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Analytical Methods

Fast HPLC-DAD quantification of nine polyphenols in honey by using second-order calibration method based on trilinear decomposition algorithm

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ABSTRACT

This paper describes the use of second-order calibration for development of HPLC-DAD method to quantify nine polyphenols in five kinds of honey samples. The sample treatment procedure was simplified effectively relative to the traditional ways. Baselines drift was also overcome by means of regarding the drift as additional factor(s) as well as the analytes of interest in the mathematical model. The contents of polyphenols obtained by the alternating trilinear decomposition (ATLD) method have been successfully used to distinguish different types of honey. This method shows good linearity ($r > 0.99$), rapidity ($t < 7.60$ min) and accuracy, which may be extremely promising as an excellent routine strategy for identification and quantification of polyphenols in the complex matrices.

1. Introduction

Honey is a wholesome natural food product well known for its high nutrition. It has been demonstrated that honey is similar to many fruits and vegetables in antioxidant activity (Gheldof & Engeseth, 2002). It could be incorporated into foods as natural preservatives, preventing lipid oxidation in meat (Nagai, Inoue, Kanamori, Suzuki, & Nagashima, 2006) as well as the browning reaction in fruits and vegetables (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000). The antioxidant ability of a number of honeys has been determined and found to be significantly correlated to the contents of polyphenols (Gheldof, Wang, & Engeseth, 2002). Polyphenols are natural antioxidants with a wide variability of structures and chemical characteristics. It is very meaningful to detect the contents of polyphenols in honeys for application purposes. Not only do polyphenols affect quality of honeys and their products, but their antioxidant activity may be beneficial for improving overall health and preventing some diseases, especially atherosclerosis, tumor, cancer and heart attack (Marini et al., 2011; Nardini & Ghiselli, 2004). Moreover, polyphenols are also used as an indicator for the geographical and botanical origin of honeys (Anklam, 1998), and to discriminate the variety of apples (Chen et al., 2011).

Numerous studies trying to analyse polyphenols in biological and food matrices have been reported in recent years, which are reviewed by Alvarez-Suarez, Tulipani, Romandini, Vidal, and Battino (2009) and Pyrzynska and Biesaga (2009). The analysis is regularly achieved on high-performance liquid chromatography with diode array detection (HPLC-DAD), which possesses a number of advantages such as general applicability of HPLC to a wide range of analytes and second-order data output of HPLC-DAD (Chen et al., 2011; Marini et al., 2011; Regos & Treutter, 2010; Vosough & Salemi, 2011). However, in the chromatographic analysis of complex samples, such as some environmental, biological and food matrices, baseline drift and peaks overlapping between matrix constituents and the compounds of interest often occur. In these cases, a lot of trivial pretreatments are necessary to separate the interfering compounds from analytes of interest, for example, solid phase extraction (Kaškonienė, Maruška, & Kornyšova, 2009; Michalkiewicz, Biesaga, & Pyrzynska, 2008). Moreover, it may take a long time and/or complex mobile phase to isolate each of the analytes to provide accurate results. But these involve time-consuming and consuming large amount of organic solvents and so on. Also the separation strongly depends upon the sample matrix.

The development of multivariate calibration techniques, especially second-order calibration methods, may bridge the gap by mathematically decomposing the overlapping profile into the pure profiles of each chemical species even the unknown interference

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(Goicoechea, Culzoni, Gil García, & Martínez Galera, 2011). This property is called 'second-order advantage' (Sanchez & Kowalski, 1986, 1990). This fact means that HPLC coupled with second-order calibration methods can use faster elution leading to an incomplete experimental separation, yet achieving accurate quantification of the analytes of interest in complex matrices. This strategy has been widely applied recently (Tan, Tan, Zhao, & Li, 2011; Vosough & Salemi, 2011). It allows one to save time and money, and reduce the use of harmful chemical solvents.

Honey samples have been shown a complex background. It contains at least 181 substances and it is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes and vitamins (Chow, 2002; Pérez, 2002; Terrab et al., 2003). So it is difficult to separate all the interfering compounds from analytes of interest by traditional chromatographic methods. Following the approach combining HPLC-DAD with a second-order calibration method based on the alternating trilinear decomposition (ATLD) algorithm, an accurate method was developed to identify and detect the real contents of polyphenols in honey. Indeed, when an isocratic elution was used, highly overlapping peaks among analytes of interest and the interferences in the complex matrix would occur. However, ATLD algorithm can supply accurate prediction of concentrations together with reasonable resolution of chromatographic and spectral profiles for polyphenols owing to the 'second-order advantage'. The baselines drift is also removed by means of regarding it as additional factor(s) as well as the analytes of interest in the mathematical model. With the quantitative results obtained by ATLD algorithm, the relationship between the contents of the polyphenols and honey varieties was also studied. The efficiency of the method was validated by figures of merit and statistic parameters. The results showed that the chromatographic and spectral profiles of each analyte were free from interference of sample matrix and the concentrations were estimated approvingly.

