Analysis of polyphenols in spruce extracts by LC-MS/MS

VITO – Mol, Belgien

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INNVENTIA

About my STSM placement

- My STSM was performed at VITO (The Flemish institute for technological research) during four weeks in August and September of 2010.

- VITO is located in Mol, Belgium.

- Supervisors at VITO was Dr. Stefan Voorspoels, Bart Noten and Domien DePaepe.

Purpose of the STSM

- Learn/adjust method used for determination of polyphenols at VITO.
- Test method with model substances and make calibration curves for compounds of interest to Innventia.
- Analyze extracts of spruce bark and eucalyptus leaves.

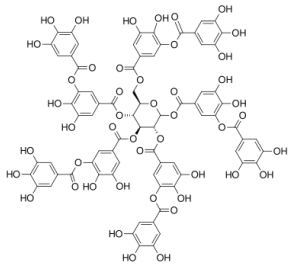
INTRODUCTION

Bark and other wood residues represent large-volume but low-value by-products in the forest industry. These fractions are a rich source of secondary metabolites and contain several compounds of potential commercial interest.

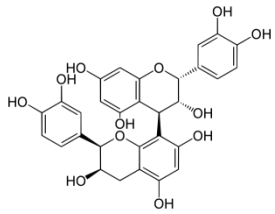
Polyphenolic compounds are one of these major groups known for their powerful antioxidant properties. These compounds have a potential use as preservatives in food and cosmetics and can also be used to prevent oxidation in different industrial products such as e.g. paints, lubricants and oils.

INTRODUCTION

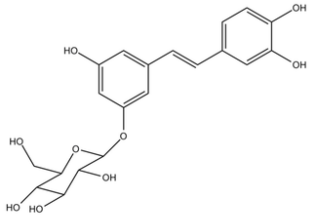
- Hydrolyzable tannins – Tannic acid



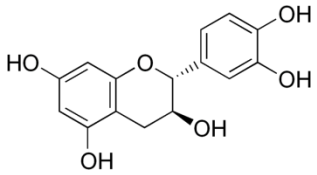
- Condensed tannin – Procyanidin B2



- Stilbene glucoside – Astringin



- Flavanol – Catechin



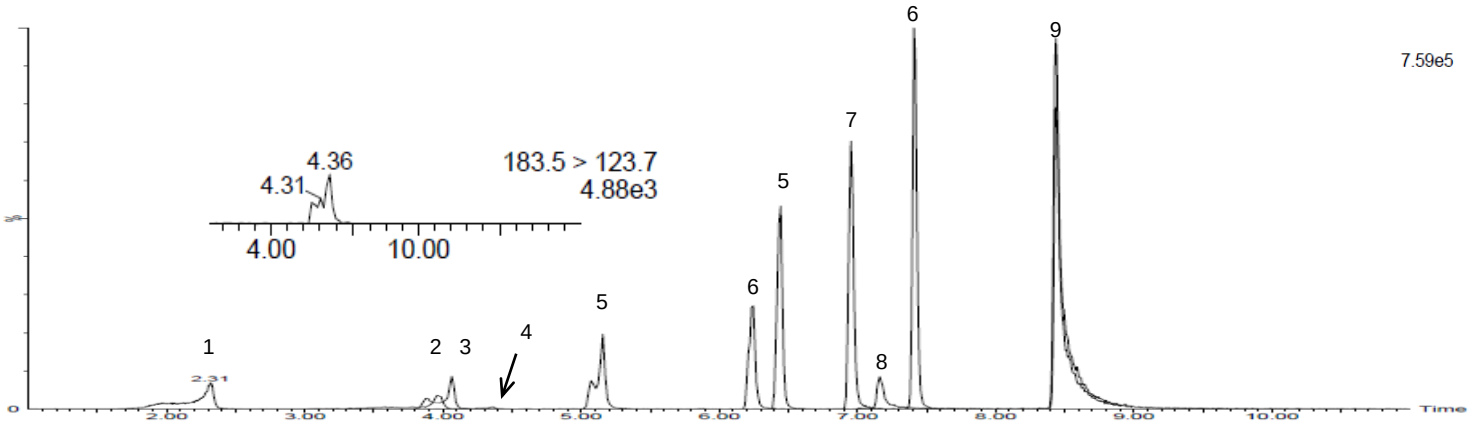
EXPERIMENTAL

- Analysis was done using LC-MS/MS (QQQ, ESI interface) in negative ion mode (MRM-mode used).
- Standard compounds used to make calibration curves (external calibration).
- Bark from spruce *(Picea abies)* and leaves from eucalyptus *(Eucalyptus globulus*) were used.
- Samples were pre-extracted with petroleum ether (Soxtec) and then extracted with methanol using an ultra sonic bath.

