Determination of sterol ester hydroperoxides by HPLC-ELSD

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Food applications enriched with phytosterols and their esters are increasing rapidly in the market. These compounds are added into food because of their known ability to lower serum cholesterol levels. Because some oxidation products may have negative health effects, it is essential to study the oxidation process. In this study a normal phase high performance liquid chromatography method with diode array and evaporative light scattering detectors (HPLC, DAD, ELSD) was developed for analyzing oxidation products of sterol fatty acid esters produced by photo-oxidation. The method was developed with cholesterol esters. Reproducible separations were achieved by silica column, gradient elution and constant cleanup and equilibration times between injections. Gradient elution was performed with 0.3-10% methyl-*tert*-butyl ether (MTBE) in heptane followed by cleanup with 30% MTBE. Compounds were detected with DAD at wavelengths 206 nm and 234 nm before ELSD. The response of ELSD was found to be cubic (r^2=0.99) but close to linear (r^2=0.96) for unoxidized cholesterol linoleate at a concentration range of 2-200 µg/ml. The response of DAD was linear with both studied wavelengths (r^2=0.99 and r^2=0.99). The limits of detection and quantification for unoxidized cholesterol linoleate with ELSD were calculated to be ≤2 µg/ml using 10 µl injections. Despite the somewhat cubical relationship between peak area responses versus concentrations, quantification with ELSD was more repeatable compared to DAD because of a stable baseline. For oxidized samples the repeatability was somewhat poorer because of partly co-eluting peaks. Four cholesterol linoleate primary oxidation products were separated as two groups: 1) 13-hydroperoxy-9,11-octadecadienoate, 12-hydroperoxy-9,13-octadecadienoate, and 2) 9-hydroperoxy-10,12-octadecadienoate and 10-hydroperoxy-8,12-octadecadienoate. In conclusion, normal phase liquid chromatography combined with DAD and ELSD enables analysis of intact sterol esters and their oxidation products.