# Fluorescence Detection by Intensity Changes for Automated Multiple Development-HPTLC separation of lipids

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Oral presentation

Automated Multiple Development (AMD), a gradient-based separation HPTLC technique, has been used to separate different families of lipids. Special attention will be paid in this presentation to the separation of sphingolipids, which are target analytes in the diagnosis and treatment monitoring of lysosomal storage diseases.

Fluorescence Detection by Intensity Changes (FDIC) allows a universal detection of lipids to be carried out, including that of saturated lipidic structures. FDIC operates through the increases in emission experienced by berberine cation, which are induced by non-specific interactions between this fluorophore and the polarizable hydrocarbon chain of the corresponding lipid, on silica gel plates when the system is excited using 365 nm-wavelength light.

The fluorescent molar responses of studied lipids, and differences in response among different families can be rationalized in the light of a previously proposed model of FDIC response, and using computational calculations based on Molecular Mechanics. An explanation for the high FDIC response of cholesterol (LOD= 5 ng; see figure) and other cholesterol-derivatives has been proposed.

Unlike derivatization techniques, FDIC sensitivity can be tailored through a simple variation of fluorophore concentration.