RP-HPLC method for simultaneous determination of retinol, α-tocopherol, γ-tocopherol, retinol-palmitate and β-carotene in human plasma

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Simple, isocratic RP-HPLC method with UV detection for measurement of liposoluble vitamins: retinol, α-tocopherol, γ-tocopherol, retinol-palmitate and β-carotene in human plasma was developed. Plasma samples were prepared for analysis by liquid-liquid extraction. Ethanol was used for precipitation of proteins. Further extraction of analytes was performed with n-hexane. Extracts were evaporated to dryness under stream of nitrogen and further reconstituted in ethanol before HPLC analysis. Separation of analytes was performed on Zorbax Eclipse XDB-C18 Rapid Resolution, 4.6x100 mm, 3.5 μm chromatographic column with mobile phase consisting of methanol and ethanol (75:25, v/v). Mobile phase flow rate was 1 mL/min. Column temperature was set on 40°C and autosampler temperature on 10°C. Total run time was 16 minutes. UV detection of retinol and retinol-palmitate was performed on 325 nm, α-tocopherol and γ-tocopherol at 292 nm and β-carotene on 450 nm. Tocopherol-acetate was used as internal standard. Calibration curves for all analytes were constructed in following ranges: retinol, α-tocopherol and γ-tocopherol (5–35 μg/mL), β-carotene and retinol-palmitate (10–35 μg/mL). Developed method was successfully used for routine determination of liposoluble vitamins in plasma samples of healthy, middle-aged volunteers.

Biography
Milkica Crevar Sakač has completed her PhD at the Department of Medicinal Chemistry, Faculty of Pharmacy, University of Belgrade. She works as Teaching Assistant and Researcher at Department of Medicinal Chemistry.

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