Determination of capsaicin and dihydrocapsaicin in topical creams using HPLC and extended standard addition calibration

A E Anghel[1] and A C Moț[2]

[1] Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, 11 Arany János Street, RO-400028 Cluj-Napoca, Romania

[2] Chemometrics and Bioanalytical Chemistry Laboratory, Analytica Research Center, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, 11 Arany János Street, RO-400028 Cluj-Napoca, Romania

E-mail: ancu.anghel@gmail.com

1. Introduction:

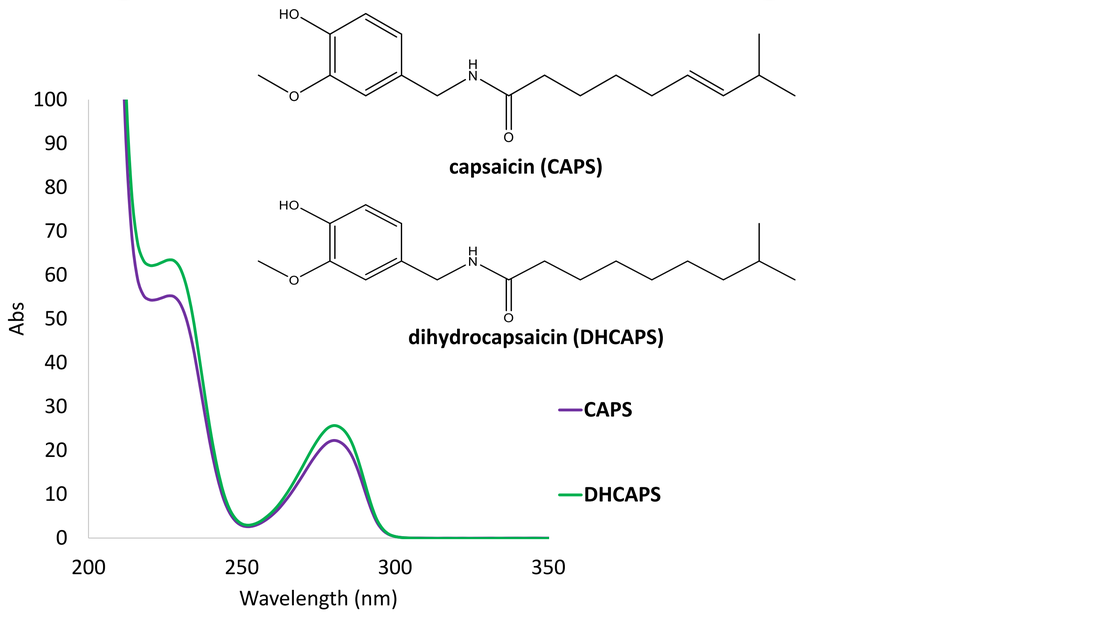
Capsaicin and dihydrocapsaicin, the pungent principles that account for 90% of total capsaicinoid content in chilli peppers, are lipophilic alkaloids formed through the condensation of vanillylamine with fatty acid derivatives [1].

Topical capsaicin selectively stimulates the release of substance P and, possibly, other neurotransmitters, from certain types of afferent neurons, accounting for the initial algesic effect associated with its use. After sustained application, however, depletion of neurotransmitter at the sensory nerve endings occurs, leading to long lasting desensitization to painful stimuli [2].

