SIMULTANEOUS QUANTITATIVE DETERMINATION OF MAJOR PHENOLICS IN ROSEMARY EXTRACT *via* DPPH REAGENT FREE-RADICAL-SCAVENGING ACTIVITY

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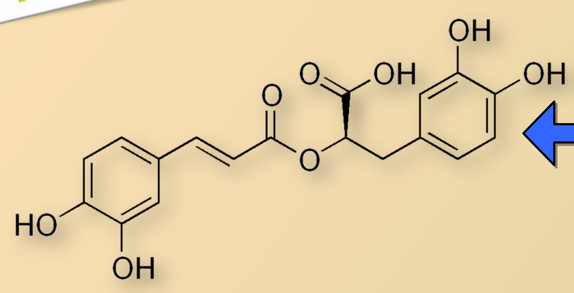
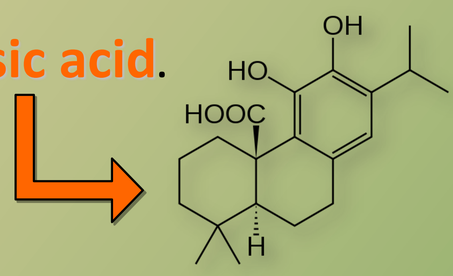
Rosemary extracts are convenient sources of natural antioxidants particularly phenolic constituents. This group is mainly constituted by several minor flavonoids, while the main components are rosmarinic acid and the diterpenoid compound known as carnosic acid.



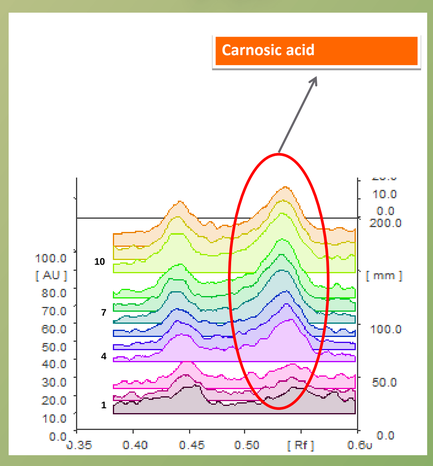


Consequently the quality of commercial extracts depends on the claimed antioxidant content calculated as rosmarinic acid (RA) and carnosic acid (CA). Therefore a rapid and selective method aiming to evaluate the above mentioned features should be highly desirable.

While rosmaric acid is trouble-free determinable by densitometric HPTLC at 330 nm, carnosic acid shows two crucial features: aptitude for degradation and low molar absorptivity (ε =1131.9±57.7 l M−1 cm−1) at λ_{max} 284 nm [1] yielding by direct densitometric scanning LOD = 886 ng

Therefore a new HPTLC-densitometric method, based on DPPH in situ derivatization was developed for routine analysis of RA and CA in rosemary extracts. This latter compound, is reported to rapidly degrade toward the oxidized forms of carnosol , carnosol quinone and rosmanol [2,3].

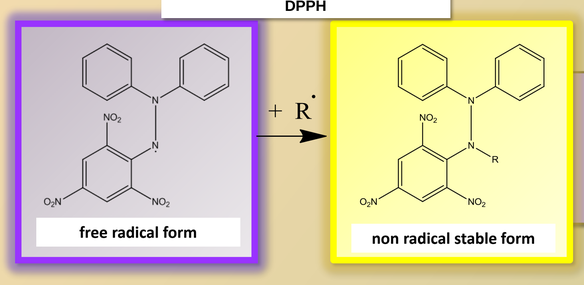
The problem of the chemical behaviour of the carnosic acid during the HPTLC analyses, must be weighed up. Experiments are in progress to look further in the stability of carnosic acid.



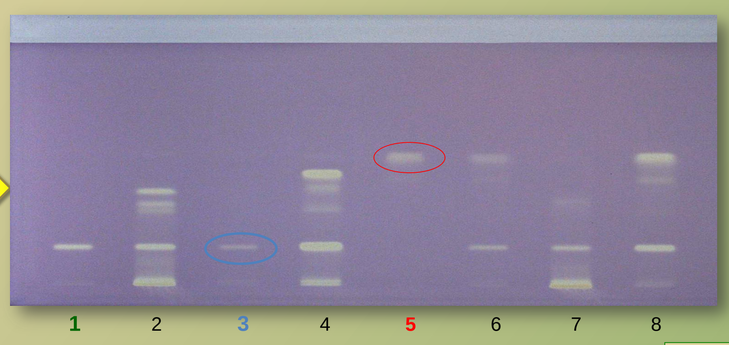
CA densitograms at 284 nm on Lichrospher F_{254}, toluene, ethyl formiate, formic acid (6:4:1 v/v).

