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Solid-phase extraction procedure to remove organic acids from honey

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Abstract

A solid-phase extraction procedure was applied to remove organic acids from honey. Malic, maleic, citric, succinic and fumaric acids were isolated with an anion-exchange cartridge. The different parameters which affected the extraction procedure were studied and optimised to establish the optimal conditions for maximum recovery of organic acids and minimum extraction of interferences. The optimised procedure used a cartridge which was activated with 10 ml of 0.1 *M* sodium hydroxide solution (percolation rate 3 ml/min). A 10 ml volume of honey solution was passed at a flow-rate of 0.5 ml/min. The cartridge was washed with 10 ml of water (3 ml/min) and organic acids were eluted with 4 ml of 0.1 *M* sulfuric acid (0.5 ml/min). This solution was injected directly into the chromatograph. When this procedure was carried out on standard solutions of organic acids, recoveries between 99.2 and 103.4% were found. If this procedure was applied to honey samples these recoveries were also satisfactory and ranged from 62.9 to 99.4%.

Keywords: Honey; Organic acids

1. Introduction

Sample preparation is a key procedure in modern chemical analysis. Solid-phase extraction (SPE) is one of the simplest, yet most effective and versatile, methods of sample preparation. Utilizing low cost, prepacked, disposable cartridges, a sample component of interest is separated from other species by applying the sample mixture to a solid chromatographic sorbent and selectively eluting the desired components [1].

Organic acids are components of several food. In the majority, they are found in low concentrations, so an extraction procedure is necessary for their later determination. SPE is very useful for isolating and concentrating these organic acids [2–7].

In honey, organic acids are also minor constituents but contribute to the flavour of honey [8]. They could be also used as indicators of deterioration on account of storage, aging or even to measure purity and authenticity. The organic acids, which were studied in this work (malic, maleic, citric, succinic and fumaric acids), were found in honey samples in quantities of mg/kg. The high-performance liquid chromatography (HPLC) determination of these organic acids in honey was difficult because of the low concentration and the interferences [9]. Several authors have been carried out SPE procedures to remove organic acids from honey [9–11].

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The purpose of this paper was to apply an SPE procedure to remove organic acids from honey.

2. Experimental

2.1. Reagents and materials

Analytical-standard grade malic, maleic, citric, succinic and fumaric acids were obtained from Sigma (St. Louis, MO, USA). Stock standard solutions were obtained by dissolution of acids in Milli-Q water and were stored at 4 °C for 1 month. The Milli-Q water was purified by passage through a Compact Milli-RO and Milli-Q water system from Millipore (Milford, MA, USA). Working standard solutions were prepared daily by dilution with Milli-Q water. Metaphosphoric acid, sulfuric acid and sodium hydroxide pellets were analytical-reagent grade and supplied by Merck (Darmstadt, Germany).

The eluent was filtered with membrane filters (0.45 μm, AFO-0504) from Phenomenex (CA, USA).

2.2. Apparatus

The HPLC system consisted of a Waters liquid chromatograph equipped with a Waters ILD on-line degasser, a Waters 600E pump, a Waters 717 plus autosampler and a Waters 996 diode-array UV detector (Waters, Milford, MA, USA).

The column was a Spherisorb ODS-2 S5 (particle size 5 μm, 250×4.6 mm I.D.) from Waters.

The data acquisition system was the Chromatography Data System Millennium 32 from Waters.

SPE cartridges—Waters C_{18} and Accell Plus QMA—were also from Waters.

The filtration system consisted of membrane filters (0.45 μm, AFO-0504) from Phenomenex.

2.3. Assay procedure

A solution of honey (7.50 g in 100 ml of Milli-Q water) was adjusted to pH 10.50 with 0.1 *M* NaOH and stirred for 15 min at room temperature. Then, this solution was adjusted to pH 5.00 with 0.1 *M* H_2SO_4. This procedure is carried out to avoid interferences in the baseline.

A 10 ml volume of this solution was filtered through a 0.45 μm cellulose acetate membrane and SPE applied. This procedure involved an ion-exchange cartridge. The cartridge was activated with 10 ml of 0.1 *M* sodium hydroxide solution (percolation rate 3 ml/min) passed at a flow-rate of 0.5 ml/min. The cartridge was washed with 10 ml of water (3 ml/min) and the organic acids were eluted with 4 ml of 0.1 *M* sulfuric acid (0.5 ml/min). This solution was injected directly into the chromatograph.

2.4. Chromatographic conditions

Chromatographic separation was achieved with the Spherisorb ODS-2 S5 column thermostated at 25 °C. The procedure was carried out isocratically using 4.5% metaphosphoric acid (pH 2.20) as the eluent at a flow-rate of 0.7 ml/min. Previously it was filtered through membrane filters (0.45 μm). This mobile phase must be prepared fresh daily. The injection volume was 20 μl. Standards and honey samples were injected in triplicate. Organic acids were detected at 215 nm.

