

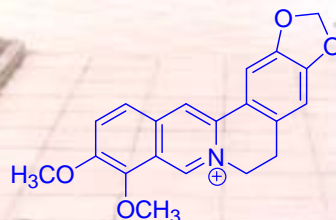
Fluorescence Detection by Intensity Changes (FDIC) for Automated Multiple Development (AMD)-HPTLC Separation of Lipids

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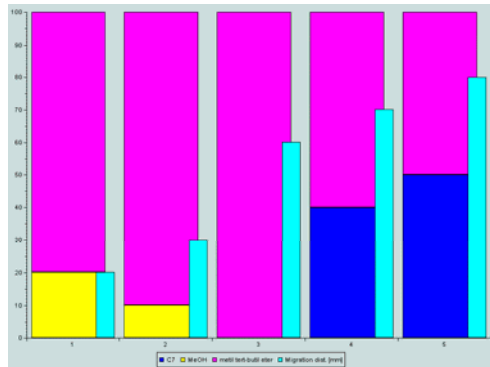
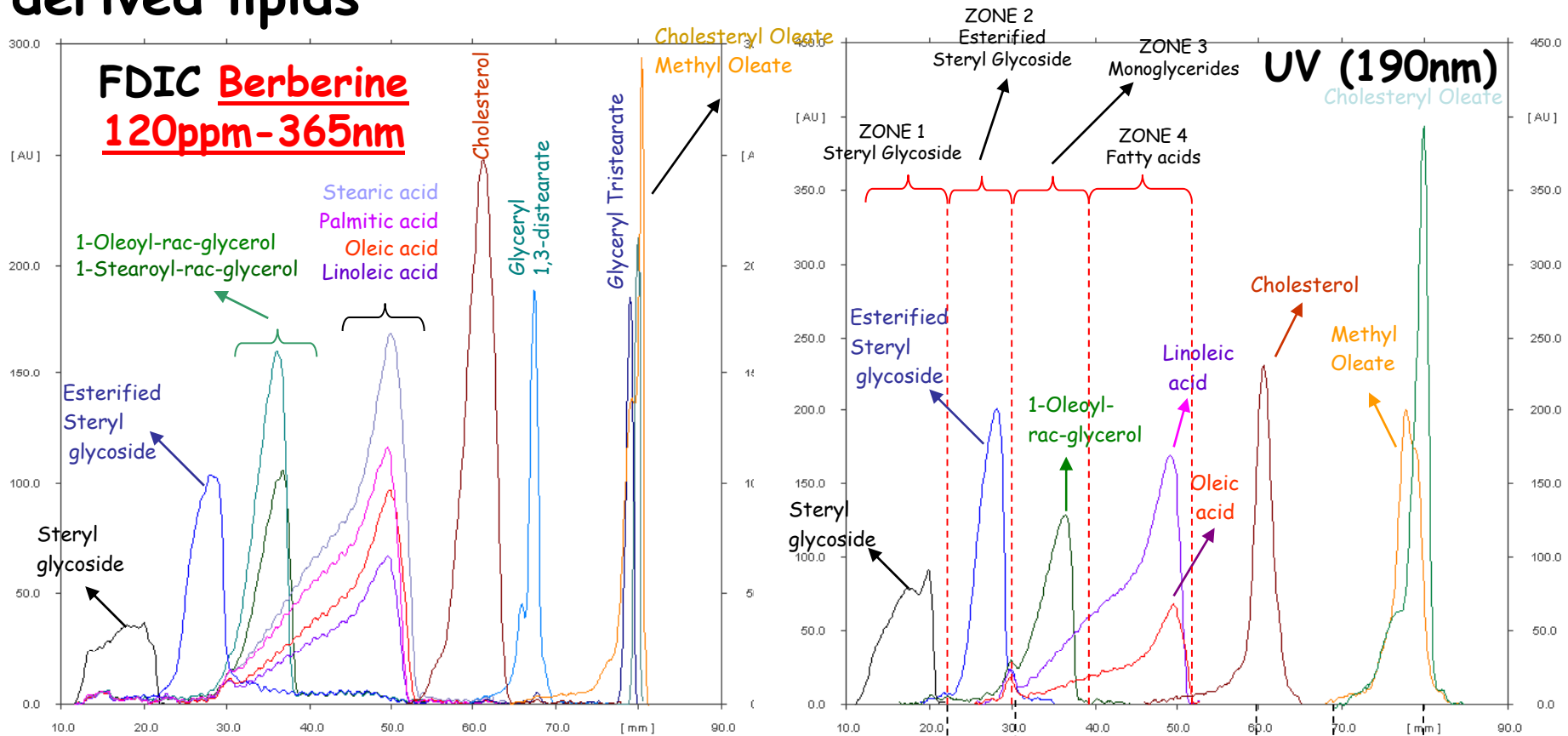
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AMD separation and FDIC detection of neutral and sterol-derived lipids