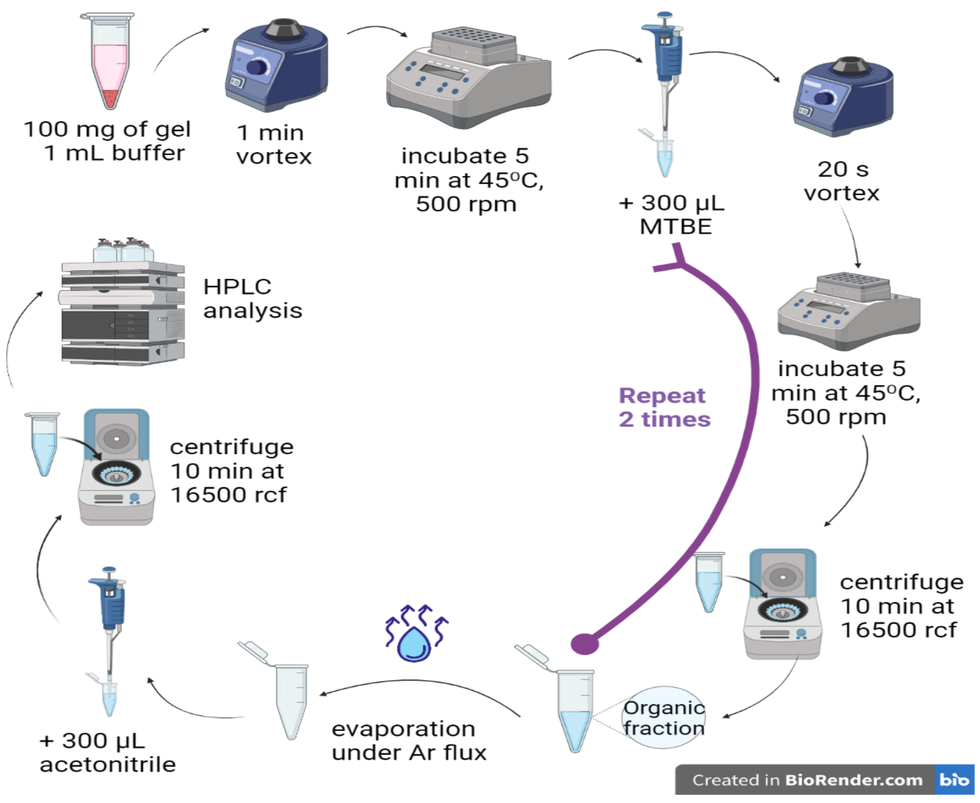
In this work, a method for the non-destructive determination of capsaicin and dihydrocapsaicin from topical creams is developed, consisting of subsequent solvent extraction steps and HPLC/UV-Vis analysis of the pooled organic fractions. Different calibration methodologies are explored to quantify both capsaicinoids.

Extended standard addition calibration combines standard addition (constant sample masses, spiked with varying amounts of analytes, subjected to the extraction procedure) and blank addition (blank and sample material subjected to extraction procedure). While both standard addition and extended standard addition are suitable when investigating analytes embedded in a complex matrix, the latter also provides information about the linearity of the calibration curve below spiking level zero [3].

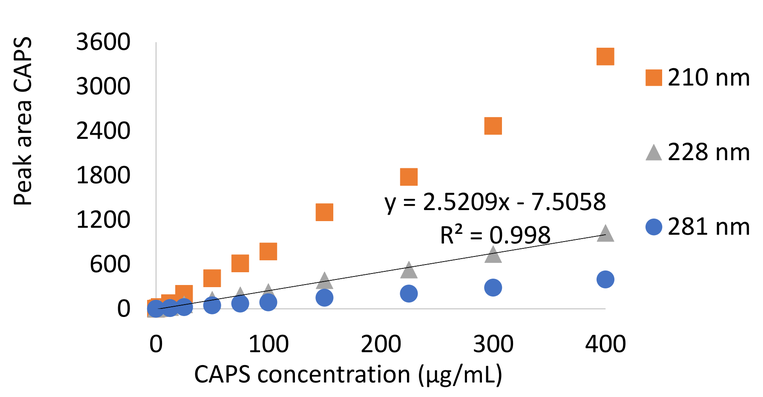
2. Capsaicinoids' structure & UV-Vis spectra

