Determination of Combretastatin A4 Influence on Human Lung Cancer and Mouse Melanoma as *in vitro* Cell Culture Models by SPME-LC-HRMS

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Introduction

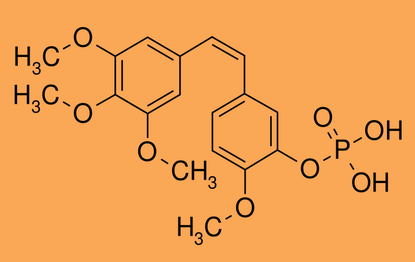
The use of solid phase microextraction (SPME) in combination with high-resolution mass spectrometry (HRMS) for cell culture metabolomics analysis allows getting more sophisticated data from in vitro assays.

Due to fact that SPME is an equilibrium based non-exhaustive microextraction technique it enables performing multiple extractions from a single sample with no influence on the tested cells. Moreover, the opportunity of time course analysis from a single sample reduces the total number of samples, and eliminates inter-batch variability, for which cell culturing can be significant.

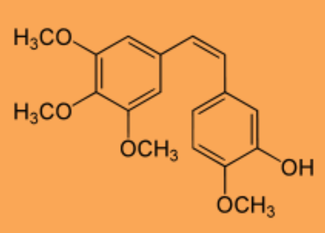
Aim

Aim of the presented study was assessing the influence of combretastatin A4 (CA4) and its pro-drug combretastatin A4 phosphate (CA4P) on cancer cell lines A549 (human non-small cell lung cancer) and B16F10 (mouse melanoma) using SPME.

Materials & Methods



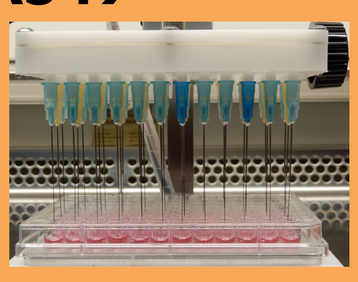
Combretastatin A4 (CA4) features potent anticancer activity but, due to low water solubility, this compound is not suitable as a drug. Therefore, combretastatin A4 phosphate disodium salt (CA4P) was synthesized. In live organism, CA4P (a prodrug) is metabolized to CA4 (active compound) rapidly by unspecific phosphatases.

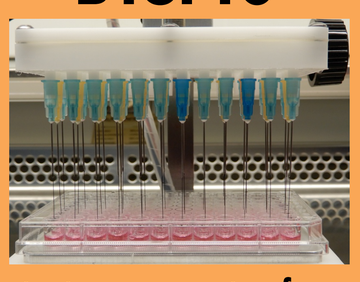


CA4P **CA4**

A549 **B16F10**

and





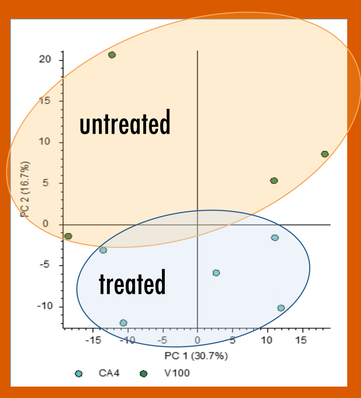
six-times extraction from the same plate

SPME Protocols		
Insert	96-well plate	
A549		B16F10
Preconditioning		
30 min, MeOH:W (50/50)		
Wash		
5 sec, water		
Extraction		
90 min, direct immersion from glass inserts, 8mm mix mode fibers, 1200 rpm agitation	30 min, direct immersion from 96-well plate, 2mm HLB fibers, no agitation	30 min, direct immersion from 96-well plate, 2mm HLB fibers, no agitation, **six-times**
Wash		
5 sec, water		
Desorption		
90 min, ACN:W (80/20), 1200 rpm agitation		

untargeted metabolomic analysis using LC-HRMS (Q-Exactive Orbitrap)