80% Tert-Butyl Methyl Ether + 20%MeOH

90% Tert-Butyl Methyl Ether + 10%MeOH

100% Tert-Butyl Methyl Ether

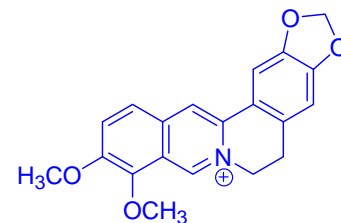
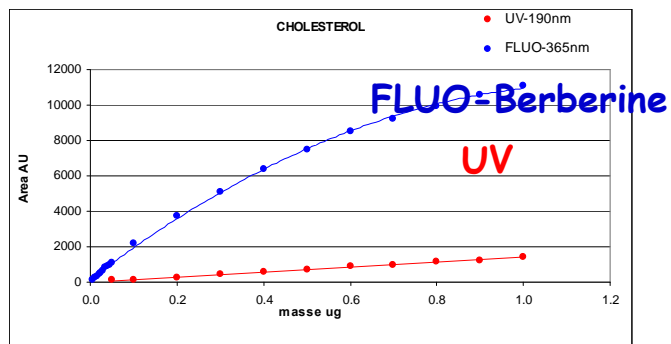
60% Tert-Butyl Methyl Ether + 40%C7