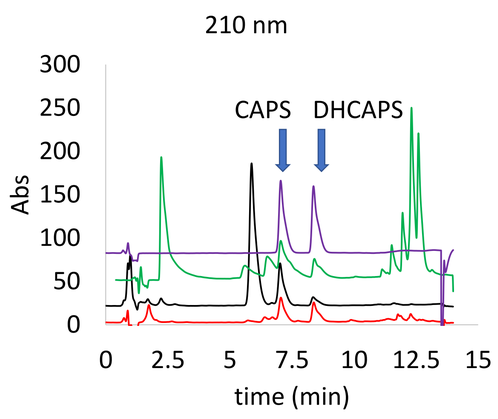


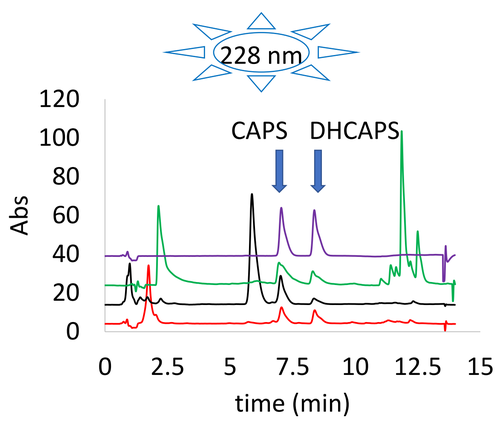
3. Capsaicinoid extraction from topical creams