mass tolerance: 3 ppm
S/N threshold: 3
RT tolerance: 0.2 min

Results



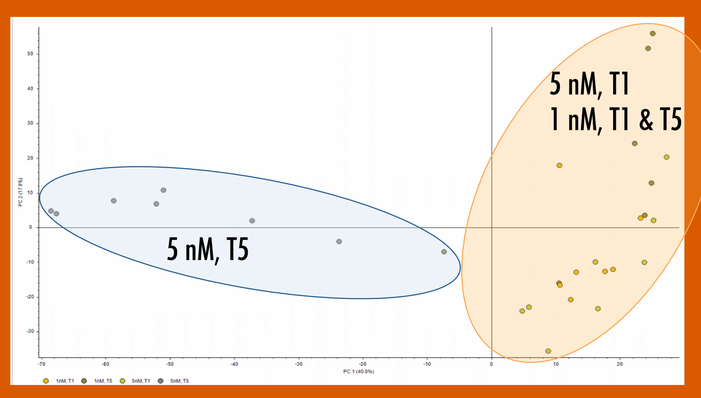


Figure 1. PCA diagrams showing difference in A549 cell line (left side) and B16F10 cell line (right side) after treatment with CA4 on PFP column in positive ionization mode.

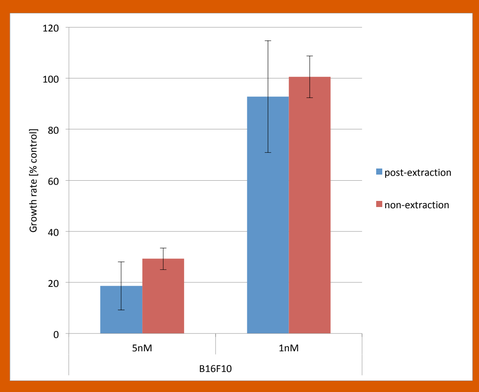
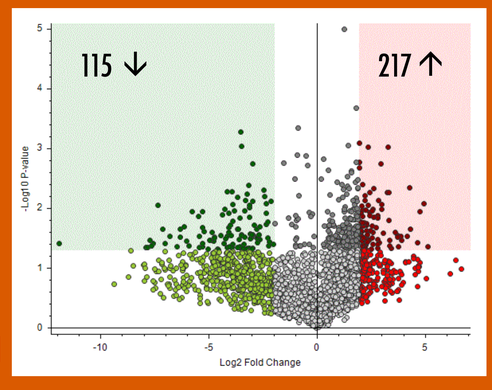


Figure 2. Growth rate of B16F10 cell line without (red column) and after extraction (blue column).



p <0.05
Log_2 change >1

Figure 3. Volcano plot for B16F10 cell line (5nM, T5 vs. T1).

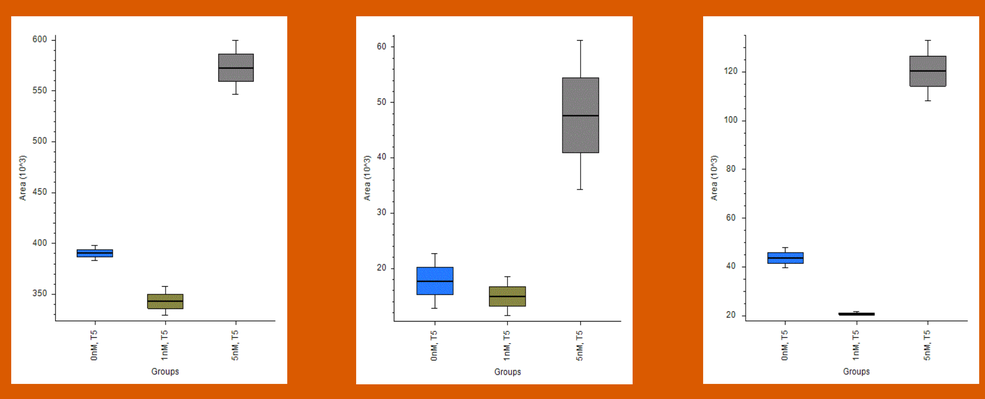


Figure 4. The box Whisker Charts representing peak areas for palmitamide (left), hypoxanthin (center) and L-tyrosine (right) (B16F10 cell line).

Conclusions

1. Using direct immersion SPME for cell cultures analysis after exposure to drug allows obtaining information about metabolites changes over time from the same wells without affecting cells growth.
2. Presented procedure can be considered as high–throughput screening (HTS) and „non-disturbing" for cells.
3. The use of SPME-(LC)-HRMS with cell cultures in a high-throughput manner were presented for the first time.

References

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Acknowledgements

Presented study was financed by Nicolaus Copernicus University (Toruń, Poland) statutory grant No. 451.