OPLC: A rapid chromatography technique to research active components from an algae extract for the cosmetic industry

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Introduction

The design and the perfecting of new natural active ingredients for skin care products is one of the missions of LVMH Research. The definition of the relation between phytochemistry and biological activity is also an important step in the development of ingredients.

The research of natural products (active ingredients) in cosmetic suggests rapid, high performance techniques for both analysis and fractionation. In this field, the Optimum Performance Laminar Chromatography (OPLC) can be a useful technique for the isolation and identification of lead candidates with interesting biological properties.

Indeed, OPLC is a pumped flow chromatography technique that combines the user-friendly interface of HPLC with the capacity of flash chromatography and the inexpensive and multidimensional aspects of TLC.

We present here results obtained from the fractionation of an algae extract containing polar compounds of interest such as amino acids and carbohydrates.

OPLC Technique

1/ Principe

OPLC is corresponding to an over pressured or forced flow TLC. Although this technique is not widespread, OPLC has been described as an analytic and semi-preparative technique that develop by Tiyhak and al. in 1979. A chromatographic pump forces the flow of a liquid mobile phase through a planar stationary phase under 50 bar applied pressure (see figure 1).

2/ Instrumentation

The Personal OPLC 50 (see figure 2) is a stand-alone instrument comprising a separation (or pressurization) chamber and a solvent delivery pump. The structure of column consist in a planar stationary phase layer on an aluminium or glass plate as for TLC but is manufactured to be used like cylindrical HPLC column. The column is inserted into a cassette between a metal support and a Teflon foil. The assembly is inserted in the separation chamber and submitted to pressure.

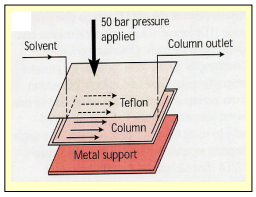


Figure 1: OPLC cassette design
1D separation (Source: Bionisis SA)

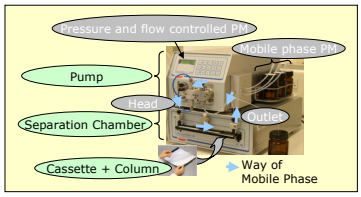


Figure 2: PersonalOPLC50

3/ Modes of Injection and detection

"OFF-LINE" is describing the way the samples are applied onto the column and "ON-LINE" is describing the way the compound are detected after their separation (see figure 3).

In a total OFF-LINE mode, the column is handled outside the development chamber for the application and the detection of the sample(s) whereas in total ON-LINE mode, as in HPLC, the column is first equilibrated inside the chamber and sample are injected with a valve and detected by modules such as UV or ELSD [1]. It is also possible to combine "ON-LINE" and "OFF-LINE" modes.

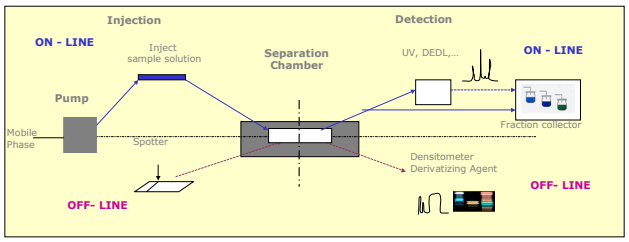


Figure 3: ON LINE and OFF LINE Modes

For this application, the analytic development was done in OFF-LINE mode. The fractionation of the algae extract was done in OFF LINE Injection, ON-LINE Collection and OFF-LINE Detection by spray-on ninhydrin and molish reagents because molecules are lacking of UV-chromophore (carbohydrates and amino acids).

Results

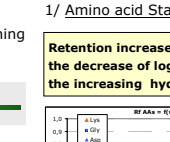
A/ Analytical Development

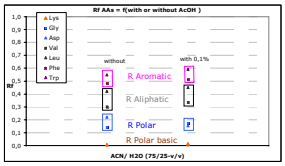
The separation of standard amino acids has been first developed on the Silica Phase in the "On-line" mode [1] with an elution of ACN/H_2O/AcOH 75/25/0.1 (v/v/v) in an isocratic mode at flow at the analytical scale. In aim of collecting fractions, a mobile phase without acetic acid (graph 1) was tested to limit the compounds degradation during drying step. Mobile phases are mixtures of water and acetonitrile at 0.5 mL/min with standard compounds (0.25g/L) of amino acids (graph 1) and carbohydrates (graph 2).

During the development phase, it is recommended to use the infusion technique [2] in the "Off-line" mode. A stopper is placed at the column outlet during this development step. This technique brings 3 benefits: no products exit the system since the migration distance of the solvent front is always the same, the Rf values are much more reproducible regardless of variations in the layer thickness; the disturbing zone effects are reduced.

