Phenolic compounds in apple juices – Method of quantification by UHPLC-UV and by UHPLC-MS/MS

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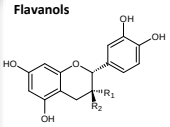
Introduction:

Cider is produced and consumed essentially in Europe and Canada. Astringency, bitterness, color and aroma of cider are traits related to the nature and the content in phenolic compounds [1-3]. The benefic properties associated with apple and cider consumption is generally linked to the high antioxidant potential of these compounds. For this reason, a great number of studies are focused on identification and quantification of phenolic compounds in apple, apple juice or cider. However, no genetic study was available for phenolic contents in cider apple, only two teams having published their work about QTL detection in two dessert apple progenies [4-5]. The first one used a UHPLC-UV method and the second one a HPLC-MS method to separate and quantify phenolic compounds. The UHPLC system allows a reduced analysis time and an increased resolution when compared with the HPLC system [6]. The UV detector allows a good repeatability whereas the mass spectrometer allows a higher sensibility and selectivity, particularly when used in the selected reaction monitoring (SRM) mode. Generally, both spectrometers are equivalent but significant differences have already been reported when comparing the phenolic compound quantifications obtained with the HPLC-UV or HPLC-MS methods. Co-elution and matrix effects are often described to be responsible for them.

The aim of this work was to develop two methods in UHPLC-UV and UHPLC-MS/MS to separate and quantify major phenolic compounds in apple juice and usable for a further genetic study on cider apple. The content of each phenolic compounds obtained for 120 cider progenies with both methods were then compared.

Phenolic compounds quantified in apple juice

Flavanols

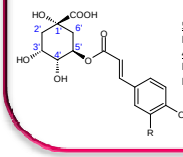


Monomers:
R_1=OH; R_2=H: (-)-epicatechin
R_1=H; R_2=OH: (+)-catechin
Procyanidins:
B1: epicatechin-(4β→8)-catechin
B2: epicatechin-(4β→8)-epicatechin
B5: epicatechin-(4β→6)-catechin
C1: [epicatechin-(4β→8)]$_2$-epicatechin

Dihydrochalcones

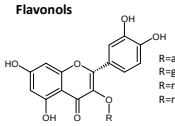
R=glucose : Phloridzin
R=glucose-xylose : Phloretin xyloglucoside

Hydroxycinnamic acids



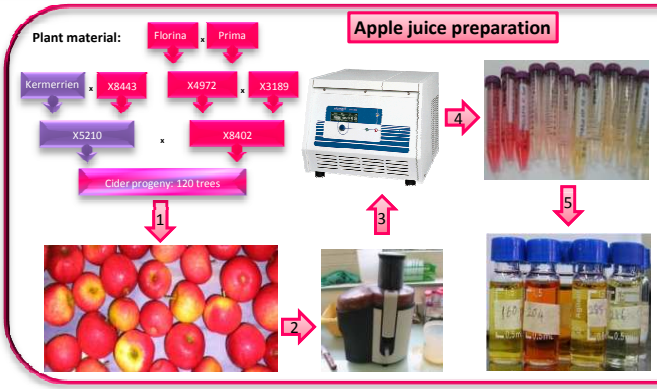
5' position:
R=OH: 5-caffeoylquinic acid
4' position:
R=H: 4-*p*-coumaroylquinic acid
R=OH: 4-caffeoylquinic acid

Flavonols



R=arabinose: Avicularin
R=galactose: Hyperoside
R=rhamnose: Quercitrin
R=rutinose: Rutin

Apple juice preparation



1. Harvest of 1 Kg of fruits per tree at the mature stage "50% of fallen fruit" and division into 3 batches
2. Coring and grinding apples with skin and addition of sodium fluoride in juice
3. Centrifugation
4. Recovery of clear apple juice
5. Addition of 1 volume of acidified methanol

Storage of juices at -80°C before chromatographic analyses

Validation tests for both methods:

- **Limits of Detection (LOD) and Quantification (LOQ):** determined by the signal to noise ratio of 3 and 10 respectively
- **Linearity:** determined by the injection of 10 volumes of the standard working solution (SWS) in 5 replicates taking into account the residual distributions
- **Recovery:** determined by the recovery percent between an apple juice and the same apple juice added with 100µL of the SWS

$$Rec.=[(amount_{juice}+amound_{added})/amount_{measured}]*100$$

- **Precision:** expressed by RSD% for intra- (5 replicates one day) and inter-day (3 replicates in 3 different days) variations

UHPLC-UV method

The UV experiments were performed with a Thermo Accela PDA Detector. Hydroxycinnamic acids were detected at 320 nm and dihydrochalcones, flavonols and flavanols were detected at 280 nm.