50% Tert-Butyl Methyl Ether + 50%C7

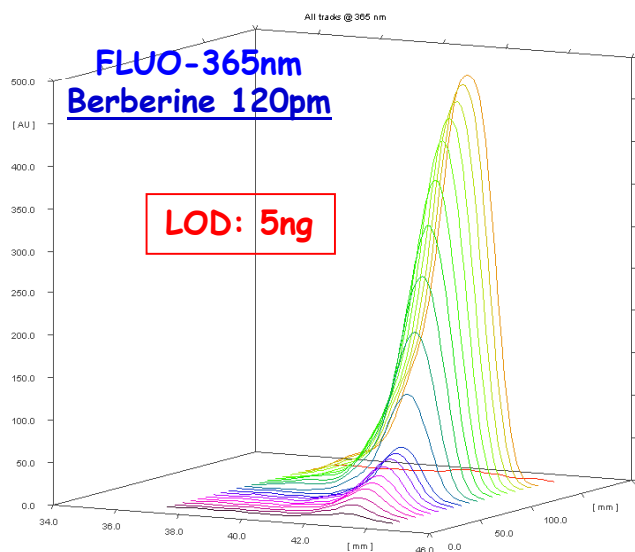


LOD of neutral lipids and steryl-derivatives using FDIC

Cholesterol



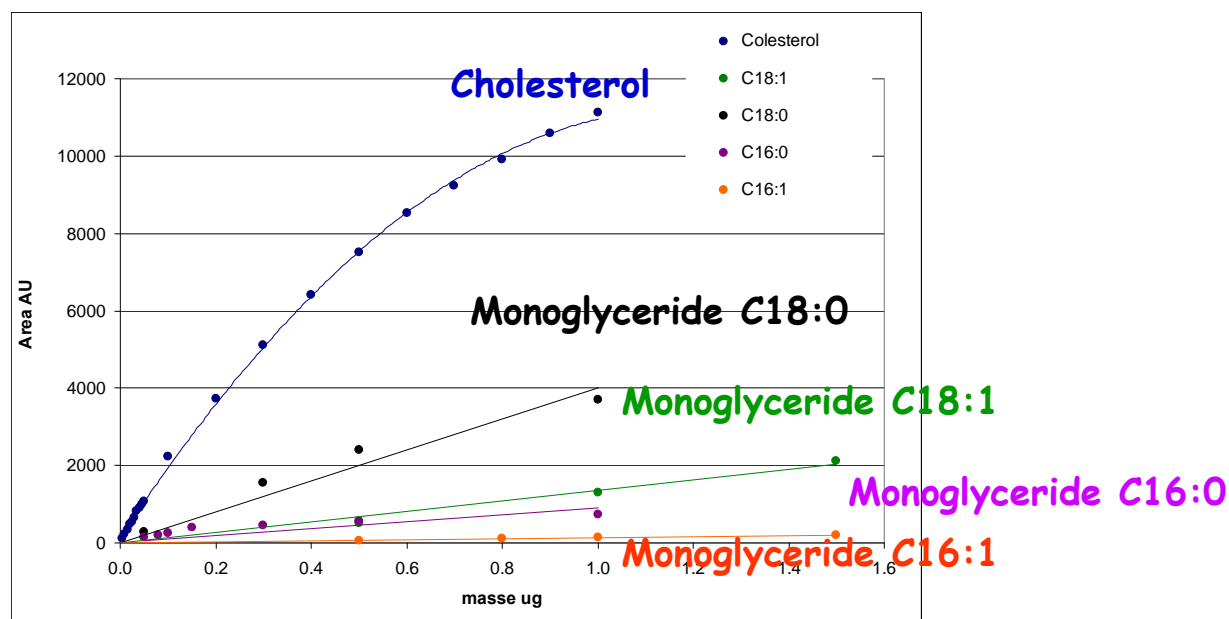
Berberine



	FDIC-berberine (μg)	UV (190 nm) (μg)
1-oleoyl glycerol	0.15	0.15
1-monopalmitoleoyl-glycerol	0.10	0.10
1-stearoyl-glycerol	0.05	Not detected
1-palmitoyl-glycerol	0.05	Not detected
Steryl glycoside	0.005	0.10
Cholesterol	0.005	0.10

FDIC responses of neutral lipids and steryl-derivatives

	Area masse ⁻¹ (A.U. μg ⁻¹)	Area mol ⁻¹	σ (Å ³)	MW
Cholesteryl oleate	16074	10.5	80.69	651.10
Esterified-steryl glycoside	8140	6.6	94.09	815.26
Cholesterol	16500	6.4	47.56	386.65
Steryl glycoside	5760	3.3	64.59	576.85

