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Application of technology new stationary phases in HPLC analysis of biologically active compounds - determination of vitamin E

(Abstract)

The aim of my graduation thesis was to find the acceptable stationary phase for pretreatment of biological material [blood plasma] and isolation of vitamin E (α-tocopherol) by SPE technique and subsequent determination by HPLC analysis by using the developed method. LLE was then altered by SPE. I used vitamin A (retinol) as standard for my own work, because there is a possibility that the simultaneous determination of both vitamins for the laboratory GMK FN HK will be an advantageous combination. The main emphasis was put on vitamin E.

Sample preparation - optimized SPE procedure:

Blood samples were drawn from the peripheral vein after twelve hours of overnight fast. The samples were then centrifuged (1600×g, 10 min, 4°C) and plasma was separated. Then 250 µl of plasma was added and deproteinized by absolute ethanol (625 µl, 10 min, 4°C). After centrifugation (1600×g, 15 min, 4°C), the supernatant was separated and applied on the SPE column. Before application of the sample, the SPE column was activated by 1 ml methanol and then washed by 1 ml of purified water. After application of the sample the precipitate was diluted in 625 µl of absolute ethanol, centrifuged (1900×g, 25 min, 4°C) and all the supernatant was again applied on the SPE column. 2 ml of n-hexane were used as eluting solution. N-hexane was evaporated to dry at 45°C. The residuum was diluted in 250 µl of methanol and applied into the analytic column under these conditions:

- mobile phase - 100% methanol
- column - Monolithic column Chromolith Performance RP-18e, 100 × 4.6mm, MERCK (Darmstadt, Germany)
- flow rate - 2,5 ml/min
- injection volume - 50 µl
- total time of analysis - 2 min
- detection - 325 nm in time 1 min. for retinol
- detection - 295 nm in time 1,8 min. for α-tocopherol

Following this process I developed 10 samples from which I extracted vitamins and, using HPLC, determined average recoveries of 80,51% and 98,97% for retinol and α-tocopherol respectively. The relative standard deviation for retinol was 7,21% and for α-tocopherol 3,18%. α-tocopherol complied with a requirement of RDS under 5%.

The accuracy of this method was confirmed by the method of standard addition to a sample of plasma. 6 samples were prepared, including 3 samples with a cool plasma and 3 samples with standard additions. The average recovery was 82,92 % for retinol and 98,26 % for α-tocopherol. The accuracy was then confirmed by BIO-RAD Laboratories control set, when the measuring quantity of retinol and α-tocopherol was in the acceptable range.

This method of SPE was successfully developed for determination of the above-mentioned vitamins in real samples of biological material which were obtained from Faculty Hospital, Hradec Králové. Levels of vitamins corresponded to the range specified by the LLE application method.