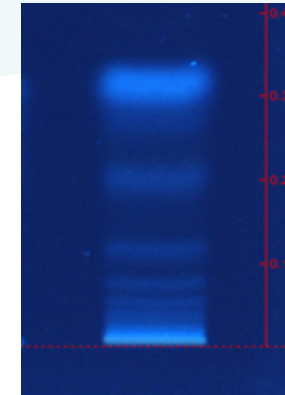




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Streamlined analysis of sugars in chicory root juice: Comparison of two chromatographic methods.

Antoine Bessin



A collaborative project

- ➔ **FLORIMOND-DESPREZ: New crop varieties and seed production: beet, chicory, wheat...**
Cappelle-en-Pévèle (France)



- ➔ **PURIFUNCTION: R&D platform for process and analytical development of natural products, Lille (France)**



- ➔ **UNIVERSITY OF GIESSEN, Department of food science,**
Pr Gertrud Morlock , Giessen (Germany)



Chicories, Asteraceae

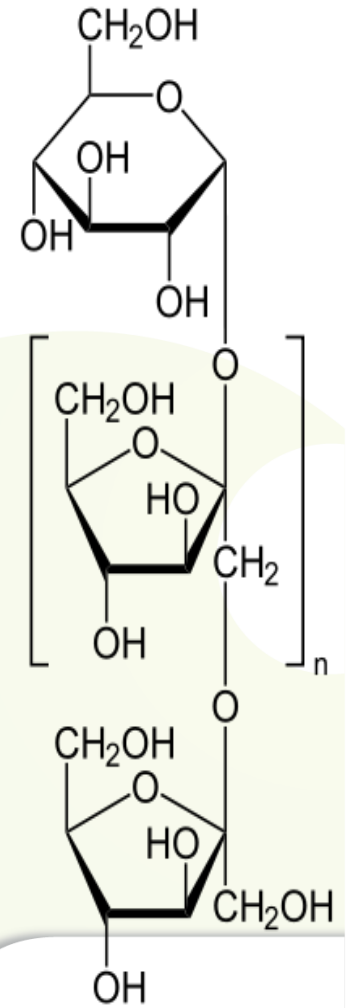
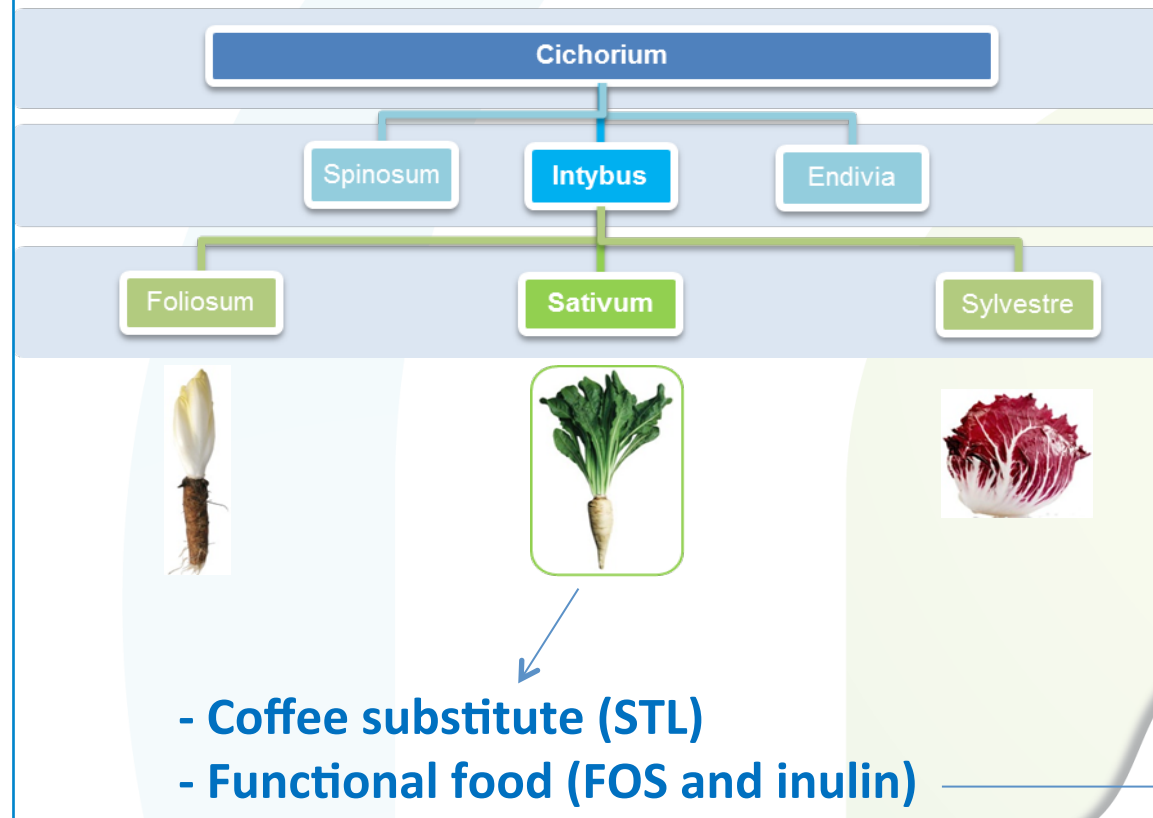
Wild chicory:
Cichorium intybus L.



