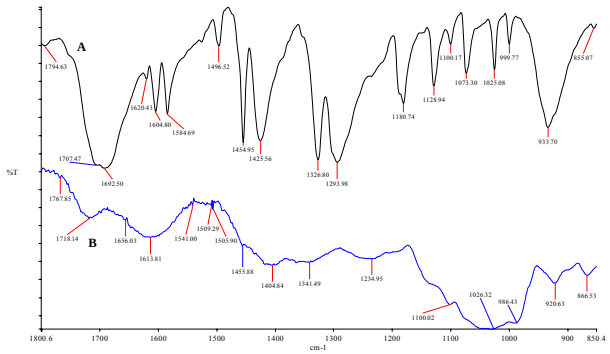

Figure 1. Benzoic acid ATR spectra

A – ATR spectrum of Benzoic Acid

B – ATR spectrum for Orange Juice sample

The significant infrared bands and their assignment are: O-H group identified by the region between 2500-3300 cm^{-1} and an absorption around 1300 cm^{-1}, C=O group was identified by an absorption between 1680-1750 cm^{-1}, stretch vibration frequencies of C = C of the benzene nucleus at 1506 cm^{-1} and 1454 cm^{-1} and absorption around 900-1100 cm^{-1} could be due to carbon-oxygen bound of the acid grouping. In all analyzed juices benzoic acid prevailing in orange juices, berries and multivitamin juices having a low amount of this preservative. Noteworthy is good correlation with HPLC analysis and spectrophotometric analysis, spectral method confirming the presence of benzoic acid in these juices.

HPLC analysis

For HPLC method validation the following performance parameters were calculated: precision, linearity, detection limit, quantification limit, also the expanded uncertainty.

The precision of the analytical method was evaluated by measuring the peak chromatographic area of benzoic acid six time on the same sample. The coefficient of variation obtained, RSD %, was 0.33%.

The external standard method was the technique used for quantification. Peak areas from HPLC chromatogram were plotted against the known concentrations of stock solutions of varying concentrations.

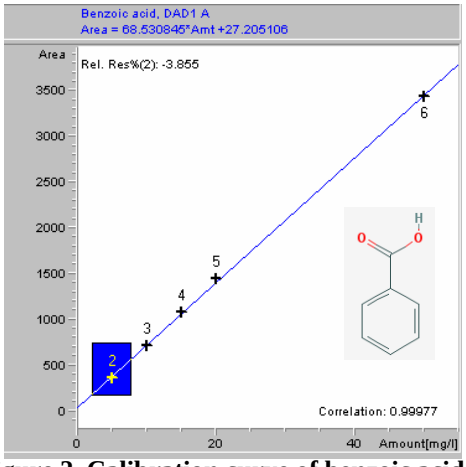

Figure 2. Calibration curve of benzoic acid

Benzoic acid structure according with PubChem Substance [15].

Under the assay conditions described, a linear relationship between the concentration of benzoic acid and the UV absorbances at 235 nm was obtained. The correlation coefficient for standard curve was 0.9997.

The detection limit (LOD) and quantification limit (LOQ) values were calculated based on standard deviation of the response and the slope of the calibration curve [13].

Detection limits was 0.97 mg·L^{-1} and quantification limit was 2.94 mg·L^{-1}.

In order to obtain the overall uncertainty each uncertainty source was estimated. The combined uncertainty u_c was calculated by combining standard

uncertainties. The overall uncertainty U was obtained with formula $U = k \cdot u_c$, k=2 for a level of confidence of 95% [12].

Concentrations of benzoic acid were determined from the calibration curve established using synthetic standards. It can be seen that orange juice was the most abundant sample with amount of benzoic acid in the range of 71 $mg \cdot L^{-1}$.

Table 1.Benzoic acid content in juice samples

Sample info	Benzoic acid, $mg \cdot L^{-1}$	U (k=2) $mg \cdot L^{-1}$
Orange juice 1	70.7	± 3
Orange juice 2	63.7	± 3
Multivitamin juice	<LOD	-
Berries juice	<LOD	-

The results obtained are in concordance with data reported [10] or [11] for this kind of sample.

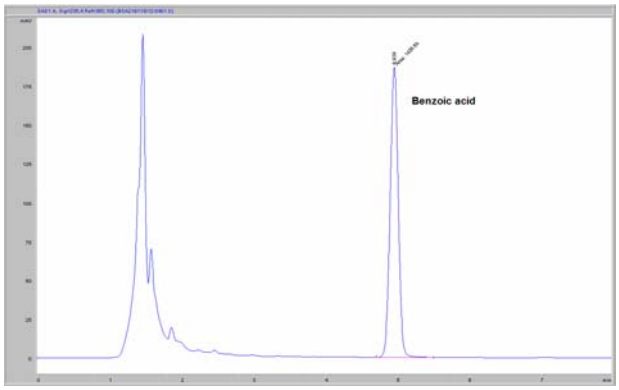

Figure 3. HPLC Benzoic Acid profile of juice sample. Detection of 235 nm; Retention time 4.939 min.

As an example, Figure 3 shows the HPLC profile of a juice sample. Results from quantification applied of juice samples are shown in Table 1. The highest concentration of benzoic acid was found in orange juice in the range of 71 $mg \cdot L^{-1}$ being in accordance with the maximum limits allowed by EU directive [14].

Conclusion

The developed method is suitable for determination of benzoic acid in juice samples. The application of the measurement system was demonstrated by analyzing four samples. The method provides good sensitivity (detection limits of 1 $mg \cdot L^{-1}$). The benzoic acid in the juice sample was separated by reverse phase chromatography on a Kromasil C18 column, detected by absorbance at the wavelength of 235 nm and quantified with calibration graph. Methods can be applied for assessing concentrations of benzoic acid from different varieties of juices.

4. ACKNOWLEDGMENTS

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