RESULTS AND DISCUSSION - CHROMATOGRAPHY

Chromatogram showing the 9 polyphenols included in the STSM.

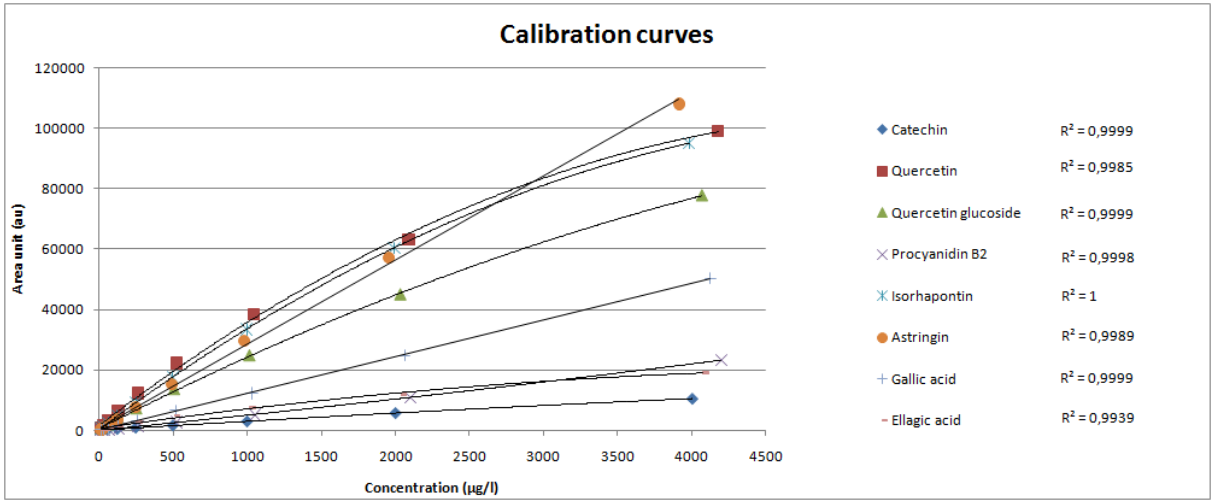
No.	CAS	Polyphenol	[M - H]$^-$	Daughter ion (Q)	Daughter ion (q)	Retention time (min)
1	59-95-86-8	Gallic acid	168.9	124.7		2.3
2	29106-49-8	Procyanidin B2	577.2	288.8	425.2	4.0
3	7295-85-4	Catechin	289.1	244.9	204.9	4.1
4	1401-55-4	Tannic acid	183.5	123.7		4.4
5	29884-49-9	*(E)*-Astringin	405.2	242.9		5.1
6	32727-29-0	*(E)*-Isorhapontin	419.2	256.9	240.9	6.3
7	482-35-9	Quercetin glucoside	463.1	300.8	299.9	7.0
8	314041-08-2	Ellagic acid	300.9	300.9		7.2
9	117-39-5	Quercetin	300.9	150.7	178.8	8.5



RESULTS AND DISCUSSION – CALIBRATION CURVES

Calibration curves for all polyphenols, except Tannic acid for which no reproducible data could be obtained.

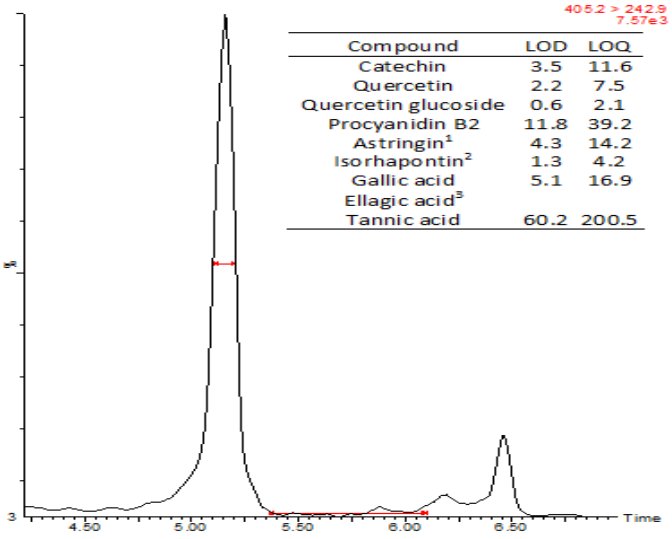
Range of the curves goes from 15 µg/L to 4000 µg/L.



