

## 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. 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. 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 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 (figure 1). Vitamin E molecule has only one pKa value of 10.8 (figure 2).

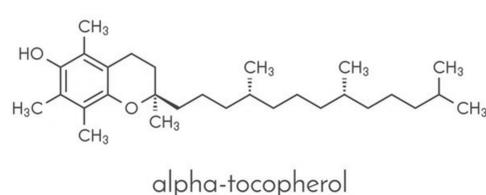


Figure 1. Chemical structure of Resveratrol

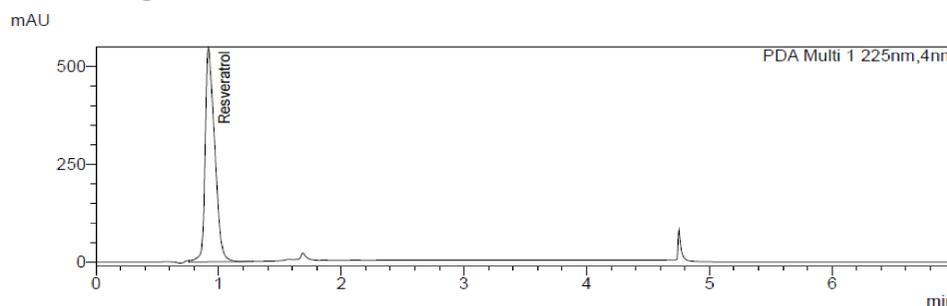
Figure 2. Chemical structure of Vitamin E (alpha-tocopherol)

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 75mm x 4mm 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°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 about 1.0 and 2.7 minute, resulting in perfect peak shapes respectively (figure 3 and 4).

#### <Chromatogram>

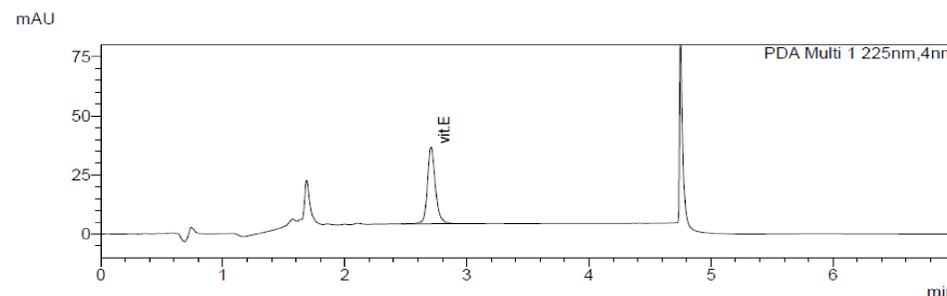


#### <Peak Table>

Peak#	Name	Ret. Time	Area	Area%	Resolution(USP)
1	Resveratrol	0.941	2937720	100.000	--
Total			2937720	100.000	

Figure 3. Chromatogram of Resveratrol

#### <Chromatogram>

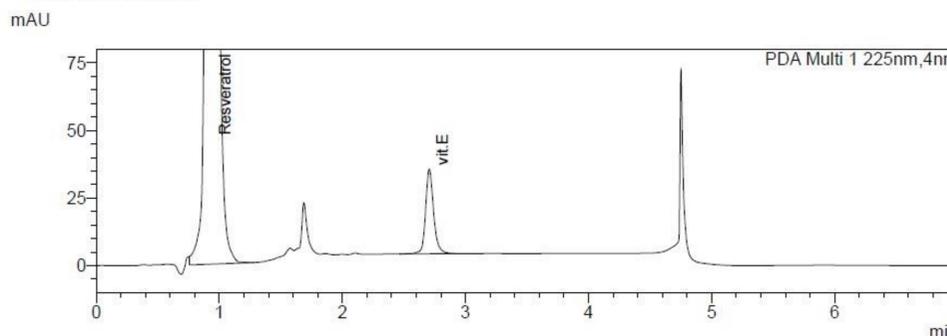


#### <Peak Table>

Peak#	Name	Ret. Time	Area	Area%	Resolution(USP)
1	vit.E	2.708	148066	100.000	--
Total			148066	100.000	

Figure 4. Chromatogram of Vitamin E

#### <Chromatogram>



#### <Peak Table>

Peak#	Name	Ret. Time	Area	Area%	Resolution(USP)
1	Resveratrol	0.941	2839454	95.226	--
2	vit.E	2.707	142365	4.774	12.773
Total			2981818	100.000	

Figure 5. Chromatogram of Resveratrol and Vitamin E

The resolution between two peaks was about 12.8 (figure 5). Relatively small retention and number of theoretical plates of first eluting resveratrol is not a problem, since its concentration and huge molar absorptive coefficient.

### 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  
 CraciunVI, Gligor FG, Juncan AM, Chis AA, Rus LL. A New, Rapid and Efficient HPLC Method to Assay Resveratrol in Food Supplements. Rev. Chim. [internet]. 2019 Sep;70(9):3202-3205. <https://doi.org/10.37358/RC.19.9.7516>  
 European Pharmacopoeia 10.0, Monograph 01/2017:2726, EDQM  
 Mehmet E, Şeker, Ali Çelik, Kenan Dost, Determination of Vitamin E Isomers of Grape Seeds By High-Performance Liquid Chromatography–UV Detection, Journal of Chromatographic Science, 2012 Feb;50(2):97–101; <https://doi.org/10.1093/chromsci/bmr032>  
 National Center for Biotechnology Information. PubChem Compound Database; CID= 445154 <https://pubchem.ncbi.nlm.nih.gov/compound/resveratrol>  
 National Center for Biotechnology Information. PubChem Compound Database; CID= 14985 <https://pubchem.ncbi.nlm.nih.gov/compound/Vitamin-E>  
 The United States Pharmacopoeia and National Formulary (USP 43-NF 38); The United States Pharmacopoeia Convention, Inc.; Rockville, MD, USA.  
 Wang C, Wang N, Li N, Yu Q and 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>