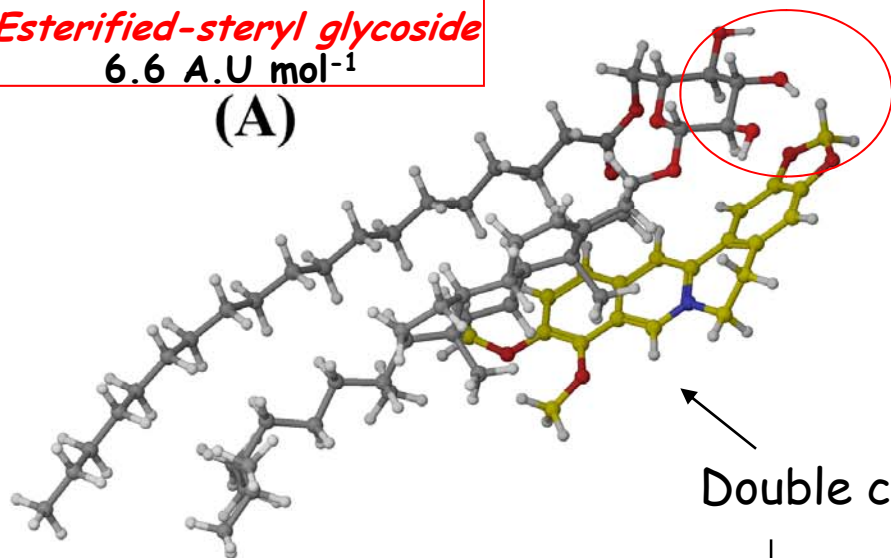


High FDIC responses

OPLS_2005 force field, MacroModel package,
Schrödinger

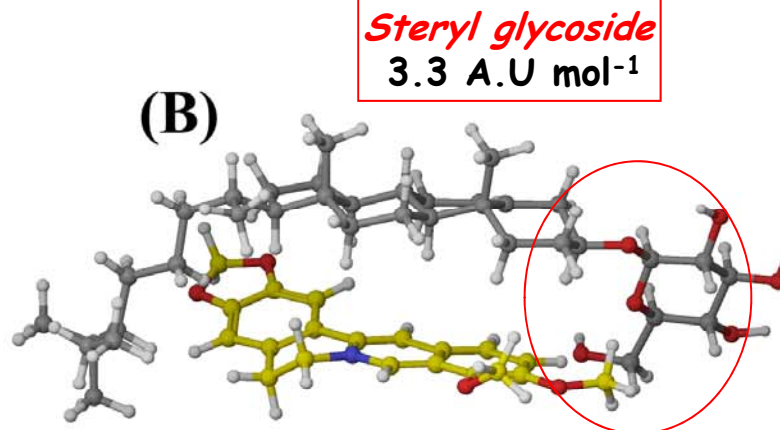
Esterified-steryl glycoside
6.6 A.U mol⁻¹

(A)



Steryl glycoside
3.3 A.U mol⁻¹

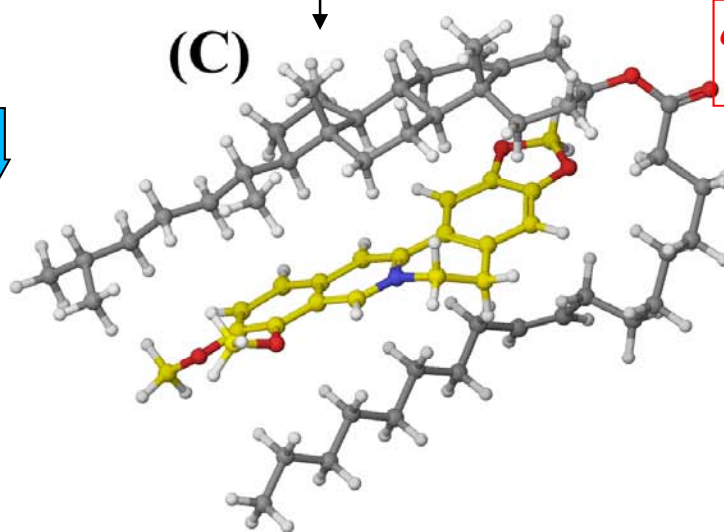
(B)



Specific interaction

$$\uparrow \phi = \frac{k_r}{k_r + k_{nr}} \downarrow$$

(C)