RESULTS AND DISCUSSION – LOD and LOQ

- The signal to noise (S/N) was determined graphically (Masslynx)

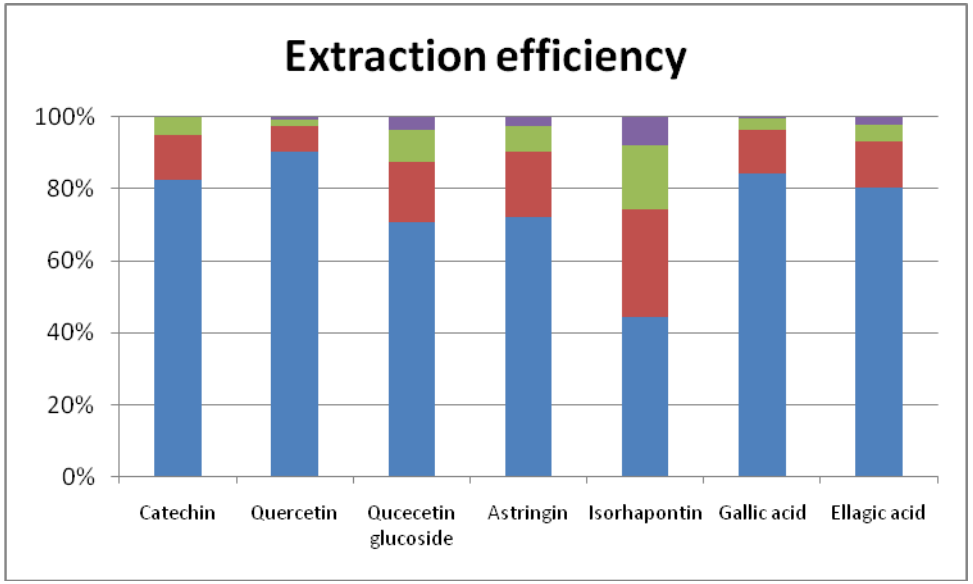
- Limit of detection (LOD) was set for a S/N of 3 × (conc(std)/(S/N)) and limit of quantification (LOQ) was set to 10 × (conc(std)/(S/N)).



Compound	LOD	LOQ
Catechin	3.5	11.6
Quercetin	2.2	7.5
Quercetin glucoside	0.6	2.1
Procyanidin B2	11.8	39.2
Astringin[1]	4.3	14.2
Isorhapontin[2]	1.3	4.2
Gallic acid	5.1	16.9
Ellagic acid[3]		
Tannic acid	60.2	200.5

RESULTS AND DISCUSSION – EXTRACTION ANALYSIS

- This chart shows the extraction efficiency of 7 out of the 9 polyphenols in this project. It is noticeable that full extraction was not obtained in just on extraction cycle, especially not for the stilbene glucosides.



Extraction parameters

- 500 mg bark sample
- 25 ml MeOH
- 1 h in ultrasonic bath
- 4 extraction cycles

RESULTS AND DISCUSSION – EXTRACTION ANALYSIS

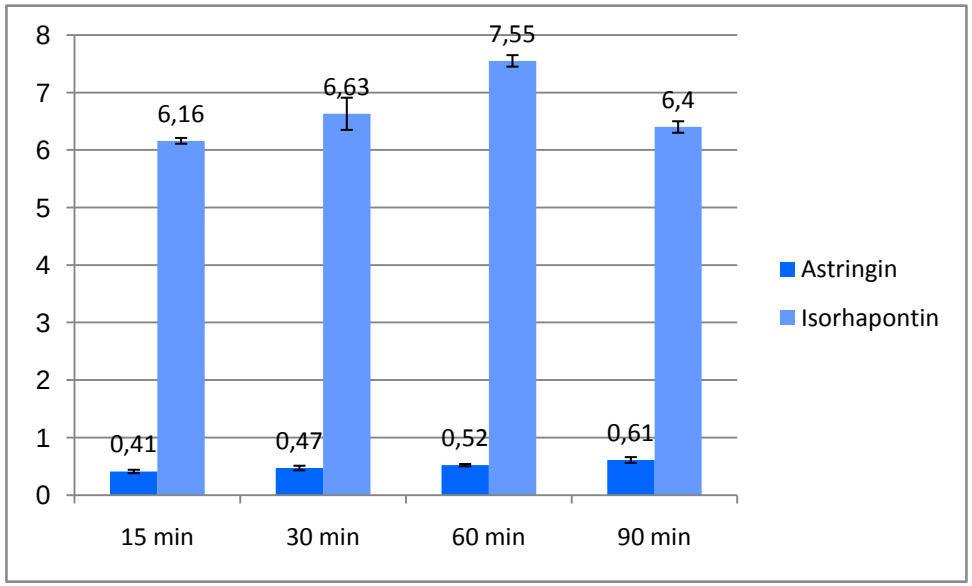
- The two stilbene glucosides, Astringin and Isorhapontin, were present in great excess compared to the other polyphenols of intrest in the spruce bark extract.

Extraction	Catechin	Quercetin	Qucecetin glucoside	Astringin	Isorhapontin	Gallic acid	Ellagic acid
A-D	970	3600	700	225000	440000	950	5800

The table above shows the actual difference in area units for the four extraction cycles.