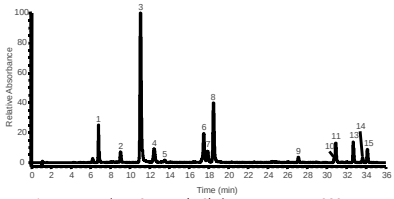


Figure 1. Total Ion Current (TIC) chromatogram at 280 nm.

	LOD (ng)	LOQ (ng)	Linearity (r^2)	Recovery %	Intra-day RSD %	Inter-day RSD %
Min	0.3	0.5	0.99	94.3	1.3	2.6
Max	1	6.7	0.9999	110.4	5.3	11.6

Table 1. Validation test results for the UHPLC-UV method for all compounds except the rutin which could not be detected in UV.

UHPLC-MS/MS method

The MS experiments were performed with a Thermo TSQ quantum access max equipped with an electrospray interface (ESI) operating in negative ionization mode. The Selective Reaction Monitoring (SRM) mode was used to quantify phenolic compounds.

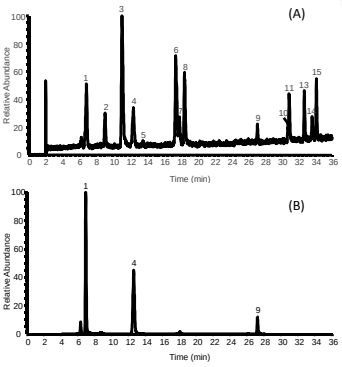


Figure 2. Total Ion Current (TIC) chromatogram in ESI(-) (A) and Single Reaction Monitoring (SRM) chromatogram in ESI(-) for procyanidin B. Parent ion: 577 m/z, fragment ion: 289 m/z and collision energy: 16 V (B).

	LOD (ng)	LOQ (ng)	Linearity (r^2)	Recovery %	Intra-day RSD %	Inter-day RSD %
Min	0.003	0.007	0.9893	91.2	2.3	3
Max	2	6.7	0.9991	113.3	6.8	10.7

Table 2. Validation test results for the UHPLC-MS/MS method for all compounds.

Analyze of 120 different apple juices and comparison between the two methods

- 120 hybrids were used to prepare apple juices which were analyzed in triplicate with the two methods.

➢ The major compound of apple juice is chlorogenic acid with concentrations comprised between 97.23 and 741.1 µg/mL of apple juice. Procyanidin B2 is the second major compound with concentrations comprised between 76.2 and 355.7 µg/mL. The less concentrated compound is rutin with a concentration comprised between 0.16 and 1.75 µg/mL.

- The 2 quantification results were then compared for 12 phenolic compounds quantified with the 2 methods (example for procyanidin B1 and phloretin xyloglucoside on the Figure 3).

➢ All compounds have shown a high correlation coefficient comprised between 0.86 and 0.99.

➢ The slopes of the linear regression obtained were comprised between 0.47 and 1.32.

- For the chlorogenic acid (slope value 1.34) a co-elution with another compound could explain the overestimation of its quantification with the UV detector as suggested by Caporossi and collaborators (2010, [7]).
- For the epicatechin, 4-p-coumaroylquinic acid, quercitrin and avicularin, the low slop values (0.63, 0.65, 0.78, 0.47 respectively) show an overestimation with the mass detector (or an underestimation with the UV detector). This has already been reported by another team, but no satisfactory explanation was found [8-9]. It could be a matrix effect that reduce the masse response in apple juice compared to standards. Further studies are needed to validate this hypothesis.

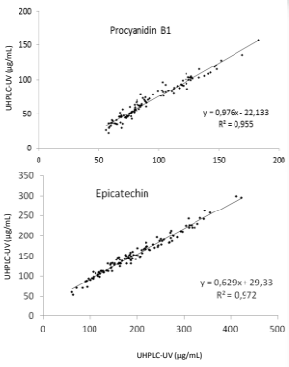


Figure 3. Comparison between UHPLC-MS/MS and UHPLC-UV quantification with the mean of quantification of 120 apple juices for procyanidin B1 and phloretin xyloglucoside compounds.

Conclusion:

Two new UHPLC-UV and UHPLC-MS/MS methods were developed to separate and quantify phenolic compounds in apple juice. Both were validated separately with the estimation of limits of detection and quantification, linearity, recovery and precision for the 15 major compounds of apple juice, except for the rutin which could not be detected with the UV detector.

These two methods were then used to quantify phenolic compounds in juices prepared from 120 hybrids of a cider apple progeny. The comparison of quantities obtained with both spectrometers showed an overestimation with the mass analyzer for four compounds. The possible matrix effect that affect the mass quantification shows that this type of analyzer should not be necessarily the reference for the phenolic compound quantification in complex matrices as apple juice.

References:

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