Cholesteryl oleate
10.5 A.U mol⁻¹

Cholesterol
6.4 A.U mol⁻¹

↓
"Dimer"
Higher α

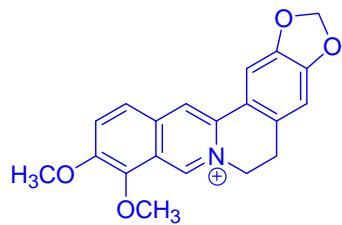
Yellow: berberine

● N atom

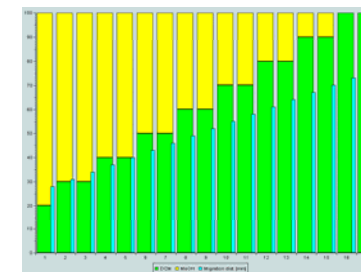
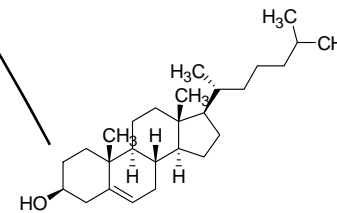
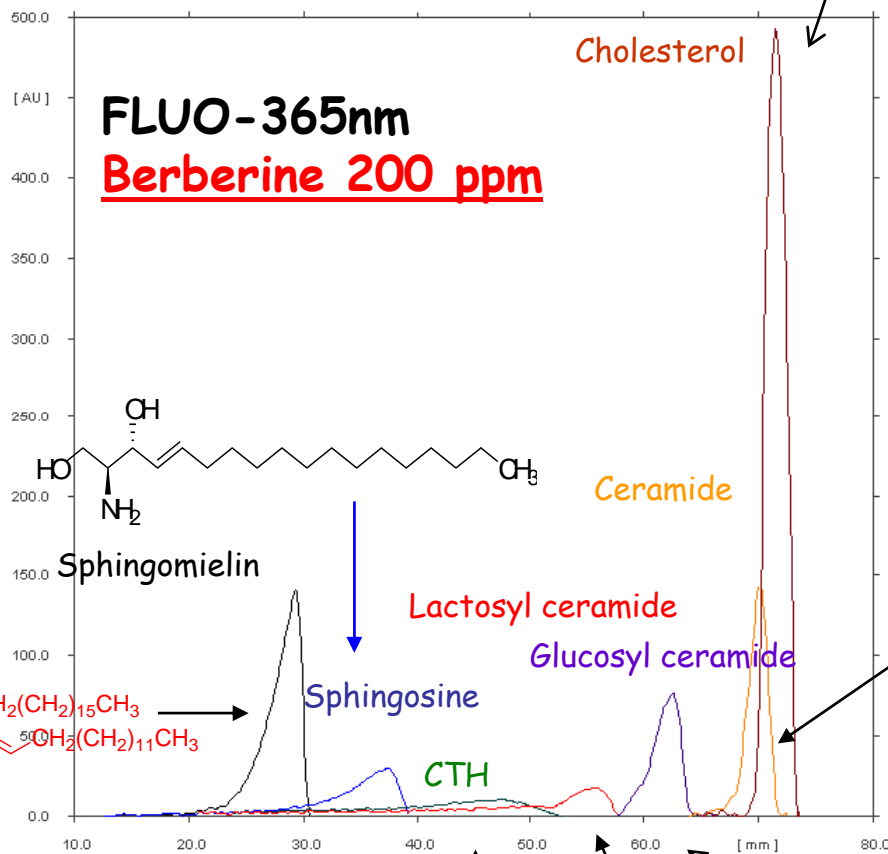
● O atom

● C atom

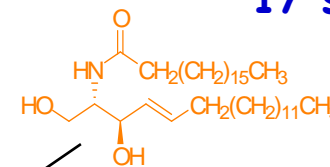
AMD separation and FDIC detection of sphingolipids



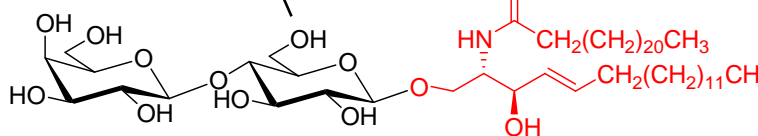
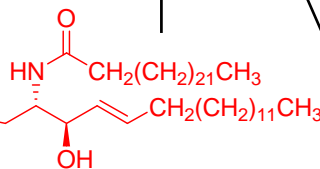
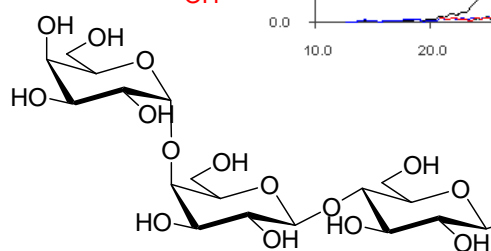
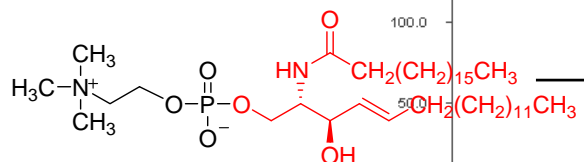
Berberine



