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Short communication

Development of RP HPLC method with gradient elution for simultaneous Resveratrol and Vitamin E determination in solid dosage forms

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Introduction

Resveratrol and vitamin E are both compounds with potent antioxidant and cytoprotective properties (Craciun et al., 2019). Trans-resveratrol (in the further text Resveratrol) is naturally occurring polyphenol component known to be present in various plant species, such as grapes, blueberries, blackberries, raspberries etc. and despite the above-mentioned capabilities has anti-inflammatory, as well as anti-aging actions (Craciun et al., 2019; Wang et al., 2021).Vitamin E is liposoluble vitamin, naturally mostly occurring in vegetable oils, nuts, seeds, green leafy vegetables etc., also positively affecting the immune system and neurological functions (Wang et al., 2021; Şeker et al., 2012). Up to date, due to lack of evidence, these compounds formulated in suitable dosage forms are not intended to be used as drugs, but as dietary supplements, with cautious use. Since there are clinical studies for confirming their synergistic or additive actions resulting in better anti-inflammation, anti-oxidant and anti-cancer properties, the formulation of a suitable dosage form containing both compounds as well as designing an analytical method for fast, simple, eco-friendly and precise method is a real challenge (Wang et al., 2021). The current edition of European Pharmacopoeia (EP) contains Monograph for assay of Polydatin (Polygonum cuspidatum rhizome and root Monograph), which is natural precursor and glycoside of resveratrol; and British Pharmacopoeia (BP) nor United States Pharmacopoeia (USP) provide Monograph for Resveratrol. For vitamin E (all forms) EP provides suitable monograph, BP does not provide one and the USP has Monograph on vitamin E, as well as for dosage forms single or combined with other chemical compounds (EP; BP; USP). So far, many studies have been made for quantitative determination of vitamin E and Resveratrol from different matrixes. These methods include HPLC with different detection wavelengths, LC-MS, HPLC with electrochemical detection, GC, voltammeter (Cranium et al., 2019; Wang et al., 2021).

The aim of our study was to develop a robust method for simultaneous determination of both compounds, Resveratrol and vitamin E in solid pharmaceutical dosage form, in order to make the quality control of such combination of compounds be easier to perform, less time consuming, environment friendly and cost effective.

Materials and methods

The following standards have been used: Trans-resveratrol CRM and vitamin E (alpha tocopherol acetate) CRM, purchased from Sigma Aldrich. The tested samples, were purchased from Replek Farm Ltd, Skopje, R.N.Macedonia.

The reagents that have been used are: Methanol (for analysis) purchased from Carlo Erba Reagents; Acetonitrile (HPLC gradient grade) purchased from Fisher Scientific and demineralized water, “in house” produced with conductivity of 0.055 µS/cm (Millipore).

The RC (regenerated cellulose) 0.45 µm syringe filters were purchased from Agilent Technologies (USA).

Regarding the instruments involved in sample and standard preparation: analytical balance Mettler Toledo AG285, ultrasonic bath and mechanical shaker. The analyzes were conducted on Shimadzu Nexera XR UPLC system with LPG quaternary pump with degasser,

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autosempler, PDA detector, column oven and controller, controlled by Lab Solutions software, version 5.97.

The chromatographic separation was best achieved on a RP Select B 75 mm x 4 mm column, with 5 µm particle size, purchased from Merck. This column has highest carbon loading and active surface of particles, enabling proper retention of fast eluting resveratrol in highly hydrophobic mobile phase compositions.

The test solution was prepared by dissolving capsule contents in methanol to obtain concentration of 0.3 mg/mL and 0.06 mg/mL for Resveratrol and vitamin E, respectively.

