CAPILLARY ELECTROPHORESIS OF APIGENIN

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Introduction

Increasing interest in phytocompounds especially flavonoids is a result of their widespread occurrence in plant kingdom and wide range of biological activity. Apigenin (4,5,7-trihydroxyflavone), a naturally occurring plant flavone is a potent antioxidant that exhibits anti-inflammatory activities, is a scavenger of free radicals, and also prevents oxidation of vitamins C, E and glutathione. Apigenin exhibit some antitumor effects by inhibiting the tyrosine kinase activity of topoisomerase, angiogenesis and protecting against oxidative damage of DNA[1].

Capillary electrophoresis (CE) is a modern analytical technique which allows effective separation of charged particles in electric field. CE is considered as a green analytical technique due to its low reagent consumption, small sample volume and short analysis time[2]. Unfortunately, important limitation on the application of capillary electrophoresis to biological samples is high limit of detection.

Very important role in the process of increasing the sensitivity of capillary electrophoresis play an analyte sweeping methods inside the capillary. One promising modality for sweeping the sample in the capillary is micellar electrokinetic chromatography (MEKC). The most suitable electrolyte for this separation was a buffer solution (pH 10.1) of 0.03 M sodium borate with addition of sodium dodecyl sulfate (0.01M) and acetonitrile (10%). Analysis of a complex matrix (plant extracts) requires precise step of sample preparation. The air-dried parsley leaves were powdered and several times extracted with methanol and ethyl acetate. The extract was in the last step centrifuged and the resulting supernatant was subjected to CE analysis. All separations were performed at 25°C with detector set to measure peaks at 390 nm.

Materials and methods

- Hewlett Packard HP 3D Capillary Electrophoresis System with diode array detector,
- Capillary length 60 cm and an internal diameter 50 microns,
- BGE: borane buffer (c = 0.03 M, pH = 10.1) with 10 % ACN content and the addition of SDS (c = 0.01 M),
- Sample introduction: 30 s, 50 mbar,
- λ = 390 nm
- Temperature of 25 ºC and a voltage of 30 kV,

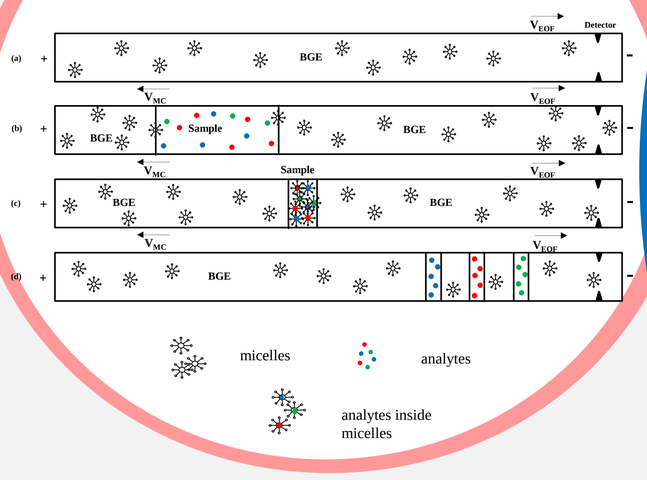
The main sources of apigenin

VEGETABLES:
- parsley
- celery
- artichokes

FRUIT:
- oranges
- cherries
- apples
- nuts
- grapes

DRINKS:
- red wine
- herbs (chamomile)
- green tea

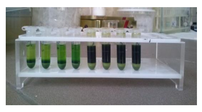
Proposed mechanism of sweeping MEKC of Apigenin



Sample preparation and the initial parameters

The air-dried parsley leaves were powdered using a mortar and extracted with methanol.

After 15 minutes samples were centrifuged (14 000 RPM, 3 min., 25ºC)



The upper layer was collected and then evaporated to dryness at 100ºC

Dry samples were dissolved in deionized water (0.25 ml) and re-extracted with ethyl acetate (1.25 ml, 15 minutes)

Acetate layer was collected into an ependorf vials and evaporated to dryness. The samples were dissolved in 0.1 ml MeOH, then 0.016 ml of deionized water was added. After centrifugation (14 000 RPM, 3 minutes, 25°C) the samples were ready for electrophoretic analysis.