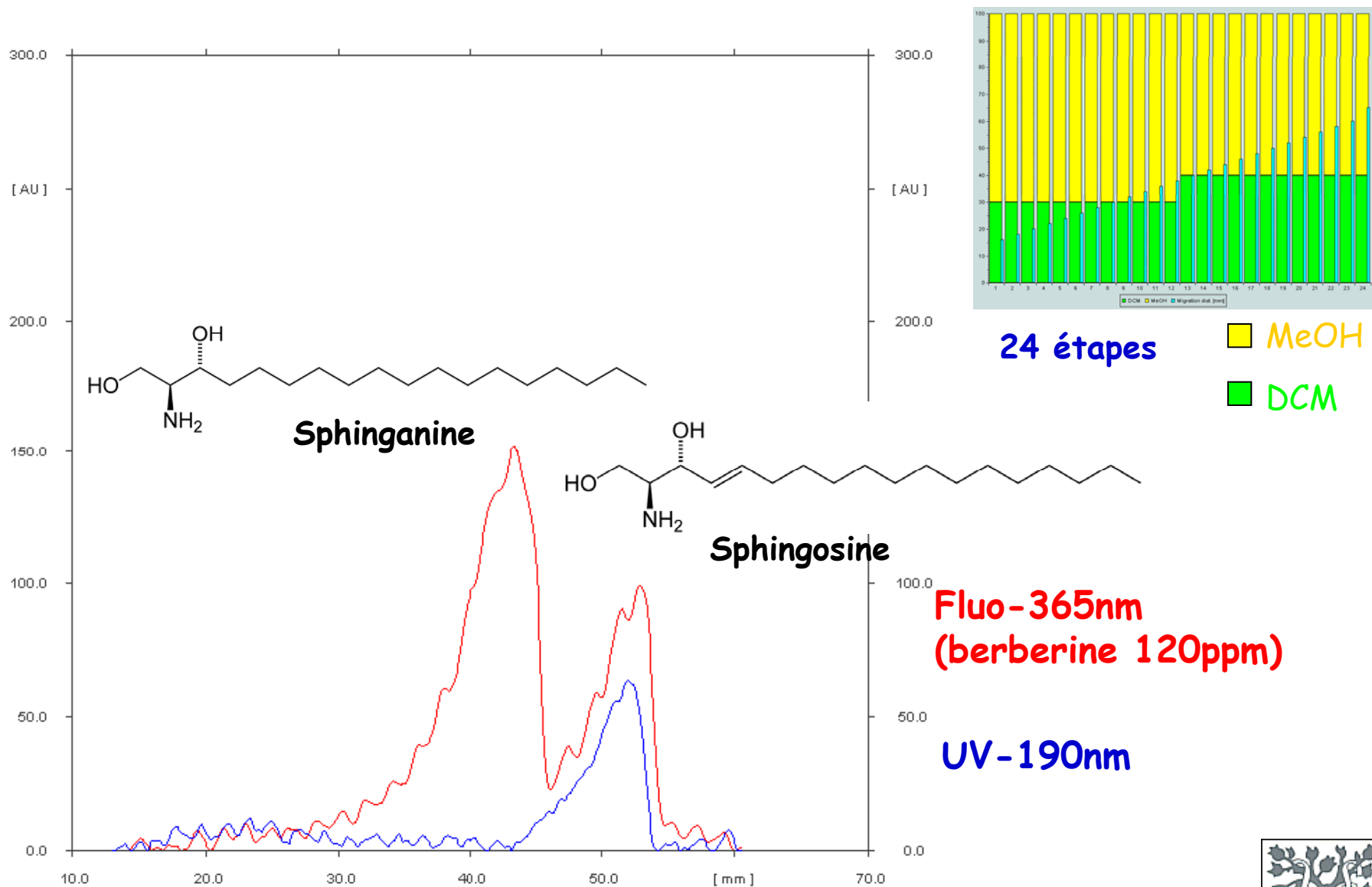
17 steps



■ MeOH
■ DCM



AMD separation of sphingosine and sphinganine





Conclusions

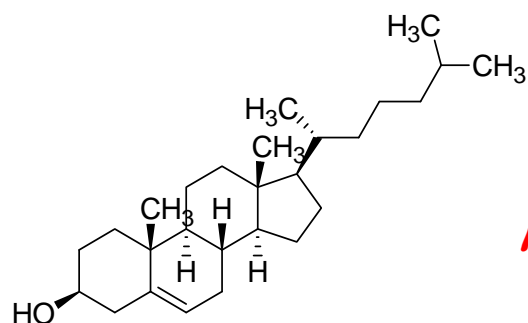
- FDIC can be used (by pre- or post-impregnation) with AMD to separate, detect and quantify all kinds of lipids. FDIC is induced by nonspecific interactions between berberine cation and the polarizable hydrocarbon chain of lipids.
- FDIC response can be explained. A mechanism has been proposed in this and previous works which includes an ion-induced dipole model and computational calculations.
- FDIC is not a derivatization reaction. Sensitivity can be modulated by a simple change of fluorophore concentration.

