HPTLC FOR A BEST KNOWLEDGE OF VINEYARD PRODUCTS

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INTRODUCTION

Vineyard products offer great biodiversity in phytochemical compounds and deserve special attention in characterization of these compounds. In fact, the quality of Cognac perceived by consumers depends on the specific components of grapes, Ugni blanc variety, corresponding wine and distillate, or even on the wood in which "eaux-de-vie" are aged. LVMH Research Laboratories have developed phytochemical screening using HPTLC with the aim of defining the phytochemical profiles of natural extracts and drawing up their corresponding 'phytochemical identity cards' (1), thereby enhancing their expertise.

This method provides an overview of biodiversity in the characterization of phytochemical compounds. It makes possible to find and identify potential specific phytochemical markers of extracts, or of biological activity or even markers of grape ripening.This approach to molecular biodiversity using HPTLC has allowed a collaboration between BNIC (Station Viticole) and LVMH Research Laboratories whose twofold aim is to characterize samples such as 'Ugni blanc' must extract and oak wood extract. Moreover, this preliminary study will determine the interest and the relevance of the HPTLC used on these samples.

Actually, HPTLC is a separative chromatographic technique fitting to study such complex samples containing a variety of metabolites. And thanks to large combination of elutive systems and specific staining reagents (figure 1), molecules will give specific answers, according to their nature. HPTLC is also an interesting method for three main points: it is a **visual** method (components will give answers in terms of reference front (Rf)) and specific colour depending on phytochemical family studied. It is also a **rapid** (up to forty samples can be deposed per plate) and **comparative** method (answers are evaluated at the same time).

METHODS

UGNI BLANC (UB)

This sample corresponds to a liquid extract obtained from bunches of grapes, pressed and freezed before fermentation. In a first time, we studied the native extract, only solubilized in a alcohol/water blend.

But carbohydrates are the main components. For this reason and especially for matter of solubilities, they could hide other present molecules.

Then, some liquid/liquid extractions were realized with solvents such as water (to have polar components) and ethyl acetate (to have the others – medium and non-polar compounds).

One of the advantage of the liquid/liquid partition is to separate molecules but also concentrate them in each phase.

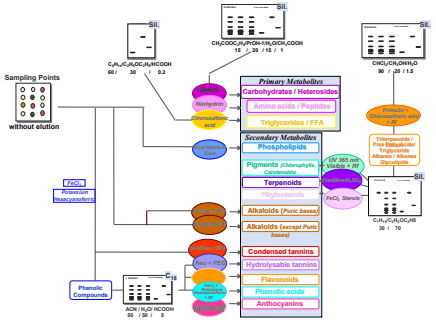


Figure 1 : HPTLC Methodology

OAK WOOD (OW)

This sample corresponds to aqueous extraction from 'heart wood', concentrated and freeze-dried.

In a first time, we studied the native extract, only solubilized in an alcohol/water blend. A liquid/liquid partition between water and diethyl ether was also realized to concentrate molecules.

To complete, an hydrolysis was made because of tanins which are suspected to be present, in reason of the origin of this extract, and to detect then monomeres. This hydrolysis was followed by liquid/liquid extraction by diethylether and ethyl acetate, giving two phases.

RESULTS

These phytochemical cards, with the two diagrams, give us a view of the different chemical families detected in these Ugni Blanc and Oak Wood extracts (polar, phenolic and/or lipidic compounds). With these separative conditions, we can evaluate the ratio between the several coloured spots seen on plates and then, estimate the potential concentration in samples. (diagrams 1 and 2).

But the interest of HPTLC is also to enable us to detect new molecules may be present in the extracts.

