

Thin-Layer Densitometry as an Alternative Tool in the Quantitative Evaluation of the Free Radical Scavenging Activity of Natural Antioxidants

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Z. Naturforsch. **60b**, 1212 – 1218 (2005); received March 29, 2005

An HPTLC system commonly used for chromatographic separations and analytical quality control has been utilized non-chromatographically as a screening tool for antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay. The accurate and precise capabilities for sample application and densitometric analysis of dry spots were applied to determine the 50% inhibition of DPPH (IC₅₀) by a number of natural antioxidants at 530 nm. Concentration-response curves were generated from 3rd degree polynomial fitting of data acquired for each antioxidant at 4 concentration levels. The IC₅₀ values ranged from 9.0 – 332.8 μ M with quercetin being the most active and silybin the least active of the pure compounds. Three antioxidant extracts were also evaluated and grapeseed extract was the most active (IC₅₀ 8.3 μ g/ml). The coefficients of variation for all IC₅₀ values were around 5% which indicated method reproducibility and the suitability of the used system for the intended purpose. Based on its densitometric evaluation of dry spots, the described technique provides an alternative to the spectrophotometric evaluation of samples in solution.

Key words: Densitometry, DPPH Assay, Free-Radical Scavenging, Antioxidants, HPTLC