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A NEW HPLC-DAD METHOD FOR STEROLS ANALYSIS IN BIOLOGICAL FLUIDS

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Introduction: Several methods were described in literature for sterols quantification. Among them gas chromatography-mass spectrometry (GC-MS) with selected-ion monitoring (SIM) is the most common one. We set up a new sensitive and specific reversed-phase high-performance liquid chromatographic method using DAD detection (HPLC-DAD) for the simultaneous quantification of sterols in biological fluids as well as cell homogenates.

Material and Methods: We used HPLC-DAD equipment (Lachrom, Merck Hitachi®) with software HSM-system 1. After an injection of 100 µl, sterols were separated at 30ºC, in a nucleosil C-18 column (250mm × 5 mm; 5µm particle size) with an isocratic elution. Mobile phase was methanol /water (100:4, v/v) and detection was performed at a wavelength of 210 nm. The flow rate used was 1 ml/min. and total running time was 40
min..

Results: We were able to identified ergosterol, 7-dehydrocholesterol, brassicasterol and cholesterol at retention times 25.41 min., 27.99 min., 28.77 min. and 32.15 min., respectively. The method was linear within the concentration range, 7.81–250 µM ($r2 > 0.9996$). The lower limit of quantification is 1nM for cholesterol, 7-dehydrocholesterol, ergosterol and brassicasterol. Within-run and between-run coefficients of variation were 1.65% and 9.99% for ergosterol and 2.55% and 10.54% for brassicasterol, respectively.

Conclusion: Our method is suitable for identification, separation and quantification of ergosterol, 7-dehydrocholesterol, brassicasterol and cholesterol in biological fluids and in cell homogenates. It was noted that our HPLC method had a better performance (for low sterol concentration) than GC-MS. Thus, this method is simple and accurate enough to be used in routine sterols analysis, and represents an interesting alternative to other methods described in literature.