Caption:samples extracts at 1% in alcohol
1 stachyose / 2 raffinose / 3 galactose
4 Native UB extract
5 UB extract - L/L – water
6 UB extract - L/L – ethyl acetate
7 α hederin / 8 glycolipid

Conditions:
Stationnary Phase: Silica gel 60 F_{254}
Mobile Phase: Ethyl acetate/Propanol 1/Water/Formic acid -15/20/15/1

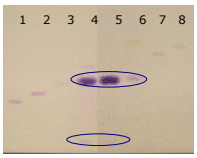


Plate 1: Molish in visible

Caption:samples extracts at 1% in alcohol
1 lupeol / 2 limonene / 3 α hederin
4 UB extract - L/L – water
5 UB extract - L/L – ethyl acetate
6 stigmasterol
7 cholesteryl palmitate
8 Native UB extract

Conditions:
Stationnary Phase: Silica gel 60 F_{254}
Mobile Phase: Chloroform/Methyl Alcohol/Water - 90/20/1,5

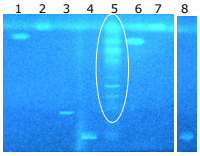


Plate 2: Primuline under 366 nm

These examples of plates illustrate the two families studied: plate 1 represents the domain of carbohydrates. We can see the main spots in purple (especially monosaccharides), but we can also detect spots stayed at the deposite line and characteristic of polysaccharids. The interesting point is that the ethyl acetate liquid/liquid partition shows far less this type of components and reveals a lot of molecules in the lipid or non polar area.

Actually, plate 2 shows a lot of white spots under UV for this sample, looking like sterols, fatty acids, terpenoids..., compared to water L/L and native extracts, which show no component in this system of migration 90/20/1,5.

Caption:
1 OW extract - L/L – water
2 OW extract - L/L – diethylether
3 α hederin / ursolic acid
4 glycolipid / stigmasterol
5 C18:2
6 OW extract hydrolyzed – ether phase
7 OW extract hydrolyzed – ethyl acetate phase
8 Native OW extract - 5% in alcohol

Conditions:
Stationnary Phase: Silica gel 60 F_{254}
Mobile Phase: Chloroform/Methyl Alcohol/Water - 90/20/1,5

Plate 3: Reading under UV 366 nm

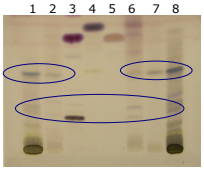


Plate 4: Chlorosulfonic acid, in visible

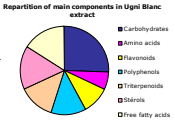
For this extract, polyphenols are major components, with relatively known compounds as tanins (gallotanins).

Also, with an adaptated preparation of samples, we can observe several spots in lipidic area as standards: saponins, terpenoids, sterols, fatty acids,... (plate 4).

Finally, the combination of different systems of staining reagents brings a lot of information and renforces some hypothesis of presence or not of particular molecules.

Actually, plates 3 and 4 were eluted in the same system but revealed differently. Depending on staining reagents, we can see, for example, some spots under UV 366 nm (fluorescent molecules at Rf 0,66) and others with chlorosulfonic reagent (blue green spots with specific Rf of 0,47 and 0,28). And these typical information are complementary and sign of potential markers.

Diagram 1



The study of native extracts, when they are rich of major components, is not sufficient.
Without this type of samples' preparation, in the case of Ugni blanc extract, no compound would be detected except carbohydrates, and for Oak Wood extract, except polyphenols, few compounds would be detected.

By this way, exploration of chromatographic answers depending on samples' preparation and elution, is a major point to make hypothesis on kinds of molecules.

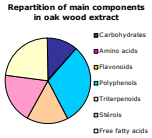


Diagram 2

CONCLUSION

At this time, results show two main points :
-The importance of vegetal material which determines the sample preparation. And so, this specific preparation allows to detect by HPTLC molecules present in these extracts, hidden by main components (liquid/liquid extraction, for example).
- The contribution of HPTLC in terms of molecular information in order to have a global composition but also to focus on a specific family with further investigations.
Actually, for Ugni blanc extract, major compounds are carbohydrates (especially monosaccharides). Their concentration is sufficient to require a sample preparation to detect minority families, possibly belonging to terpenols. And for Oak Wood extract, sample preparation brings out minority families in lipidic field.

The aim is now to determine specific phytochemical markers of extracts (differenciation markers), or markers of grape ripening.

This preliminary approach constitutes a first step in phytochemical research and therefore, we plan to complete our preliminary findings by comparing batches of these different samples in order to optimize the results and to grasp these vineyard products in terms of varietal flavor characteristics. And the next step will be the use of complementary techniques such as densitometry, Automated Multiple Development (AMD), liquid or gas chromatography, mass spectrometry,... and even some specific extraction methods.

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Bureau National Interprofessionnel du Cognac

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