Development of a simple quantification method of tocopherols and phytosterols using fast chromatography - Tandem mass spectrometry.

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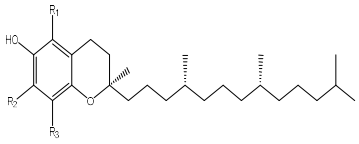
1) INTRODUCTION

Tocopherols (Fig. 1.) and **Phytosterols (Fig. 2.)** are highly abundant compounds in waste products resulting from Canola Oil production. They have significant **antioxidant** and **cholesterol lowering properties**, respectively. Since Canola is a major crop product in Canada, effective extraction of these metabolites has economical impact. Hence, there is a need for the development of a fast and easy quantification method of these active metabolites.



Compound	R1	R2	R3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Fig. 1. Structure for tocopherols (α, β, γ, and δ).

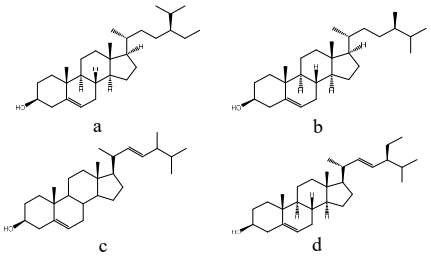


Fig. 2. Phytosterol structures; (a)- β-sitosterol, (b)- campesterol, (c)- brassicasterol, (d)- stigmasterol

Equipment	AB Sciex quadrupole linear ion trap (QTRAP 6500)
Ionization	APCI positive mode
Temperature	380 °C
Nebulizer current	2.5 µA
Curtain gas	40 psi
Gas 1	30 psi

Table 1. MS parameters

2) RESULTS

A guard column (Agilent Poroshell C18, 2.1 mm × 4.7 mm, 2.7 µm) was used to achieve fast separation between tocopherols and phytosterols **(Fig. 3.)** . The mobile phase consisted of acetonitrile:methanol (99:1) with 0.1% acetic acid. MS parameters are described in **Table 1**. Rac-tocol and cholestanol were used as internal standard for tocopherols and phytosterols, respectively. Compounds were quantified in MRM mode. Two transitions were followed for each compound **(Table 2)**. Cross talks among analytes prevented the full removal of the column **(Table 3)**. Analytes could be resolved in 2 minutes, except for campesterol and stigmasterol. They were quantified using specific transitions. β and γ tocopherol, which are isomers, were not resolved on the column either and thus quantified collectively. Calibration curves were established and a good linearity was achieved in the range of 0.25-10 µg/ml with reported R^2 of at least 0.996 for tocopherols and 0.997 for phytosterols. The method was compared to the use of conventional C18 column (Agilent Poroshell C18, 2.1 mm × 150 mm, 5µm) where the separation was achieved in 6.5 minutes (except for β-γ tocopherol) **(Fig. 4.)**.

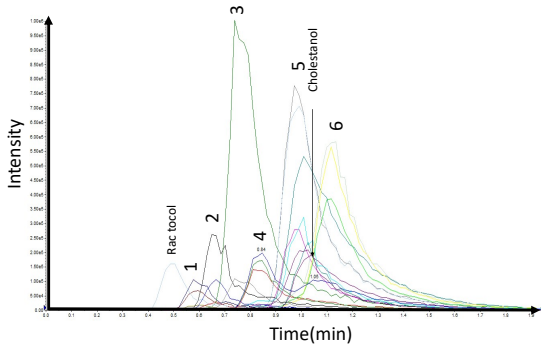


Fig. 3. Spectra of **1** δ-tocopherol **2** β-γ tocopherol **3** α-tocopherol **4** brassicasterol, **5** campesterol- stigmasterol, **6** β-sitosterol using **a guard column**. Run time of 2 minutes.

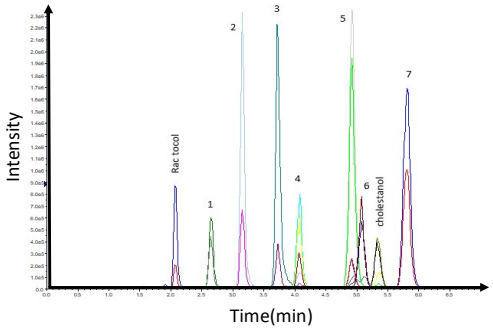


Fig. 4. Spectra of **1** δ-tocopherol **2** β-γ tocopherol **3** α-tocopherol **4** brassicasterol, **5** campesterol, **6** stigmasterol, **7** β-sitosterol using a **conventional C18 column**. Run time of 6.5 minutes.

Compounds	Precursor ion (m/z)	Quantifier ion	Qualifier ion
Delta Tocopherol (δ)	402.5	177	137
Gamma Tocopherol (γ)	416	151.3	191
Beta Tocopherol (β)	416	151.3	191
Alpha Tocopherol (α)	431.3	165.2	137
Rac Tocol	388.4	163.2	122.9
Brassicasterol	381.3	105.2	147.2
Campesterol	383.4	161.1	147.2
Stigmasterol	395.5	83.0	147.1
β-sitosterol	397.4	147.1	161.2
Cholestanol	371.4	95.2	109.1

	Molecular weight	$[M+H-H20]^+$	$[M+H-4H]^+$
Stigmasterol	412.7	**395.5**	409.7
Brasicasterol	398.7	381.3	**395.7**
β-sitosterol	414.7	**397.4**	411.7
Campesterol	400.7	383.4	**397.7**

Table 3. Molecular weight and precursor ions ($[M+H-H20]^+$ and $[M+H-4H]^+$) for phytosterols

3) CONCLUSIONS

- A fast and simple FC-MS/MS method for simultaneous quantitation of phytosterols and tocopherols was developed.
- A good linearity was achieved (0.25 -10 µg/ml).

4) ACKNOWLEDGEMENT

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