An accurate and cost-effective method for the quantification of total triterpenoid and steroidal saponins in raw plant materials

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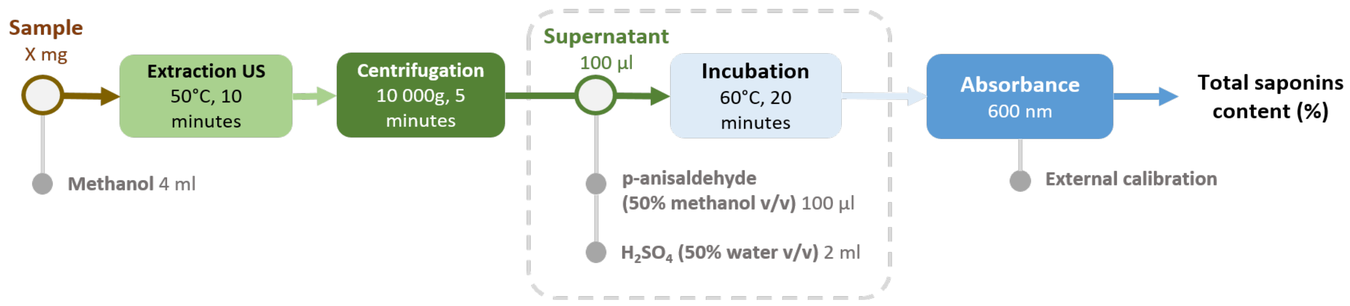
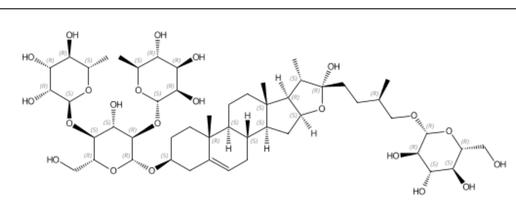
Introduction

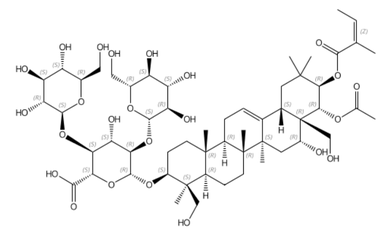
Saponins are heterosides widely distributed in the plant kingdom. Their properties are used in many industrial sectors such as food, cosmetics, agricultural, pharmaceutical and animal nutrition [1]. Although many techniques exist to quantify saponins: gravimetric, foaming, spectrophotometric or chromatographic [2], none of these methods makes it possible to be at the same time accurate, fast, inexpensive with a response similar for the triterpenoid and steroidal saponins. A colorimetric method constituted of p-anisaldehyde and sulfuric acid was developed and avoids all the above disadvantages. The method was validated and assayed over four saponin plants: Camellia , Quinoa (triterpenoid saponins) and Fenugreek, Yucca (steroidal saponins).

Materials and methods



Quantity of sample is depending of the amount of saponins present in the sample. External calibration can be performed with any saponin standard. Method is patented under the registration number EP3742153A1 [3].

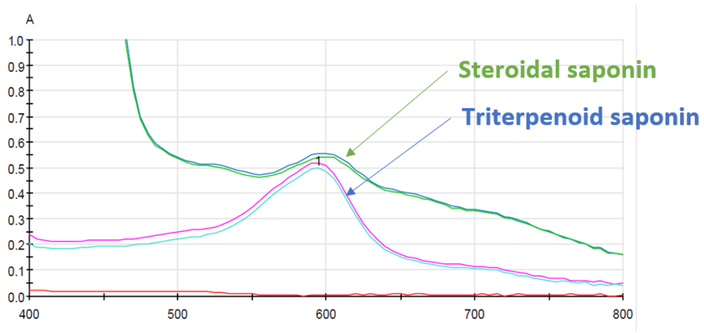

Standard Protodioscin (Steroidal saponin)


Standard Escin IB (triterpenoid saponin)

Results and discussion

Specificity and Selectivity

The colorimetric method allows the development of a chromophore at 600 nm. The response obtained is similar for steroidal and triterpenoid saponins making it possible to quantify them with any standard of saponin (e.g. protodioscin, escin IB or aescin). Due to the high price of saponin standards, we recommend the use of aescin, an inexpensive triterpenoid saponin mixture.



$$\varepsilon_{Escin\ IB\ 600nm} = 2744\ L.mol^{-1}.cm^{-1}$$
$$\varepsilon_{Protodioscin\ 600nm} = 2745\ L.mol^{-1}.cm^{-1}$$
$$\varepsilon_{Aescin\ 600nm} = 2630\ L.mol^{-1}.cm^{-1}$$

The chromogenic reaction is specific for saponins at this wavelength, making it possible to quantify directly in a complex extract and so reduces considerably the stages of sample preparation.