Traditional medicinal herb:
 Liver, digestion.

Cultivated chicories:

↳ **Industrial chicory: variety *sativum***



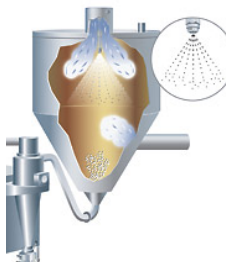
GFn

Quality markers of industrial chicory (regarding sugars)

- Glucose, Fructose, Sucrose contents
- Polyfructosaccharides average degree of polymerisation



Drying issues



Poor fiber quality



Desirable DPs



Solubility issues



DP ↓ and GFS ↑ : depends on weather and variety

⇒ Harvest in a short period => Reduce variability from weather

⇒ Analysis in max 2 months => Seeds selection for next year

Original method...

Automatised colorimetric method	
Advantage	Drawbacks
High Throughput: 25 min./analysis but 1½ min./sample in the system	Matrix interferences
	High maintenance
	Consumables out of stock

...New analytical requirements

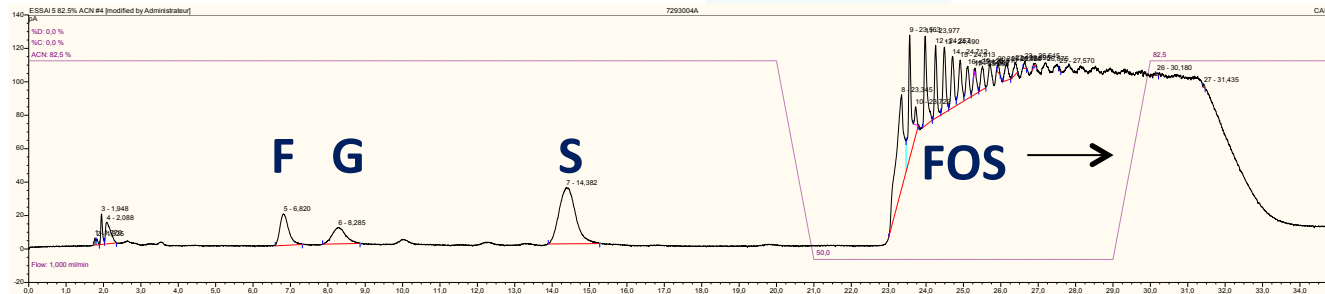
Requirements for the new methods	
Throughput	5000 batches in 2 months = 125 batches / day = 6 min./batch (1 analytical system) = 12 min./batch (2 analytical systems)
Sample prep	Compatible with the throughput of analysis and a low level of operator qualification
Safety	No use of highly hazardous reagents or solvents (CMR; STOT)
Validation	No need for high precision and accuracy. Statistical evaluation of populations