Track 1, 495 ng; track 4, 660 ng; track 7 825 ng; track 10, 990 ng.

2,2-diphenyl-1-picrylhydrazyl
DPPH

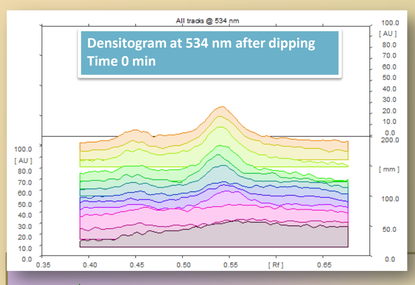


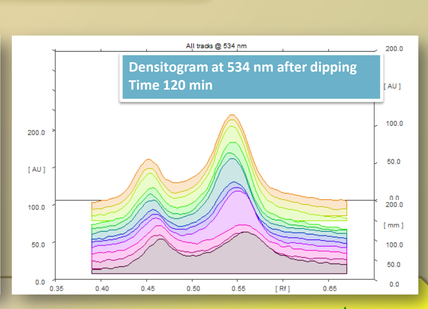
Derivatization by dipping in DPPH in AcCN 0.5 mM and densitometric scanning at 534 nm



RA std, CA std and *Rosmarinus officinalis* L. extracts on Lichrospher F_{254}, DPPH reagent; UV white light;

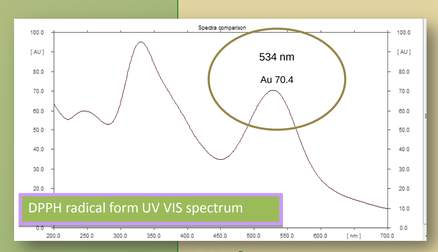
Track 1,3 RA std; 2 Leaves one year old ethanolic extract, 4 hydroalcoholic extract A; 5 CA std; 6,8 Ethanolic extracts; 7 hydroalcoholic extract B

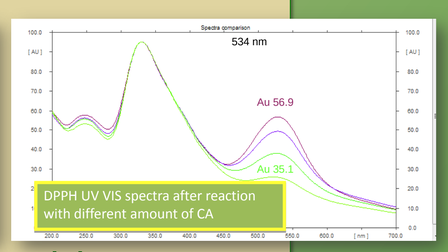




As the outcome of the derivatization is affected by time, the quantification by scanning at 534 nm could be performed after 90-120 min with consequent baseline worsening and decrease of precision and accuracy.

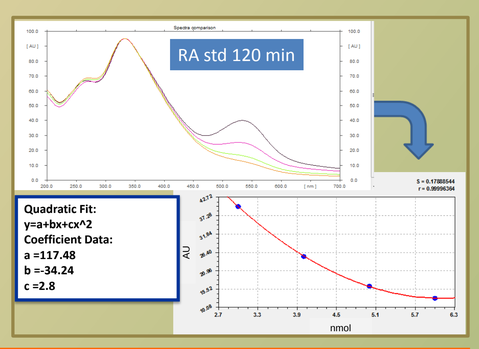
Here we propose a novel approach exploiting the features of the DPPH UV-VIS spectra before and after the reactions with CA and RA





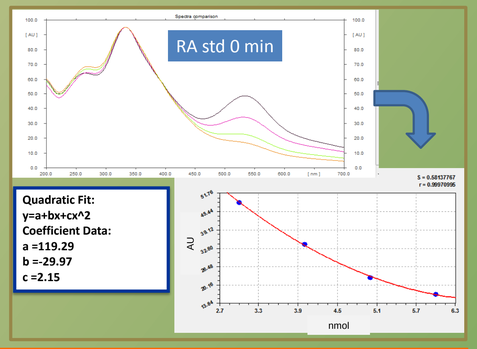
AU (arbitrary absorbance units) decrements were found to be proportional to analyte amounts, allowing to perform a calibration curve

TIME 120 min			
nmol	AU at 534 nm	ng	Sample
0.3	40	106.5	Rosmarinic Acid Std
0.4	25.2	142	Rosmarinic Acid Std
0.5	16.4	177.5	Rosmarinic Acid Std
0.6	12.8	213	Rosmarinic Acid Std
0.443	20.7	*159.61*	RA in Ethanolic Extract
0.435	21.5	*156.73*	
1.5	49	495	Carnosic Acid Std
2	38.7	660	Carnosic Acid Std
2.5	26.3	825	Carnosic Acid Std
3	14.6	990	Carnosic Acid Std
2.97	15.2	*981.34*	CA in Ethanolic Extract
2.95	15.6	*974.73*	

