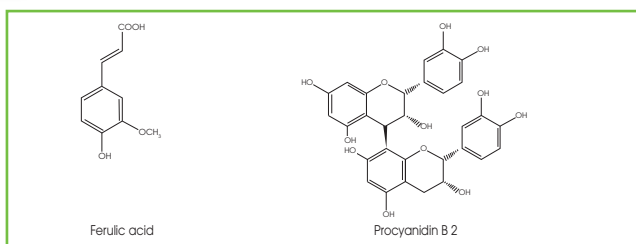


1 - INTRODUCTION

Among the cosmetic active ingredients, plant extracts are today more and more used for their biologic properties and their natural origin. The solvent used for the preparation of such plant extract can be glycol or propanediol-1,3.

HPTLC is particularly suitable to study the complex molecular composition of plant extracts. But, due to their viscosity, glycolic solvents lead to difficult HPTLC analysis. Indeed, they disturb the substances migration. Moreover, when the plant extract is diluted before analysis, the separation of substances is not improved.

In order to perform a relevant HPTLC analysis, we have developed a specific protocol using Solid Phase Extraction to remove the glycolic solvent of plant extracts. This technical approach is applied to analyse polyphenolic compounds of the last active ingredient developed by Gattefossé R&D, MALT SECRETS a rich polyphenols extract from malted barley.



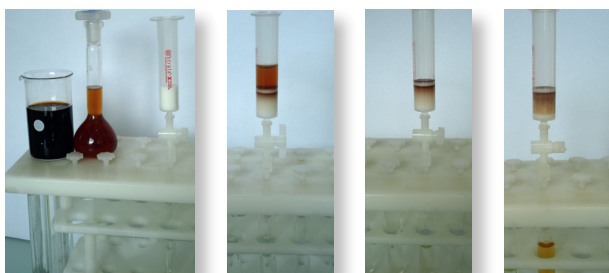
2 - SAMPLE PREPARATION PROTOCOL BY SOLID PHASE EXTRACTION

• Materials

- Glycolic plant extract containing polyphenolic compounds (MALT SECRETS)
- SPE cartridge: Polymeric Reversed Phase, Strata X 33 µ, 500 mg/6 ml, Phenomenex
- Solvent: methanol (Carlo Erba).

• SPE protocol

- Cartridge activating with 3 ml methanol and 3 ml water successively.
- Water dilution of glycolic extract. Application to the cartridge.
- Cartridge washing step with 3 ml 5% methanol in water. Glycol eluted while polyphenolic compounds adsorbed.
- Then, polyphenolic compounds eluted by 10 ml methanol.



Tests with C18 reversed phase don't allow the recovery of total polyphenols content. Some polyphenols are eluted simultaneously with glycol. Only polymeric reversed phase is able to retain phenolic acids, flavan-3-ols or procyanidin polymers (condensed tannins).

3 - SCREENING OF POLYPHENOLIC COMPOUNDS BY HPTLC

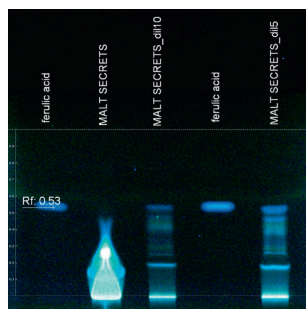
MALT SECRETS mainly contains phenolic acids and condensed tannins. Polyphenolic pattern is performed through four steps.

- Stationary phase: HPTLC plates Si 60 F₂₅₄ 10x10 cm, Merck
- Application: with Automated TLC Sampler ATS4 (CAMAG), band length 8 mm
- Development: in Automatic Development Chamber 2 ADC2 (CAMAG) with twin-trough chamber
- Migration distance: 70 mm
- Documentation: using TLC Visualizer (CAMAG)
- Reference substances: catechin, procyanidin A2, procyanidin B1, procyanidin B2 (Extrasynthèse) - ferulic acid, epicatechin (Sigma-Aldrich®) - procyanidin C (ChromaDex™).