Liquid chromatography

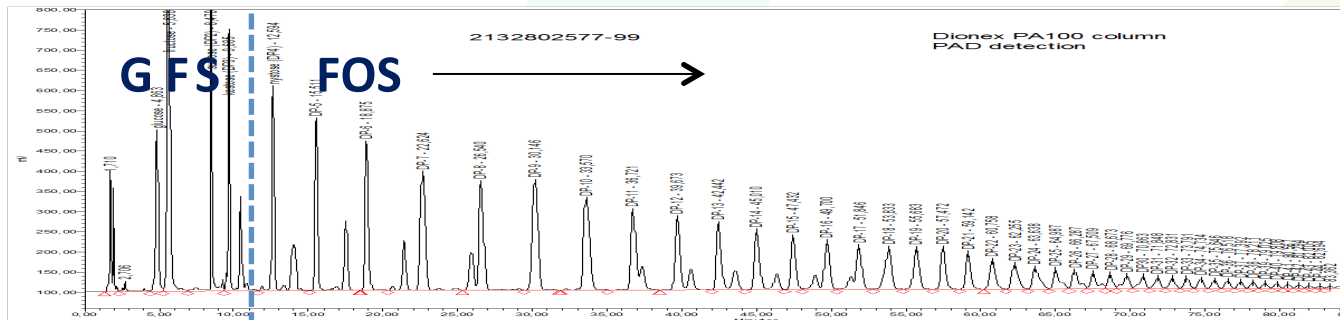
Main difficulties: ① Polysaccharides ② Salts

After optimisation:

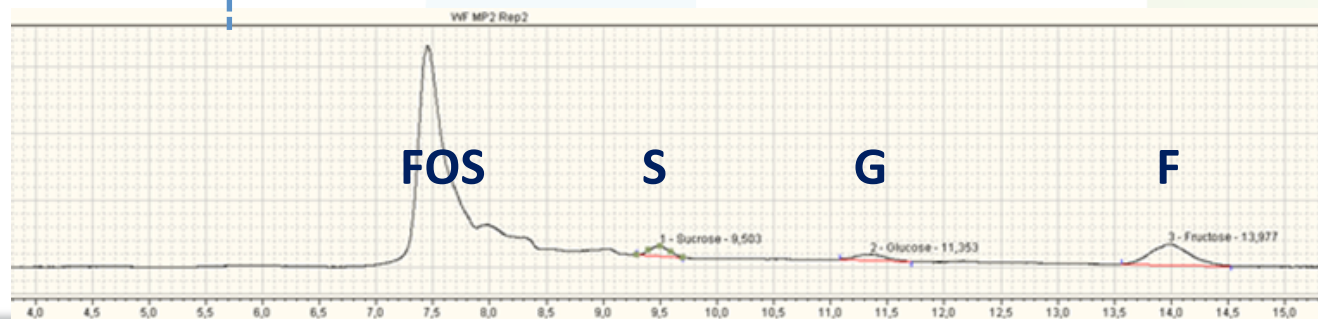
HILIC
CAD



AEC
PAD



IEC
RI



Run = 25 min.
Back pressure
> 300 bars
100 runs/col.

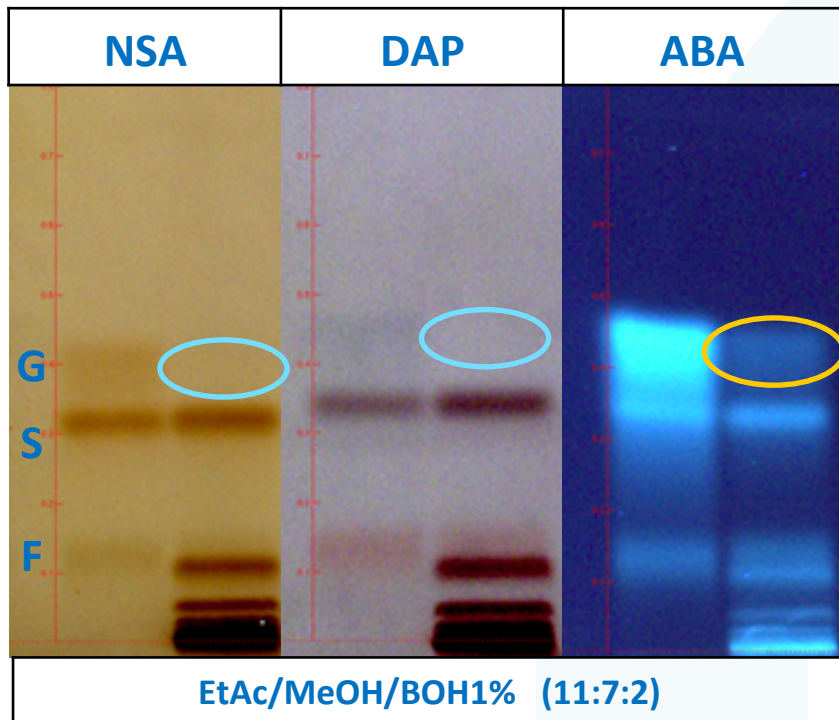
Run = 18 min.
Gradient mode

Run = 14 min.
Isocratic mode
UPW only
500 runs/col.
50 runs/precol.