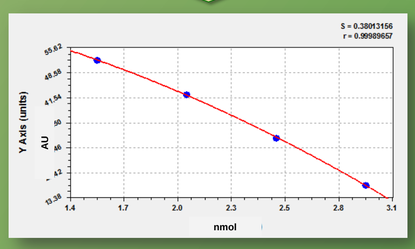


Quadratic Fit:
y=a+bx+cx^2
Coefficient Data:
a =117.48
b =-34.24
c =2.8

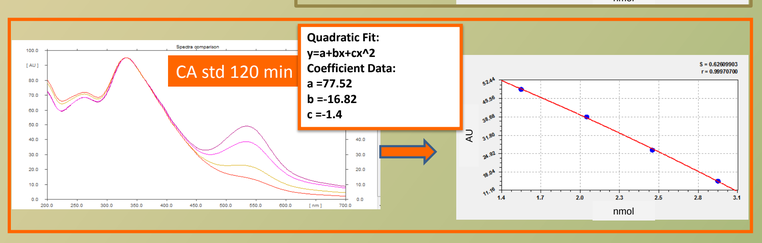
TIME 0 min			
nmol	AU at 534 nm	ng	Sample
0.3	48.6	106.5	Rosmarinic Acid Std
0.4	34.2	142	Rosmarinic Acid Std
0.5	22.8	177.5	Rosmarinic Acid Std
0.6	17	213	Rosmarinic Acid Std
0.438	29.2	*157.81*	RA in Ethanolic Extract
0.442	28.8	*159.25*	
1.5	56	495	Carnosic Acid Std
2	47.4	660	Carnosic Acid Std
2.5	35.6	825	Carnosic Acid Std
3	20.6	990	Carnosic Acid Std
2.92	23.2	*964.82*	CA in Ethanolic Extract
2.94	22.4	*971.43*	



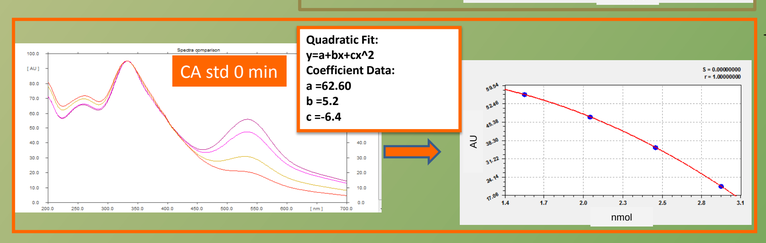
Quadratic Fit:
y=a+bx+cx^2
Coefficient Data:
a =119.29
b =-29.97
c =2.15



This behaviour resulted unaffected by variables as time, temperature, humidity, giving rise to a reliable quantitative procedure.



Quadratic Fit:
y=a+bx+cx^2
Coefficient Data:
a =77.52
b =-16.82
c =-1.4



Quadratic Fit:
y=a+bx+cx^2
Coefficient Data:
a =62.60
b =5.2
c =-6.4

Carnosic acid and rosmarinic acid were simultaneously determined in the range of 495-990 ng and 106-213 ng respectively with RSD of repeatability and intermediate precision not exceeding 1.0% .
LOQ for CA was 495 ng

	Min 0		Min 60		Min 120		Min 180		Min 240	
Sample	nmol	ng	nmol	ng	nmol	ng	nmol	ng	nmol	ng
RA in Ethanolic Extract	0.438	157.81	0.443	157.81	0.443	159.61	0.442	159.25	0.442	159.25
	0.442	159.25	0.437	159.25	0.435	156.73	0.434	156.37	0.434	156.37
CA in Ethanolic Extract	2.92	964.82	2.97	981.34	2.97	981.34	2.98	984.65	2.99	987.95
	2.94	971.43	2.96	978.04	2.95	974.73	2.97	981.34	2.97	981.34

	Mean ng (n=10)	CV %
RA in Ethanolic Extract	158.17	0.87%
CA in Ethanolic Extract	978.70	0.69%

The proposed approach is suitable for determination of other antioxidants: poliphenolic compounds and flavonoids.

[1] M. Pelillo, M.E. Cuvelier, B. Biguzzi, T. Gallina Toschi, C. Berset, G. Lercker, J. Chromatogr., A 1023 (2004) 225-229.
[2] T. Masuda, T. Kirikihira, Y. Takeda, J. Agric. Food Chem. 53 (2005) 6831-6834.
[3] C.T. Ho, T. Ferraro, Q. Chen, R.T. Rosen, M.T. Huang ACS Symposium series 547, Washington DC: American Chemical Society, (1994) 2-19.

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