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Short communication

A study of retention characteristics and quality control of nutraceuticals containing resveratrol and polydatin using fused-core column chromatography

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ABSTRACT

A new high-performance liquid chromatography method using fused-core column for fast separation of resveratrol and polydatin has been developed and used for quality control of nutraceuticals with resveratrol and polydatin content. Retention characteristics (log k) were studied under different conditions on C-18, RP-Amide C-18, Phenyl–hexyl, Pentafluorophenyl (F5) and Cyano stationary phases for both compounds. The effect of the volume fraction of acetonitrile on a retention factors log k of resveratrol and polydatin were evaluated. The optimal separation conditions for resveratrol, polydatin and internal standard *p*-nitrophenol were found on the fused-core column Ascentis Express ES-Cyano (100 × 3.0 mm), particle size 2.7 μm, with mobile phase acetonitrile/water solution with 0.5% acetic acid pH 3 (20:80, v/v) at a flow rate of 1.0 mL/min and at 60 °C. The detection wavelength was set at 305 nm. Under the optimal chromatographic conditions, good linearity with regression coefficients in the range (r = 0.9992–0.9998; n = 10) for both compounds was achieved. Commercial samples of nutraceuticals were extracted with methanol using ultrasound bath for 15 min. A 5 μL sample volume of the filtered solution was directly injected into the HPLC system. Accuracy of the method defined as a mean recovery was in the range 83.2–107.3% for both nutraceuticals. The intraday method precision was found satisfactory and relative standard deviations of sample analysis were in the range 0.8–4.7%. The developed method has shown high sample throughput during sample preparation process, modern separation approach, and short time (3 min) of analysis. The results of study showed that the declared content of resveratrol and polydatin varied widely in different nutraceuticals according the producers (71.50–115.00% of declared content).

1. Introduction

Resveratrol (3, 5, 4′-trihydroxystilbene) is polyphenolic phytoalexin, which is produced by several plant species, such as grapes, mulberries and peanuts. It occurs in many plants, but grapes and related products are considered the most important dietary sources of the polyphenolic substances. Currently, resveratrol is usually extracted from red grapes and Japanese knotweed (*Fallopia japonica*, also known as *Polygonum cuspidatum*). Resveratrol exists in the *cis*- and *trans*- isomeric forms. The *trans*-form is the preferred steric form in nature and is relatively stable. It can undergo isomerisation to the *cis*-form when exposed to the ultraviolet irradiation. Usually, this polyphenol is present in dietary products in glycosylated forms known as polydatin (piceid, resveratrol-3-O-β-D-glucoside) [1]. Due to the relatively low level of resveratrol present in grape skin, other plants have been investigated as potential and commercially viable natural sources of resveratrol for the growing nutraceutical market. The rhizome or root of *F. japonica* is currently a major source for the natural resveratrol ingredients sold in dietary supplement markets worldwide [2]. It is well-known that different growth environments have an enormous impact on the content of active ingredients in plants. Therefore the natural plant extracts used in nutraceutical production can widely differ in total content of resveratrol.

Resveratrol and polydatin show cardioprotective properties, which are associated with their ability to exert vasorelaxation, anti-inflammatory response and scavenging of reactive oxygen species. The interest of these compounds has begun after the studies on the "French Paradox", in which cardioprotective effect of wine due to its polyphenolic composition, including resveratrol showed positive biological properties. Among other health benefits, resveratrol possesses anti-tumor, anti-diabetic, anti-neurodegenerative and anti-obesity activities [3,4]. Due to these properties resveratrol is used in commercial preparations, as dietary supplements and

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herbal products. Despite the benefits of resveratrol and polydatin in the civilization disease auxiliary treatment, only few analytical methods for quality control of these substances in nutraceutical products has been proposed.

Nutraceutical products together with functional food belong to the most rapidly growing sectors in the food and personal care product industry. This development is due to the loss of consumer confidence in the modern diet, the aging population, the increased trend toward self-medication, and finally, an overall enhancement in health awareness and disease prevention among customers. The main problem associated with nutraceutical products is their legal classification. Being neither food nor pharmaceuticals, they often inhabit a gray area between the two, which makes quality control and regulation very difficult [5]. The majority of consumers trust in the safety, positive biological activity and efficacy of these products. For these reasons, a quality control is required, and new modern analytical methodologies for quality control must be developed.

At present, liquid chromatography coupled with diode array detector [6,7], UV detector or tandem mass spectrometry [8–10], is the most used method for analysis of nutraceuticals. In HPLC, reversed-phase columns are the most commonly used to analyze phenolic compounds [11]. Up to now, analysis of resveratrol and polydatin in different matrices, such as wine [12–15], plasma [16], urine [17], aqueous solutions [18] or grapes [19] and nutraceuticals [20,21] has been accomplished mostly by using high-performance liquid chromatography. Several methods using C18 columns, detection wavelength in range of 303–310 nm and mobile phase consisting of diluted acetic acid and acetonitrile or methanol for analysis of resveratrol and polydatin have been described in the literature [12–16,18,19,22].