1/ Amino acid Standards

Retention increases with the decrease of log D and the increasing hydrophily of lateral chain





Graph 1: Rf Amino acids function of presence or not of AcOH

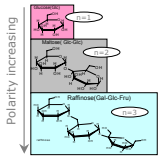
A little less Retention With AcOH in the MP except Asp (log D= -3,61)

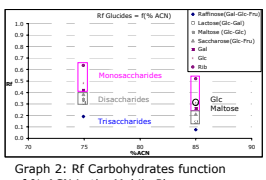
Better selectivity Without AcOH in the MP between Asp and Gly

No Elution of Lys

2/ Carbohydrate Standards

Retention increases with the increasing polarity of solutes and the increase of monosaccharidic units





Graph 2: Rf Carbohydrates function of % ACN in the Mobile Phase

Retention is increasing with the ACN percentage Rf between 0,1 et 0,5

Better selectivity with 75% ACN between Glc and Maltose

Regarding these results, 3 fractions can be expected with the mobile phase ACN/H_2O 75/25 (v/v):
- the amino acids with aromatic residues and monosaccharides
- the amino acids with long aliphatic residues and disaccharides
- the amino acids with polar and small aliphatic residues and the trisaccharides.

Best separation in 3 groups of Amino acids and Carbohydrates with the mobile binary phase ACN/H_2O 75/25 (v/v)

3/ Estimation of the retention times for fractionation

The Over running technique is also used to collect fractions of pumped solvents through the column (more than 1 Column Volumn). It is possible to determine the theoretical retention time and the theoretical volume for the elution of a given components [2].

B/ Application

1/ Analysis of Algae extract lot 06/105 (1% w/v) by HPTLC (figure 4)

Conditions:
Stationary Phase: Silica (10x10cm),
Deposited manually of Standards Amino acids and carbohydrates 0.5g/L and Extract 10g/L
MP : AcOEt/ PrOH-1/ H2O/ AcOH (15:20:15:1 - v/v/v/v)
Migration on 6cm, Analysis Time: 1H15

HPTLC analysis show the presence of Phe and/or Leu, Val, polar and basic amino acids in this extract as well as some mono- and disaccharides.

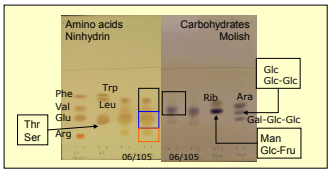


Figure 4: HPTLC plates

2/ Analysis of Algae extract lot 06/105 (1% w/v) by OPLC (figure 5)

Conditions:
Stationary Phase: Silica (BSLA 002 – 10x20cm)
Deposited with an automatic spotter: Standards 0.25g/L and Extract 10g/L
MP isocratic : ACN/H_2O - 75/25 in Infusion Mode, Flow: 0.5ml/min
Migration on 16.2 cm, Pressure 28 bar, CV: 2.184 ml, Analysis Time: 4.3 min

This analysis shows the presence of Phe (aromatic R), monosaccharides, Leu and Val (aliphatic R), disaccharides, Pro and Gly (polar R) and amino acids with basic residues (not eluted).
According to the analytical development and the theoretical estimation, less than one hour and 30ml of eluant is required to obtain the 3 expected fractions (Table 1): Fraction 1 from 9.0 to 9.8 min (pink), fraction 2 from 10.4 to 14 min (black) and fraction 3 from 16.4 to 42 min (blue).

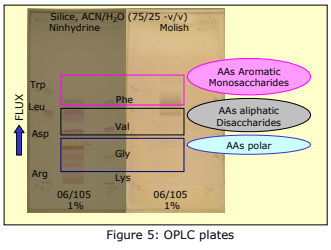


Figure 5: OPLC plates

3/ Fractionation of the Algae extract lot 06/105 (6% w/v) by OPLC

Conditions:
Stationary Phase: Silica (BSLA 002 – 10x20cm), Off line spotter of 15mg (Vspot=250µl),
MP: ACN/H_2O - 75/25 (v/v), Flow: 0.5ml/min, On line Collection, Time 1H, Volume of MP: 30ml,
Off line detection by reagents spraying on HPTLC silica plates

The end of collect is corresponding to the theoretical retention time of the last eluted components of interest. Three fractions are rapidly isolated in sufficient quantity for biological activity tests. However, a silica bleeding is observed in such conditions and it is thus difficult to determine precisely the collecting yield.

Fractions	Tr (min)	m (mg)
Fraction 1	8.5 to 12.5	5.9
Fraction 2	12.5 to 16.5	5.3
Fraction 3	16.5 to 42.5	4.6

Table 1

Conclusion

Once a separation has been developed by OPLC at the analytical scale with standards compounds, the fractionation of the complex matrix under the same conditions is simple and rapid. Moreover, OPLC columns are less expensive than classical HPLC columns, the volume of mobile phase in OPLC is lower than in HPLC and thus the cost of the development can be reduced.

One great interest of OPLC is the possible combination of "On-Line" and "Off-line" injection and detection modes to analyse and fractionate some complex matrix like natural extracts. The method was first rapidly developed in the "On-line" mode [1]. The "Off-line" mode also allows the overloading of the column to use OPLC at a semi preparative scale. Although, OPLC conditions could be transposed to preparative HPLC (binary mobile phase, isocratic elution).

It appears that OPLC is an interesting technique to define the relation between structural/ biological activity for LVMH Research.
The silica bleeding is still a drawback which could limited the structural studies (NMR). Therefore, one of perspectives of this work is to increase extract quantity of spotter, use a 20x20cm OPLC plate to increase the weight collects and so decrease the impact of bleeding.

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Bibliography

[1] P. Chaimbault, C. Elfakir and M. Lafosse. Poster presentation,1er Symposium de Chimie et Biologie Analytiques, September 2005 - Montpellier (France)

[2] PersonalOPLC50, Method development Guide, OPLC-NIT Ltd., December 2005

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