RESULTS AND DISCUSSION – EXTRACTION ANALYSIS

- This chart shows the extraction efficiency over increased extraction time for the stilbene glucosides.



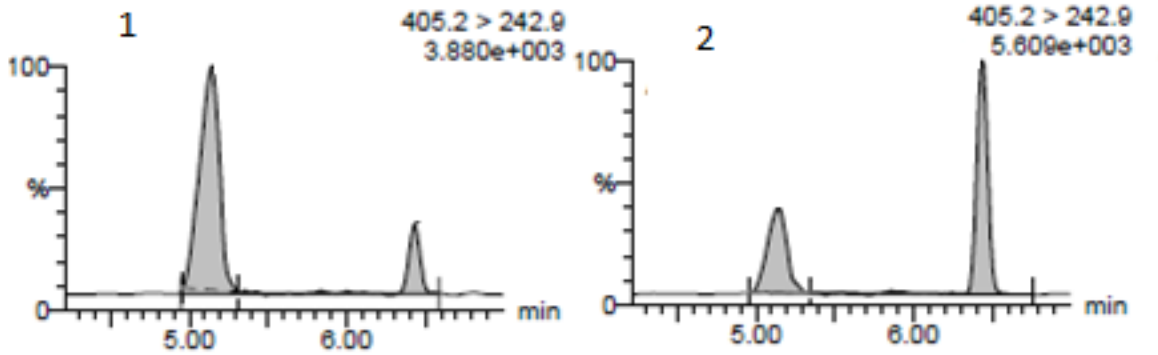
Extraction parameters

- 20 mg bark sample
- 25 ml MeOH
- Method of extraction: ultrasonic bath
- n = 2
- Conc: mg/g

RESULTS AND DISCUSSION – COMPOUND STABILITY

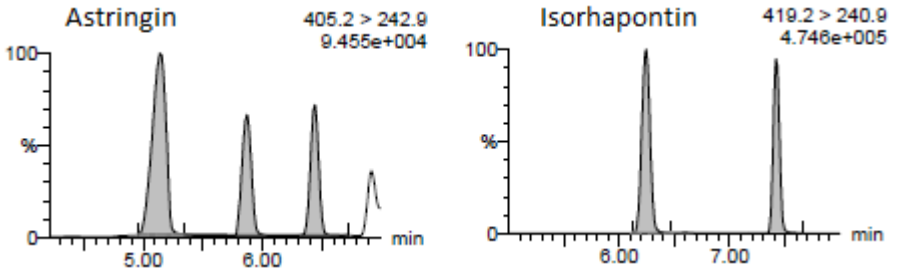
- It was noticed that the stilbene glucosides easily underwent isomerization, especially after being exposed to UV light.

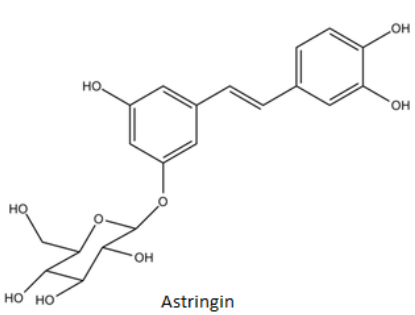
- In the first figure (1) the Astringin sample was prepared under a fume hood while in the second figure (2) the same sample was prepared on the lab counter (exposed to sunlight).



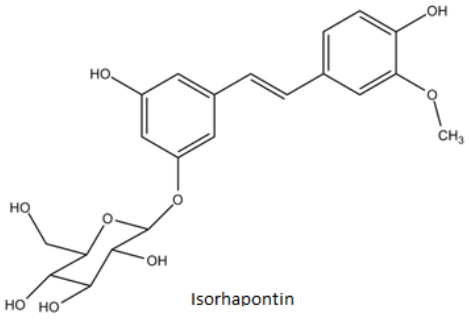
RESULTS AND DISCUSSION - ISOMERISATION

- In the LC-MS/MS chromatograms of the spruce bark extracts four different peaks corresponding to Astringin and two peaks corresponding to Isorhapontin were seen.





Astringin



Isorhapontin

RESULTS AND DISCUSSION - CONCLUSIONS

- The method used was able to separate and measure all nine polyphenols included in this STSM project.
- No parent ion could be detected for the hydrolysable tannin Tannic acid and the response for that particular polyphenol was insufficient and without satisfactory repeatability.
- For future work other solvents might be considered for the extraction.
- Other methods for securing total extraction of the polyphenolic compounds in spruce bark and eucalyptus leaves would be necessary. For example ASE (accelerated solvent extraction).

I would like to thank this COST action for allowing me the opportunity to participate in a STSM project and also thank you for letting me come here to Paris today and give this presentation!

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