Fused-core particle columns have recently been introduced as an alternative to using sub-2 μm particles in chromatographic separations. Fused-core particles are composed of a 1.7 μm solid core surrounded by a 0.5-μm porous silica layer (total particle diameter is 2.7 μm) [23]. Porous layer is characterized by 9 nm pores, and its volume is about 87% of total particle volume. This technology allows to reduce mass transfer and to increase peak efficiency. Along with the improvement in peak efficiency, fused-core particle columns offer lower flow resistance in HPLC system, higher resolution, shorter analysis times, and lower limits of detection compared to traditional HPLC particles with 3–5 μm diameters. The Ascentis Express columns packed with 2.7 μm fused-core particles offer a really high-separation power with modest operating pressure. The performance achieved under both gradient and isocratic condition, is comparable to those obtained with totally porous sub-2 μm particles [24]. Therefore the using of fused core particle columns shows to be a good alternative to UHPLC (ultra high-performance liquid chromatography) technique to achieve better separation efficiency in common HPLC systems.

This paper presents for the first time a retention study of resveratrol and polydatin on different stationary phases (non-polar and middle polar) and new HPLC method for the determination of resveratrol and polydatin in nutraceuticals employing modern fused-core particle column and easy sample preparation. Developed method was validated and successfully applied for the determination of resveratrol and polydatin in different nutraceuticals on local market.

2. Materials and methods

2.1. Chemicals and Materials

Standards of resveratrol (purity 99.0%), polydatin (purity 95%) and organic solvents (gradient grade) acetonitrile, methanol and acetic acid were obtained from Sigma–Aldrich Chemie GmbH, Germany. Internal standard *p*-nitrophenol was obtained from Lachema, Czech Republic. The ultrapure water used for mobile phase preparation was purified through a Milli-Q system (Millipore, Bedford, MA, USA). All other materials were of analytical grade. The nutraceuticals with content of resveratrol and polydatin (tablets and capsules) were purchased on local market in the Czech Republic.

The analysed preparations are mentioned in Table 3 and are as follows: Resveratrol MAX (120 tablets, batch no. not available); A1-Resveratrol 800 RX (30 tablets, batch no. not available); Evelor Resveratrol 50 mg (30 capsules, batch no. FS6C02); Walmark Lecithin with resveratrol (90 tablets, batch no. MFG161110); Resveratrol Antiaging (60 capsules, batch no. 4599B1); and Indonal Partner for Woman (90 capsules, batch no. 0290113).

2.2. Equipment and chromatographic system

HPLC chromatographic apparatus consisted of a Shimadzu LC-10 system (Shimadzu Corporation, Kyoto, Japan), equipped with solvent delivery system LC-10 AD, with SIL-HTA autosampler, DGU-14A on-line degasser, SPD-M10A DAD detector, CTO-10 AC column oven and communication module. The system control, data acquisition and data evaluation were performed using the Shimadzu "LC Lab-Solution" software (Shimadzu Corporation, Kyoto, Japan).

2.3. Preparation of stock solutions and nutraceuticals preparation

Resveratrol and polydatin standard stock solution was prepared by dissolving 25 mg of resveratrol and 25 mg of polydatin into 25 mL of methanol. Standard stock solution was stored at −18 °C in dark. Standard stock solution was further diluted with methanol to obtain working standard solution in a concentration 25 μg mL^{-1} of resveratrol and 25 μg mL^{-1} of polydatin for method development and validation. Stock solution of internal standard was prepared by dissolving 1.0 g of *p*-nitrophenol into 100 mL of methanol. This solution was further diluted with working solutions of resveratrol and polydatin for method development and validation. The calibration standard solutions were prepared over the concentration range of 5–500 μg mL^{-1}, using ten calibration points.

Nutraceutical samples, 0.1–0.4 g of pulverized tablets or capsules content (depending on the type of commercial preparation), were accurately weighed into a 25 mL volumetric flask. Internal standard *p*-nitrophenol (2 mL) was added and the sample was subsequently filled to the mark with methanol. The mixture was extracted for 15 min with the help of ultrasound bath. This extracted sample solution was filtered through a 0.45 μm PTFE filter. A 25 μL of filtered solution was diluted with 975 μL of methanol directly into a glass vial. A 5 μL of this diluted sample solution was injected directly into the HPLC system. All food supplement samples and working standard solutions were prepared fresh daily.

2.4. Chromatography procedure

Analysis of all compounds-resveratrol, polydatin, and internal standard *p*-nitrophenol was carried out using simple isocratic elution that consisted of mobile phase acetonitrile/water solution of acetic acid pH 3 (20:80, v/v) that was pumped at a flow rate of 1.0 mL min^{-1}. Separation was performed on the fused-core column Ascentis Express ES-Cyano (100 × 3.0 mm), particle size 2.7 μm at 60 °C. The detector was set at 305 nm. The detection wavelength was selected according to the absorption spectra of resveratrol, polydatin and internal standard.