3. Results and discussion

The proposed SPE procedure allowed the isolation of a standard solution of malic, maleic, citric, succinic and fumaric acids with recovery values comprised between 99.2 and 103.4% (Table 1). Fig. 1 shows a chromatogram of a standard solution of organic acids submitted to the proposed solid-phase extraction procedure.

When this procedure was applied to honey samples, recovery results were from 62.9 to 99.4%

Table 1
Recoveries of a mixture of standards of organic acids after solid-phase extraction procedure (n=3)

Organic acid	Mean (%)±SD	RSD (%)
Malic	101.8±0.18	0.18
Maleic	103.3±0.099	0.10
Citric	100.8±0.085	0.08
Succinic	99.2±0.34	0.34
Fumaric	103.4±1.43	1.38

Mean values±standard deviations.

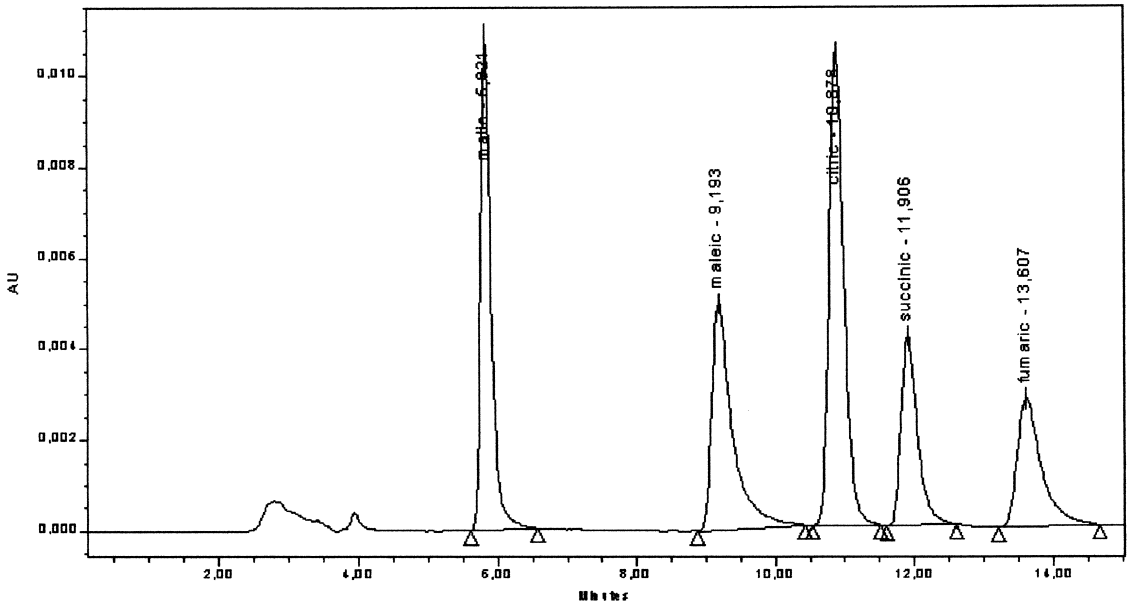

Fig. 1. Chromatogram of a solution of standards of organic acids submitted to the proposed solid-phase extraction procedure.

(Table 2). Fig. 2 shows a chromatogram of organic acids extracted from a honey sample by the same procedure.

The influence of parameters that potentially affected the extraction process was studied in order to establish the optimal conditions for maximum recovery of organic acids and minimum extraction of interferences.

3.1. Cartridge

A solid-phase extraction with a C_{18} cartridge and an anion-exchange cartridge was assayed. A mixture of standards of organic acids was dissolved in a 4.5% metaphosphoric acid solution (pH 2.2, acidic solution) and in Milli-Q water.

To hold organic acids in the C_{18} cartridge they must be in their non ionic forms. Although acidic conditions were expected to be the best conditions with this cartridge, the recoveries for the C_{18} cartridge were better when the organic acids were dissolved in Milli-Q water. Nevertheless, the obtained results were unsatisfactory with this C_{18} cartridge (Fig. 3).

To hold organic acids in the anion-exchange cartridge they must be in ionic form so the best results were achieved with organic acids dissolved in Milli-Q water. These conditions were selected because of the best recoveries for all organic acids (Fig. 3).

Table 2
Analytical recovery of organic acids added to honey after solid-phase extraction procedure ($n=3$)

Organic acid	Mean (%)±SD	RSD (%)
Malic	62.9±4.4	7.0
Maleic	93.4±8.2	8.8
Citric	99.4±1.5	1.5
Succinic	75.0±5.0	6.7
Fumaric	94.4±4.6	4.9

Mean values±standard deviations.