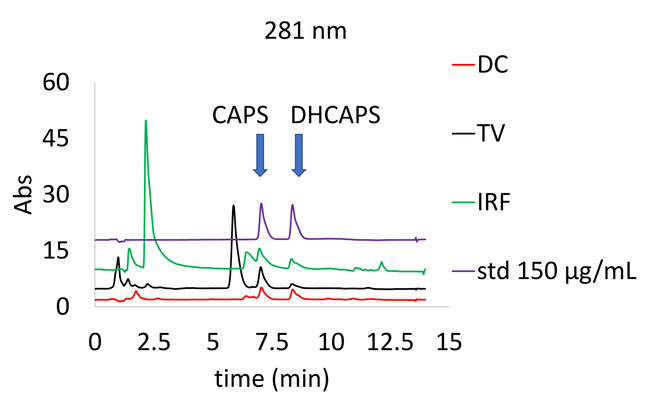


4. External Standard Calibration



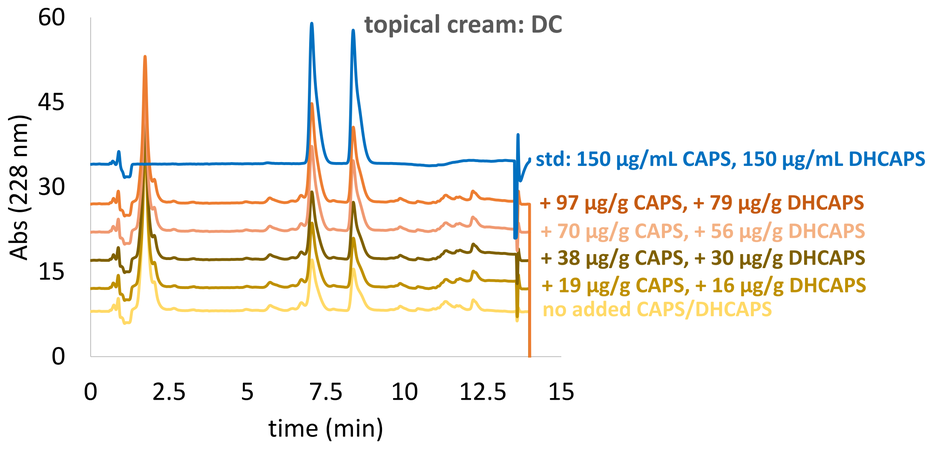


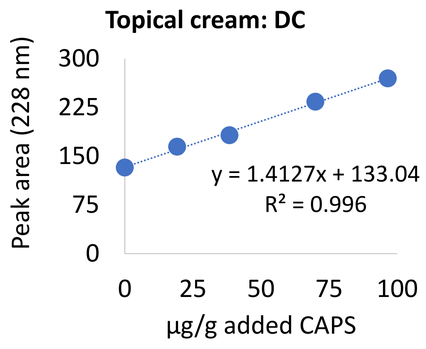


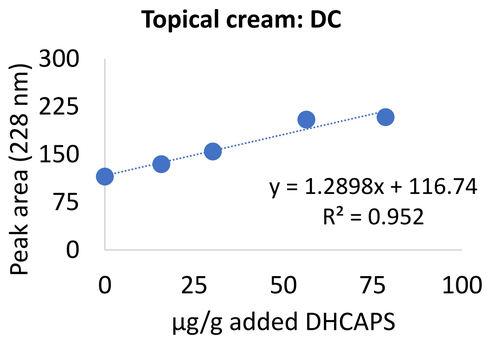


Capsaicinoid	y-intercept	slope	R^2	LOD (µg/mL)	RSD (%)			Capsaicinoid content (µg/g)		
					DC	TV	IRF	DC	TV	IRF
CAPS	-7.50	2.52	0.998	1.8	8.0	5.4	8.6	83,1 ± 16,4	139,0 ± 18,5	167,8 ± 38,1
DHCAPS	-4.63	2.57	0.998	3.1	4.3	6.4	7.9	69,9 ± 7,5	34,6 ± 5,5	71,0 ± 15,8

5. Standard addition calibration

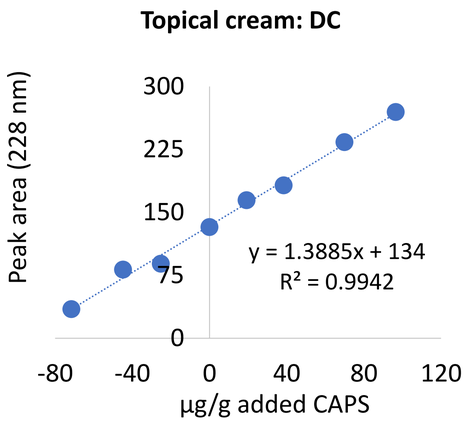


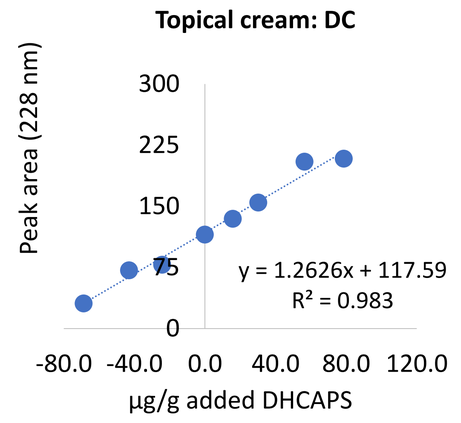




Topical cream	R^2		CAPS content (µg/g)	DHCAPS content (µg/g)	CAPS Recovery (%)			
	CAPS	DHCAPS			SA1	SA2	SA3	SA4
DC	0.996	0.952	94.2±5.3	90.5 ±16.8	130.0	84.7	103.8	99.3
TV	0.976	0.953	202.1±31.3	50.3±11.4	100.1	100.5	110.2	94.6
IRF	0.989	0.983	496.9±48.6	226.0±27.5	74.4	101.1	98.8	101.1

6. Extended standard addition calibration





Topical cream	R^2		CAPS content (µg/g)	DHCAPS content (µg/g)
	CAPS	DHCAPS		
DC	0.994	0.983	100,0±3.4	94.7 ±5.0
TV	0.991	0.985	204.1±9.7	46.0±3.0
IRF	0.991	0.992	558.8±22.1	252.9±9.3

7. Conclusions:

- A non-destructive method for the determination of capsaicin and dihydro-capsaicin from topical creams, using HPLC/UV-Vis was developed.
- Extended standard addition calibration led to narrower confidence intervals for both capsaicinoids (given the fact that more data points were taken into consideration for the calibration curve), while also accounting for the matrix effect.

8. References:

1. Reyes-Escogido, M., Gonzalez-Mondragon, E. G., & Vazquez-Tzompantzi, E. (2011). *Molecules (Basel, Switzerland)*, **16** (2), 1253–1270. **2.** Rains, C. & Bryson, H.M. (1995). *Drugs & Aging*, *7*, 317–328. **3.** Steliopoulos P. (2015). *MethodsX*, **2**, 353–359.

9. Acknowledgements:

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