Linearity

The linearity was demonstrated in the chromogenic solution for the three different standards cited above. Results shown that the absorbance for each standard was highly linear in the range 50 – 230 µg.ml-1 (Lack-of-fit test with α 5%)

Limit of detection/quantification

Six blank solutions were subjected to the whole analytical procedure six times. The standard deviation δ_0 for saponins was determined.

The limit of detection (LOD) and limit of quantification (LOQ) were determined as follow:

$$LD = 3 \times \delta_0$$
$$LD = 10 \times \delta_0$$

For the three standards, LD and LQ were respectively 6 and 20 µg.ml^{-1}

Precision and accuracy

The precision experiment was performed on Camellia and Fenugreek extracts. Repeatability is determined by measuring the same sample six times and intermediate precision by repeating it over another day. Accuracy is evaluated in triplicate with spiked standard solution at 100% of the nominal value of saponins.

Camellia extract

Day	Saponins (%m/m) (n=6)	RSD	Saponins (%m/m) (n=12)	RSD	Recovery
1	29.9	2.0%	30.5%	2.6%	97 ± 1%
2	31.1	1.4%			

Fenugreek extract

Day	Saponins (%m/m) (n=6)	RSD	Saponins (%m/m) (n=12)	RSD	Recovery
1	28.2	0.7%	28.7%	2.3%	102 ± 2%
2	29.3	1.7%			

Relative standard deviation for repeatability and intermediate fidelity did not exceed respectively 2.0% and 2.6% for both extracts indicating a good precision of the method. Recoveries were in the range [97 – 102%] for both extracts corresponding to a good accuracy of the method.

Comparison with HPLC-ELSD

Applicability of the method was tested over four saponins plants: Camellia, Fenugreek, Yucca and Quinoa. Results were compared with an HPLC-ELSD method developped in-house. Although this chromatrographic technic is expensive, it permit to measure total saponins with a great precision and accuracy.

	Colorimetric method		HPLC-ELSD method	
Saponin plants extracts	Saponins (%m/m) (n=3)	RSD	Saponins (%m/m) (n=3)	RSD
Camellia	30.5	1.7%	30.1	2.6%
Fenugreek	28.7	1.2%	28.7	2.8%
Quinoa	9.5	1.2%	9.6	4.7%
Yucca	28.1	2.7%	29.9	4.1%

Results obtained for both methods are quite similar and justify the use of the inexpensive colorimetric method for the total saponins quantification.

Quantification of saponins blends

The method gives an identical response for steroidal and triterpenoid saponins allowing the quantification of mixtures of both types. The methods was then assayed on various blends of Camellia and Fenugreek.

Blend Camellia/Fenugreek	Expected value (%m/m)	Results (%m/m)	%Bias
20/80	26.8	27.1	1.2%
50/50	27.8	26.9	-3.2%
80/20	28.7	28.0	-2.6%

Values obtained with the method were relatively close of the expected values. Theses results clearly support the applicability of the method for the quantification of blends.

Conclusion

A new, simple, fast and inexpensive spectrophotometric method for the total quantification of saponins in plant was developed. The proposed method exhibited good performances in term of linearity, precision and accuracy. The specificity of the method allows the utilization of a cost-effective extraction step by using ultrasonic bath reducing drastically the time of analysis. The response similar for both saponins types permitting to use any standard of saponins for the quantification and allowing the quantification of blends of saponins.

References

[1] Güçlü-Üstündağ Ö, Mazza G. Critical Reviews in Food Science and Nutrition. 2007; 47: 231–258
[2] Cheok CY, Salman HAK, Sulaiman R. Food Research International. 2014; 59: 16–4
[3] Le Bot M, Guilet D. EP3742153A1. 2020