3.2. Activation solution

As activation solutions methanol, sodium hydroxide, 0.05 *M* ammonium dihydrogenphosphate and acetic acid at different pH values were used. Methanol generated separation interferences when the procedure was applied to a honey sample. Ammonium dihydrogenphosphate (0.05 *M*) solution was used by other authors [10] but recoveries obtained in

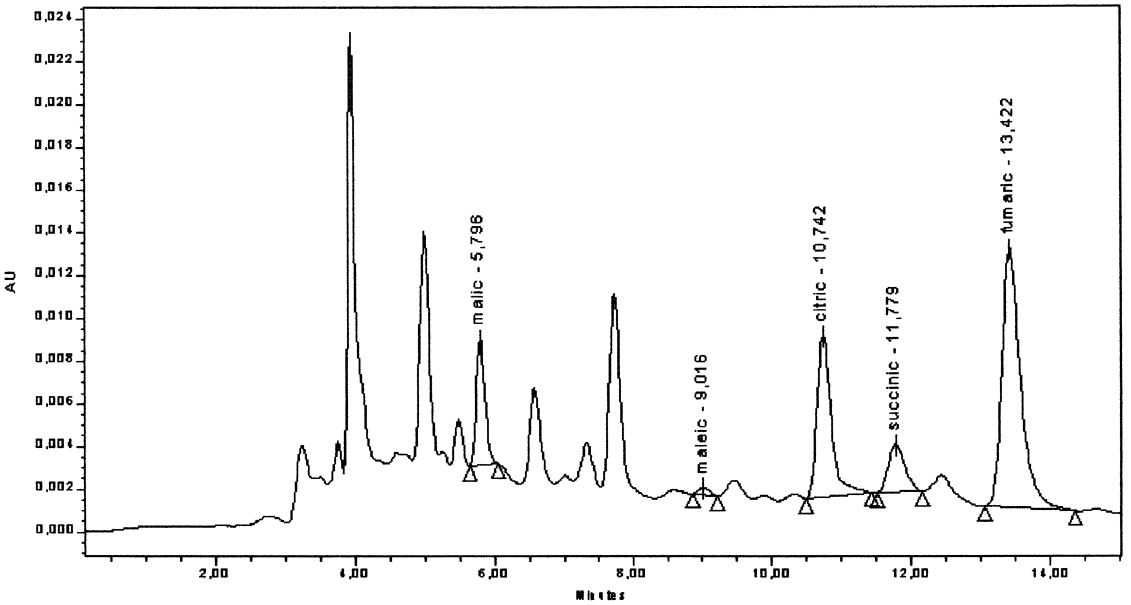

Fig. 2. Chromatogram of organic acids extracted from a *Castanea sativa* honey by the proposed procedure.

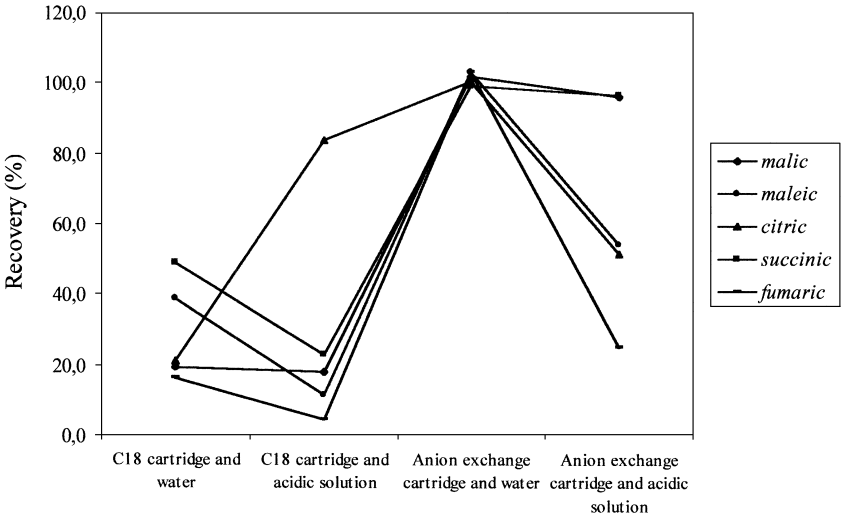

Fig. 3. Recovery results (%) obtained with a C_{18} cartridge and with an anion-exchange cartridge after submitting a mixture of standards of organic acids to the extraction procedure. The mixture was dissolved in Milli-Q water and in an acidic solution (metaphosphoric acid 4.5%).