Results and discussion

Resveratrol molecule has three pKa values, 8.99, 9.63 and 10.64, corresponding to the phenolic groups. Vitamin E molecule has only one pKa value of 10.8. These facts allow use of mobile phase without buffers, acids or other pH stabilizers. Both molecules have large differences in their hydrophobicity and in their UV absorbing molar extinctions. The solubility differences induce very different hydrophobic interactions with alkyl reversed phase column, resulting enormous differences in retention times on isocratic chromatograms, with early eluting huge peak of Resveratrol and very late and wide spread small peak of vitamin E. Linear gradient for separation is solution, but still yielding long run times. These problems imposed the use of octyl silane (C-8) over octadecylsilane (C-18) chromatographic columns. The use of acetonitrile was better choice since vitamin E has long elution time even with 100% methanol, which has even larger viscosity, generating wider peaks of analytes. Small sized column lengths were an imperative for decent run times.

The developed method, carried out on RP Select B 75 mm x 4 mm column with 5 µm particle size, under gradient conditions, with mobile phase consisted of acetonitrile and purified water, flow rate of 1.0 mL/min, detection wavelength at 225 nm, column temperature of 32^{o}C and injection volume of 2 µL generated good separation of the two active substances in total run time of 7 min, with retention time of Resveratrol and vitamin E on 1.0 and 3.85 minutes, resulting with perfect peak shapes respectively. The resolution between two peaks was 19.3. Relatively small retention and number of theoretical plates of first eluting resveratrol is not a problem, since its concentration and huge molar absorptive coefficient.

The concept and development of chromatographic HPLC method for these analytes, will appear two main obstacles, very distinctive hydrophobicity as results of molecule structures, and very significant UV absorbing characteristics, based with molar absorptivity. This discrepancies problem with UV absorptions is additionally complicated with big differences of quantities of resveratrol and vitamin E in formulations, generate chromatogram with two very different peak sizes, and appearing in worse combination of significantly smaller quantity of vitamin E, compared with giant peak of much strongly absorbing molecule which is significantly more present in samples.

The possibility of application of other separating mechanisms with other type of columns, like more polar Phenyl, Hexafluorophenyl, cyanopropyl, HILIC, Diol and most polar bear Silica, for simplification in isocratic mode of elution is in our laboratories in progress.

Conclusion

The reversed phase step gradient HPLC method was found to be simple, fast and cost-effective, thus suitable for high-throughput routine determination of both Resveratrol and vitamin E in solid dosage forms in pharmaceutical quality control laboratories. The method could be further improved and upgraded if needed for higher sensitivity and higher throughput of analysis per day.

References

British Pharmacopoeia, 2022. British Pharmacopoeia Commission 2022; The Stationery Office, London UK.

Craciun, V.I., Gligor F.G., Juncan A.M., Chis, A.A., Rus L.L., 2019. A New, Rapid and Efficient HPLC Method to Assay Resveratrol in Food Supplements. Rev. Chim. 70(9), 3202-3205. https://doi.org/10.37358/RC.19.9.7516

European Parmacopoeia 10.0, Monograph 01/2017:2726, EDQM

National Center for Biotechnology Information. PubChem Compound Database; CID= 445154 https://pubchem.ncbi.nlm.nih.gov/compound/resveratrol (accessed on May 1st 2022).

National Center for Biotechnology Information. PubChem Compound Database; CID= 14985 https://pubchem.ncbi.nlm.nih.gov/compound/Vitamin-E (accessed on May 1st 2022).

Şeker, E.M., Çelik, A., Dost, K., 2012. Determination of Vitamin E Isomers of Grape Seeds By High-Performance Liquid Chromatography–UV Detection. Journal of Chromatographic Science 50(2), 97–101. https://doi.org/10.1093/chromsci/bmr032

The United States Pharmacopeia and National Formulary (USP 43-NF 38); The United States Pharmacopeia Convention, Inc.: Rockville, MD, USA.

Wang, C., Wang, N., Li, N., Yu, Q., Wang, F., 2021. Combined Effects of Resveratrol and Vitamin E From Peanut Seeds and Sprouts on Colorectal Cancer Cells. Front. Pharmacol. 12, 760919. https://doi.org/10.3389/fphar.2021.760919