Thank you for your attention



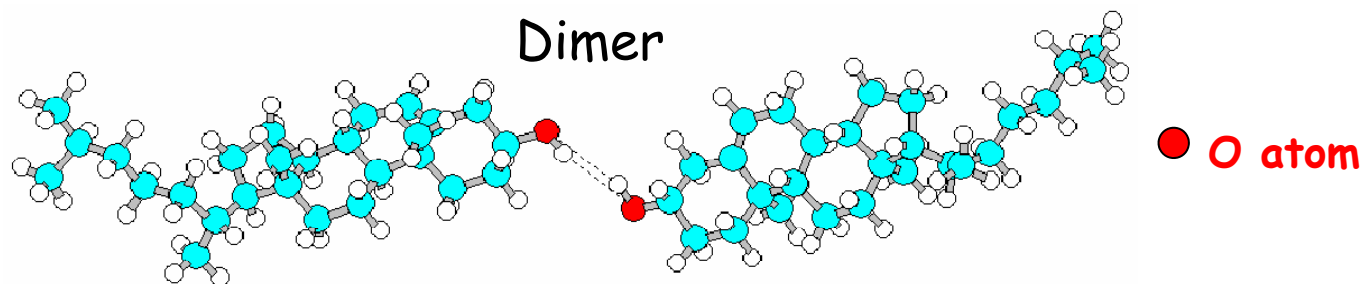
Supporting Information

The high FDIC response of cholesterol



Cholesterol 6.4 A.U mol⁻¹ $\alpha = 47.56 \text{ \AA}^3$ 27 C

n-octacosane 1.2 A.U mol⁻¹ $\alpha = 52.23 \text{ \AA}^3$ 28 C

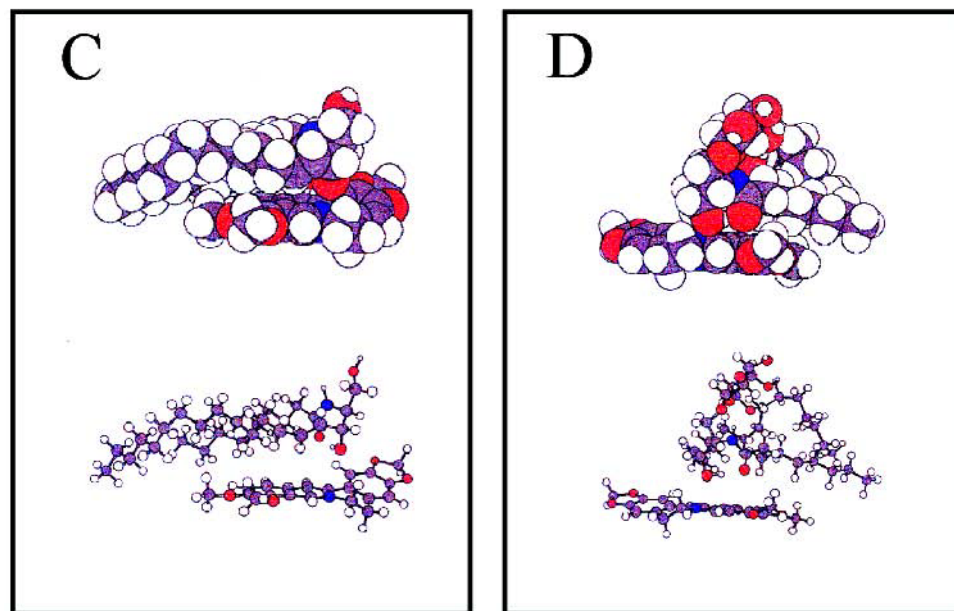


Properties of associated cholesterol as calculated by Chiosa using MM+ and AM1

	Monomer	Dimer	Trimer	Tetramer	Hexamer
Polarizabilité (α , \AA^3)	47.56	95.34	141.73	188.76	282.19
Energie d'interaction (ΔE , kcal mol ⁻¹)	-	-5.27	-23.16	-152.24	-19.28

Supporting Information

Weak FDIC responses



C Berberine-ceramide **D** Berberine-galactocerebroside

Specific N-O interactions

AMBER force field, ΔE (kcal mol⁻¹)
MacroModel package, Schrödinger

Sphingolipids

$$\downarrow \phi = \frac{k_r}{k_r + k_{nr}} \uparrow$$

- Decrease in response (+)
- Glucosyl > lactosyl > CTH
- The weakest response (CTH)
- possibility: a net quenching