2. Materials and methods

2.1. Chemicals

Five standards of protocatechuic acid, ferulic acid, gallic acid, chlorogenic acid and rutin were from the National Institute for Control of Biological and Pharmaceutical products (Changsha, China). Three standards of syringic acid, *p*-coumaric acid and caffeic acid were from Aladdin (Shanghai, China). The standard of *p*-hydroxybenzoic acid was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Stock solutions (117–819 μg ml^{-1}) were prepared in methyl alcohol. Standard working solutions were prepared by spiking stock solutions into 10 ml of mobile phase solution (methyl alcohol–formic acid in water (pH 2.54), 43:57, v/v) with concentrations range from 2.78 to 32.8 μg ml^{-1} and stored at 2.0 °C. Methyl alcohol (TEDIA Company, USA) and formic acid (Adamas Reagent Co., Ltd, China) were of HPLC grade.

2.2. HPLC analysis

Liquid chromatography was performed on a LC-20AT liquid chromatographic system (Shimadzu, Japan), equipped with a diode array detector (DAD). The analytical column used was a Wonda-Sil™ C_{18} column (150 × 4.6 mm, 5.0 μm). The operating conditions were as follows: column temperature, 30 °C; flow rate, 1.00 ml/min; injection volume, 20 μl; monitoring wavelength range, 190–450 nm.

The nature of analytes, especially phenolic acid, requires the use of acidic mobile phase in order to get satisfactory peak shape. Generally formic acid (Guillarme, Casetta, Bicchi, & Veuthey, 2010; Simirgiotis & Schmeda-Hirschmann, 2010), acetic acid (Boros et al., 2010; Marini et al., 2011) or phosphoric acid (Zgórka & Kawka, 2001) are used to adjust the pH value of the mobile phase. In our work, formic acid was chosen to obtain suitable pH for assurance of good conditions. A satisfactory chromatographic behaviour was obtained with a mobile phase consisting of methyl alcohol, pH 2.54 formic acid in water (43:57, v/v).

2.3. Sample preparation

2.3.1. Calibration samples

For sake of simplicity of solution preparation, the concentration levels of each analyte in calibration set were designed by adding standard working solution as follows: 0, 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, 0.700, 0.800, 0.900, 1.00 ml, and then constant volume to 10.0 ml with mobile phase solution. The concentrations of the calibration samples were randomly arranged within the linear range of each analyte and to avoid interference among the analytes of interest. ATLD algorithm was used to establish the calibration model for the nine substances. A fairly good performance was obtained in the prediction of the concentrations of the calibration set data, the linear correlation coefficient (R) was varying from 0.9949 to 0.9999 for all the substances, as shown in Table 1. Duplicate analysis was performed for each sample, and HPLC-DAD was measured in random order according to the sample number.

2.3.2. Honey samples

Five kinds of honey including two milk vetch honeys, one wild chrysanthemum honey, one jujube flower honey and one acacia honey were investigated in this report. They were collected from three popular manufacturers in China. Different weights of honey for each kind were diluted to 10.0 ml with mobile phase solution. For each variety of honey, five honey samples were constructed. These samples were homogenised by ultrasonic vibration for 30.0 min, filtered through 0.45 μm membranes and injected into the chromatographic system. To further validate the performance of the proposed method, recovery experiments were designed by

Table 1
Region, retention time, linear range and linear correlation coefficient (R) of each analyte in the calibration samples.

Analyte	Region	Retention time (min)	Linear range (μg ml^{-1})	R
Gallic acid	r-1	2.21	0–3.28	0.9987
Chlorogenic acid	r-2	2.70	0–1.19	0.9949
Protocatechuic acid	r-2	2.79	0–0.924	0.9994
Caffeic acid	r-3	3.70	0–0.676	0.9992
p-Hydroxybenzoic acid	r-3	3.79	0–0.656	0.9999
Syringic acid	r-3	3.96	0–0.936	0.9995
p-Coumaric acid	r-4	5.43	0–0.278	0.9994
Ferulic acid	r-4	5.74	0–0.343	0.9994
Rutin	r-5	6.94	0–1.18	0.9988