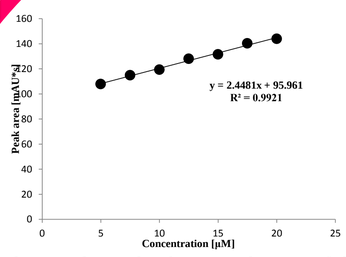
Analytical procedure

Conditioning of the capillary:
1. Double destilled water (2 minutes)
2. NaOH c=0.1 M (1 minute)
3. Double destilled water (2 minutes)
4. BGE (10 minutes)

Hydrodynamic introduction of the sample (30 s, 50 mbar)

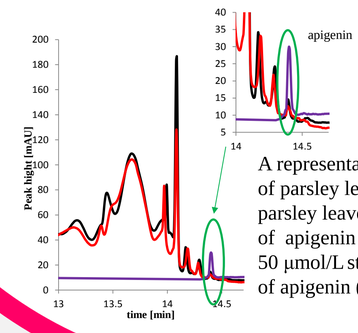
Analysis were conducted with a voltage of 30 kV using the BGE: borane buffer (c = 0.03 M, pH = 10.1) with 10 % ACN content and the addition of SDS (c = 0.01 M)

Calibration



Amound added [µM]	Average peak area [mAU*s]	SD [mAU]	RSD [%]	Recovery [%]
5	107.87	2.51	2.33	97.27
7.5	114.9	2.16	1.88	103.15
10	119.37	1.16	0.97	95.61
12.5	127.97	1.62	1.27	104.59
15	131.5	0.87	0.66	96.78
17.5	140.37	1.3	0.93	103.65
20	143.97	1.58	1.1	98.05

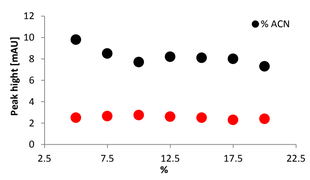
There is a linear relationship between the area of the peak and the concentration of the analyte. The calibration curve was performed on the parsley leaves sample to which various amounts of apigenin (5.0-20.0 µM) were added.



A representative electropherograms of parsley leaves sample (red line), parsley leaves spiked with 15 µmol/L of apigenin (black line) and 50 µmol/L standard sample of apigenin (purple line).

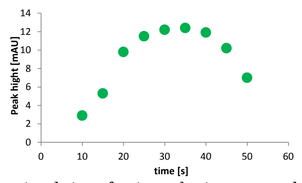
The results obtained during analytes focusing

The effect of the quantity of ACN or MeOH in BGE on the peak hight



To obtain the relatively short time of analysis and good separation of the peaks, the addition of 10% ACN for further analysis was chosen.

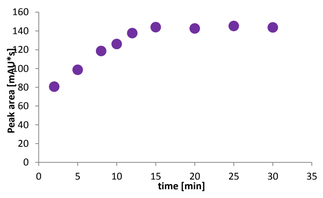
The effect of the sample introduction time on the peak height



The optimal time for introducing a sample into the capillary was 30 s. Further increasing the time of injection, results in the lowering of the peak height and broadening / splitting of the signal.

Effect of the extraction time on the peak area

Time of extraction has a significant impact on the area (and hight) of CE signals. The optimal extraction time ranged from 12 to 20 minutes.



Conclusions

- ✓ **Application of an appropriate mode of extraction allowed to use on-line stacking method for the determination of apigenin in plant extracts,**
- ✓ **The low limit of detection was 5 nmol in 1 mL of plant extract,**
- ✓ **Presented procedure allows approximately 20-fold increase of sensitivity,**
- ✓ **Elaborated assay can be useful for the determination of apigenin in biological samples.**

References

[1] S. Shukla, S. Gupta, Apigenin: a promising molecule for cancer prevention, Pharm. Res. 27, 2010, 962–978.

[2] Kubalczyk P., Bald E., Methods of Analyte Concentration in a Capillary [in]: Electromigration Techniques [Red.] B. Buszewski et al., Springer Series in Chemical Physics 105, Springer-Verlag Berlin Heidelberg 2013, ISBN 978-3-642-35042-9.

Acknowledgments

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