Thin-Layer Chromatography

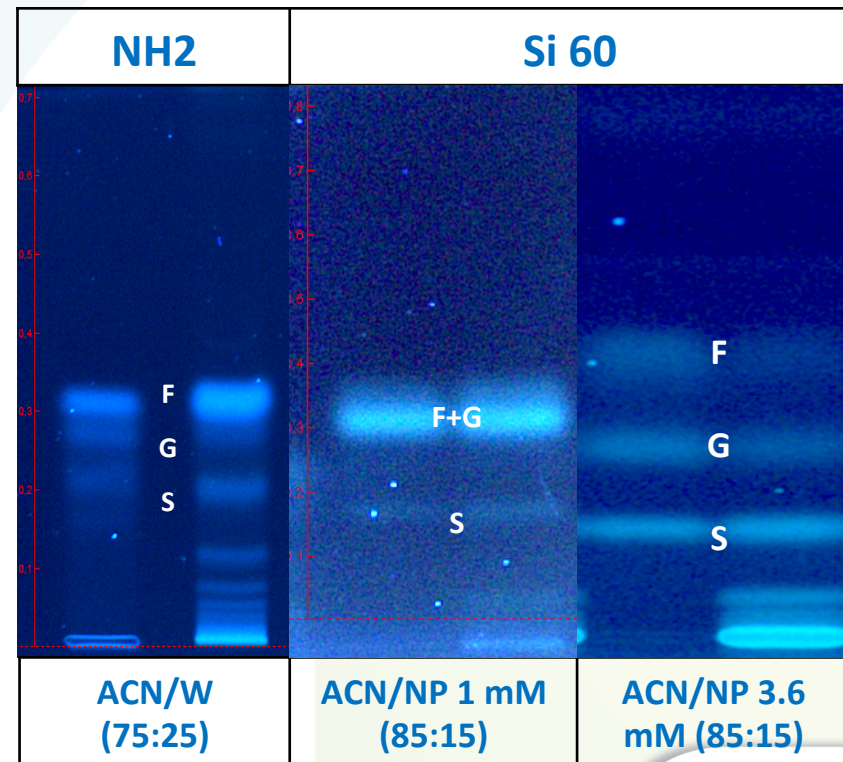
Main difficulties

① derivatization



⇒ Compensation of content gaps

② separation

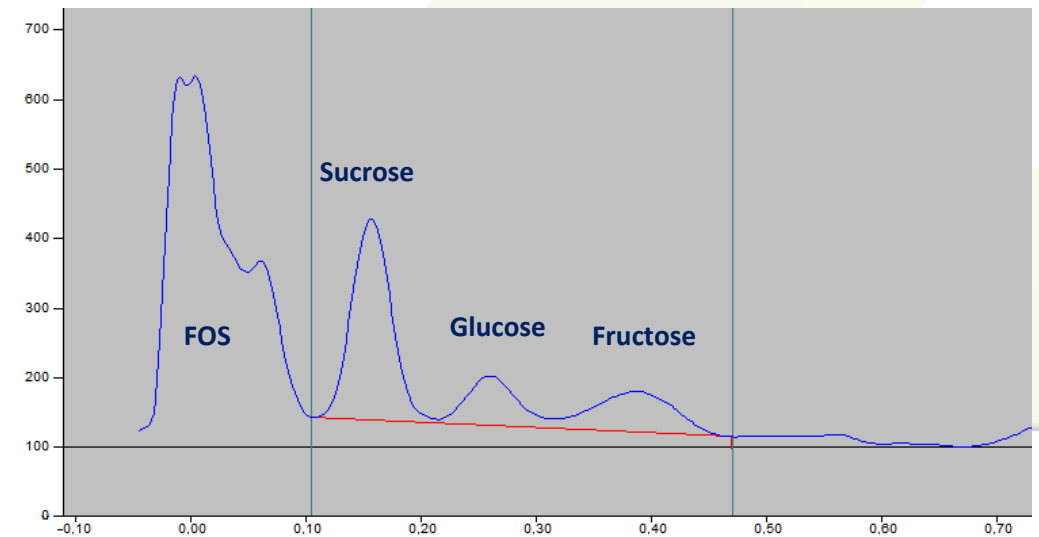
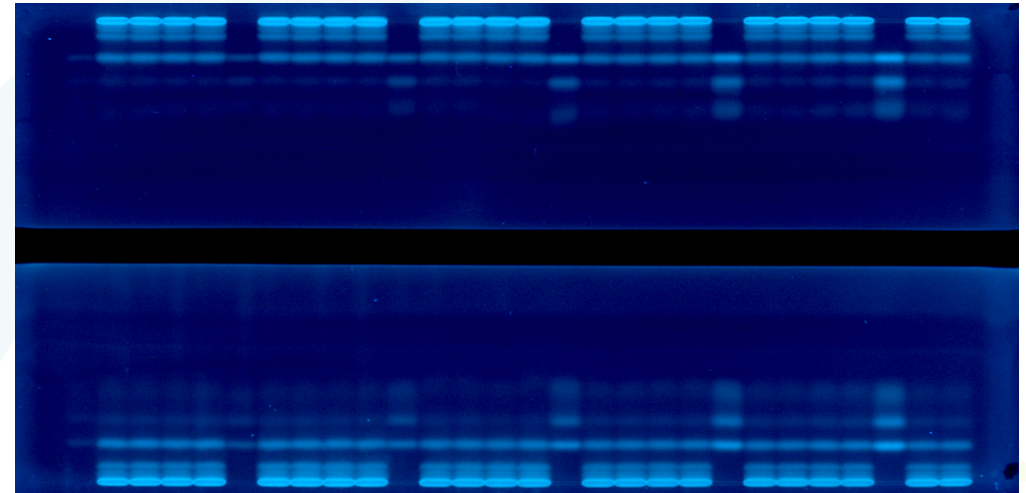