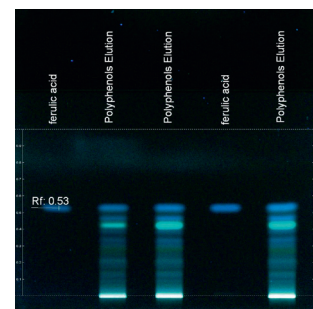
• HPTLC of phenolic acids

- Mobile phase: toluene - ethylformate - formic acid, 50:40:10, v/v/v
- ADC2 saturated 10 min.
- Derivatization by dipping the plate in Natural Product/PEG reagent (aminoethylidiphenylborinate 1% in methanol)
- Visualization under UV (366 nm).

blue color = ferulic acid, blue to blue-green color = other phenolic acids



Crude plant extract in propanediol. Despite dilution, propanediol disturbs migration of substances (flame or chimney shape).



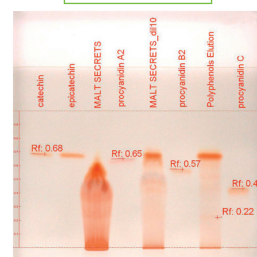
After SPE, the great diversity of phenolic compounds is now revealed.

• HPTLC fingerprint of procyanidins content

- Mobile phase: toluene - acetone - formic acid, 30:60:10, v/v/v
- ADC2 saturated 10 min.
- Derivatization by dipping the plate into a 1% vanillin solution in methanol-HCl 80:20 v/v.
- Visualization under daylight.

This mobile phase provides quickly indication about the degree of polymerization (DP) of condensed tannins: the more the increasing DP, the lower the Rf values.

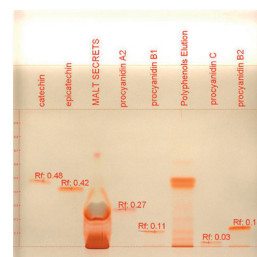
red color = procyanidins



• HPTLC of flavan-3-ols and procyanidin dimers or trimers

- Mobile phase: ethylacetate - toluene - formic acid, 9:9:2, v/v/v
- ADC2 unsaturated
- Derivatization by dipping the plate into a 1% vanillin solution in methanol-HCl 80:20 v/v
- Visualization under daylight.

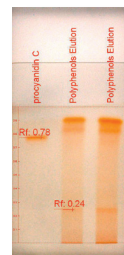
Epicatechin and catechin are particularly well separated.



• HPTLC of procyanidin oligomers with degree of polymerization > 3

- Mobile phase: ethylacetate - methyl ethyl ketone - formic acid - water, 15:9:3:3, v/v/v/v
- ADC2 saturated 10 min.
- Derivatization by dipping the plate into a 1% solution of vanillin in methanol-HCl 80:20 v/v
- Visualization under daylight.

When Rf values < 0.78, bands indicate the occurrence of procyanidin oligomers with few units (estimated > 3 units). Higher molecular weight procyanidins remain in sample application.



4 - CONCLUSION

HPTLC analysis of crude glycolic plant extract is disturbed by glycolic matrix. Sample preparation with SPE is needed to remove glycol. Polymeric reversed phase led to the best recovery of polyphenolic compounds.

This SPE protocol on polymeric reversed phase can also be applied successfully for other glycolic plant extract containing alkaloids or flavonoids. HPTLC analysis is then easily performed; right separation and resolution of substances are obtained.

5 - REFERENCES

- Kabrodt, K., Richter, J., Schellenberg I. (2002) Characterization of tannins from rhubarb by TLC/HPTLC. Camag Bibliography Service, 88, 4-6.
- Wagner, H., Bladt, S. (1996) Plant Drug Analysis - A thin layer chromatography atlas. Springer-Verlag Ed. 2nd Ed, 384 pp.