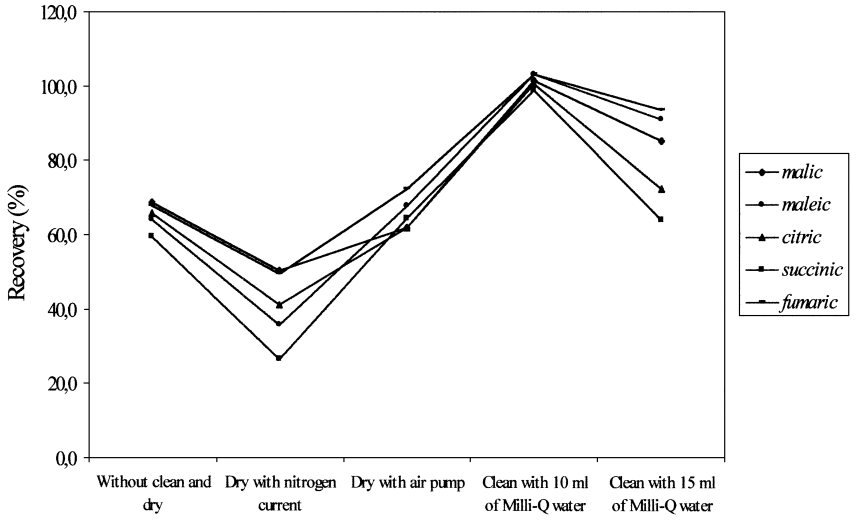

Fig. 4. Recovery results (%) obtained with a mixture of standards of organic acids submitted to different washing procedures.

standard solution of organic acids were very low for malic (85.4%) and fumaric acid (36.4%). Results obtained with acetic acid solutions at different pH values [11] were not reproducible. By using sodium hydroxide solutions at several concentrations the best results were obtained. When the concentration of sodium hydroxide increased recovery results decreased so a 0.1 *M* solution of sodium hydroxide was selected as the activation solution.

3.3. Washing procedure

To remove interferences several procedures of cleaning the cartridge were carried out. Fig. 4 shows

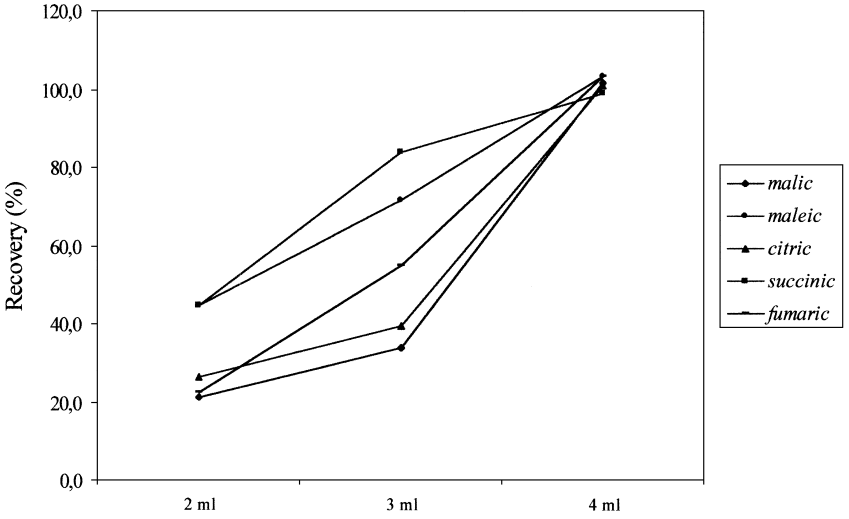

Fig. 5. Recovery results (%) obtained with a mixture of standards of organic acids submitted to different elution volumes.

the recovery results obtained with a mixture solution of standards of organic acids submitted to these different washing procedures. The cartridge was dried with a nitrogen current [2] and with a compression air pump [6,11] but recovery results were not satisfactory. The cartridge was also cleaned with different volumes of water. The best recoveries were obtained cleaning the cartridge with 10 ml of Milli-Q water. Higher volumes (for example, 15 ml) did not lead to higher recoveries.

3.4. Elution solution

To elute the organic acids from cartridge, solutions of sulfuric and metaphosforic acids were used. Sulfuric acid was selected for removing organic acids from the cartridge because it was stronger than metaphosphoric acid.

With regard to the elution volume several quantities were assayed. It was observed that volumes lower than 4 ml did not allow the total recuperation of the acids. The highest recovery obtained with 2 ml of sulfuric acid was 44.9% for maleic acid. Volumes higher than 4 ml gave the same recovery results but diluted the sample unnecessarily (Fig. 5).

The concentration of eluting sulfuric acid did not have a great influence on the recovery, so a value of 0.1 *M* was selected.

3.5. Percolation rate

First, assays were made without a percolation rate control and no reproducible results were obtained. It was also observed that the percolation rates of sample solution and sulfuric acid must be low because fast percolation rates decreased the acid recoveries. After different assays, 0.5 ml/min for sample solution and acids elution and 3.0 ml/min for activation and washing procedures were selected.

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