⇒ Resolution on Silica plates



HPTLC: optimized analytical method

Application (ATS4)	
5.5 mm bands 0.8 mm distance 6 calibration points => 44 samples /plate	45 min./plate
Chromatography (Horizontal)	
ACN/NP 3,6mM (85:15)	8 min./plate
Derivatization (immersion)	
Speed 4 cm/s Time: 0 sec Heat: 140°C ; 5 min.	12 min./plate
Documentation:	
UV 366 nm	3 min./plate
Densitometry (Scanner 4)	
Fluo 366/> 400 nm Distance: 35 mm Slit 3 x 0.20 mm Speed 20 mm/s	15 min./plate



➔ **45 min./plate ≈ 1 min./sample**

Optimized sample preparation:



① Thawing, homogenization and partial inactivation

- Frozen samples stored in 2 ml centrifuge tubes
- Shaking block heater: 5 min; 80°C



② Centrifugation

- Separation of the precipitate (proteins and fibers)
- 18 000 G; 10 min



③ Dilution

- 2 dilutions for HPTLC; 1 dilution for HPLC
- Solvent = Water
- Multiple automatic pipetting
- Deepwell plates (2 ml)



③ Centrifugation




- Directly in deepwell plates with sealing mats
- 4 000 G; 10 min.

Times and costs comparison (analysis in duplicate)

		2 HPLC-RI	1 HPTLC
Sample prep	Consumables	3 000	2 000
	Equipments	2 000	2 000
Analysis	Consumables	33 000	5 000
	Equipments	22 000	16 000
Total materials		60 000 €	25 000 €
Human resources	Weeks	14	7
	Technicians	1	2
Total HR		10 000 €	10 000 €
Total/year		70 000 €	35 000 €
Total/sample		14 €	7 €



Conclusion

	HPLC method	HPTLC method
Hazards	<ul style="list-style-type: none"> No organic solvents Ultrapure water only 	<ul style="list-style-type: none"> Solvents in small amounts No SGH06 or 08  
Sample prep	One step dilution	Two steps dilution
Time of analysis	14 minutes	1 minute
Equipments	Cheaper system (purchase)	Cheaper maintenance
Consumables	Expensive columns	Cheaper plates
Automation	Fully automatised	Application and scanning only
Validation	Good repeatability (CV < 5%) Accuracy < 10%	Lower repeatability and accuracy (up to 15-20 %)
Results/costs	Single analysis/sample => 7 weeks; 48 k€/year	Duplicated sample prep + analysis => 7 weeks; 35 k€/year

⇒ **HPTLC is the profitable technique!**



Thank you for your attention



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