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Optimization of method for study of influence of fluoranthene on the cell

Ondrej Zitka, Ondrej Vodicka, Petr Babula, Ladislav Havel, Marie Kummerova, Miroslava Beklova, Rene Kizek*

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Abstract

Polycyclic aromatics hydrocarbones (PAHs) belong to the group of the most occurring pollutants of living environment. They are able to enter ecosystems and subsequently live organisms due to their lipophilic properties. Their influence on living organisms is well known especially in the case of animals and especially human, influence of PAHs on plants is not recognized and described for the present. For this purpose, we developed rapid method for separation and detection of fluoranthene and similar PAHS, such as pyrene and benzo[*a*]pyrene, in plant extracts. Method consists in extraction and detection, where high performance liquid chromatography (HPLC) system with UV detection was used.

Ondrej Zitka, Rene Kizek*

Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic.

*Tel: +420 545 133 350, Fax: +420 545 212 044
E-mail: kizek@sci.muni.cz

Ladislav Havel

Department of Plant Biology, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic.

Ondrej Vodicka, Petr Babula

Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic.

Miroslava Beklova

Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic.

Marie Kummerova.

Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlarska 2, CZ-611 37 Brno, Czech Republic.

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Introduction

PAHs demonstrated mutagenic effect on many cell lines based on interferences with DNA. This fact is well evident especially after activation of PAHs by UV, when reactive forms – radicals originate. Ability of PAHs to generate reactive oxygenic species (ROS) is discussed too. ROS can subsequently oxidatively damage large scale of biomolecules and can serve as apoptotic signals. In area of plant physiology, knowledge about PAHs effects and their mechanisms of actions are still missing and are only limited. Moreover, induction of oxidative stress by several PAHs was demonstrated. Fluoranthene represents one of the PAHs models, which is generally used in *in vitro* as well as *in vivo* experiments. For analysis of fluoranthene and other non-polar compounds, HPLC-UV system is very helpful in this type of experiments (Kummerova et al. 2006; Wu et al. 1998).

Materials and Methods

Aim of this work consisted in developing of method for separation and detection of PAHs in tobacco cells treated by fluoranthene. HPLC-ED system consisted of two chromatographic pumps (Model 582, ESA Inc., Chelmsford, MA, working range 0.001-9.999 ml min^{-1}), chromatographic column with reverse phase Phenomenex Gemini NX C18 (100 × 2,0; 3 µm particles, Phenomenex, USA) and UV detector (Model 528, ESA, USA). Sample (20 µl) was injected by autosampler (Model 542, ESA, USA), which has thermostated space for column. All BY-2 cell samples were first destructed in liguid nitrogen and then extracted in acetonitrile. Samples of matrixes were then centrifuged at 14 000 G by time of 20 minutes. Supernatant was then directly analyzed by HPLC.

Results and discussion

We searched the best parameters and compromises in optimizing of separation method. Most suitable flow rate of mobile phase was determined as 0.3 ml min^{-1}. Mobile phase consisted of A: (acetic acid, 100 mM) and B (acetic acid, 100 mM, in methanol). Compounds of interest were eluted by linearly increasing gradient:

0-6 min (70% B), 6-10 min (100 % B), 10-13 min (100 % B). Chromatographic column

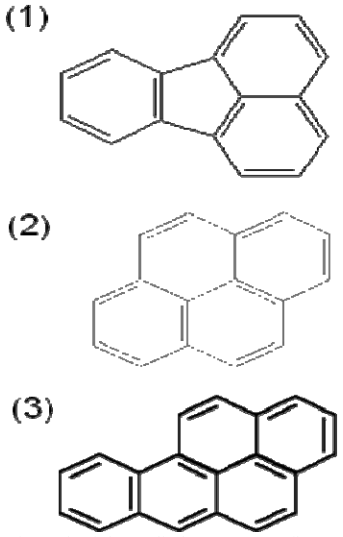

Figure 1: Structure of tree basicly studied PAH´s. (1) fluoranthene, (2) pyrene and (3) benz[a]pyrene

was thermostated to 25°C. Thanks to developing and optimizing of separation method, we were able to effectively separate non-polar compounds, as fluoranthene, pyrene, benzo[*a*]pyrene, naphtalene, anthracene, methyl-anthracene, benzylanthracene, triphenyl and coronen. All compounds were detected at one universal wavelength 275nm which was the most suitable and enough sensitive with regards to absorption maximum for all studied compounds without discrimination.

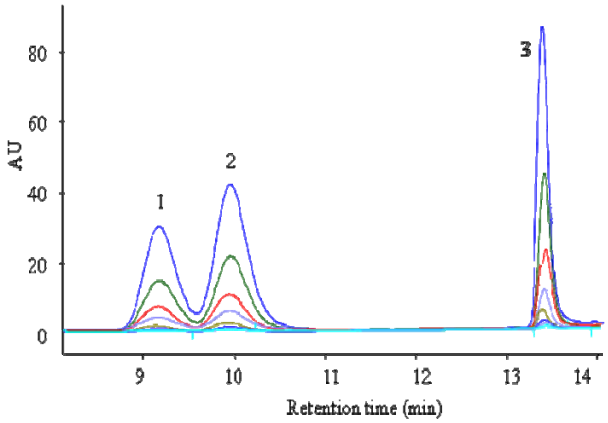

Figure 2: Chromatograms of calibration curve of three PAH´s fluoranthene (1), pyrene (2) and benz[a]pyrene.

The loose of resolution for peaks 1 and 2 due to usage of short column is shown in Fig.1. We found compromise of temperature and accurate adjusted isocratic ration of MF and thus we were able to avoid to bigger coelution. Anyway there is obvious effect of increasing of organic part of MF on peak 3 which has comparatively better resolution.

Conclusion

We achieved compromise in fast and robust separation of above-mentioned nonpolar compounds using short column with small

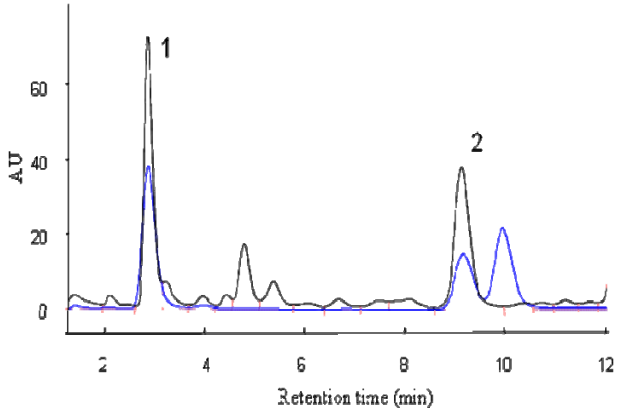

Figure 3: Comparison of standard (blue – line) and sample of extract of BY-2 cells treated by fluorantene (dark-line). Peak (1) internal standard-Toluene. Peak (2) fluorantene.

inner diameter. All of compounds of interest can be simultaneously determined in time interval shorter than 25 minutes including regeneration of column and equilibration of the system. The major benefits of this method are application during study of concentration of PAH´s in the plants after treatment. Due to simple preparation of the sample and or relatively fast separation and universal detection.this method is easy to use and robust for a high throughput data analysis.

Acknowledgements

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References

Kummerova M, Krulova J, Zezulka S, Triska J (2006) Evaluation of fluoranthene phytotoxicity in pea plants by Hill reaction and chlorophyll fluorescence. Chemosphere 65(3):489-496

Wu PF, Chiang TA, Wang LF, Chang CS, Ko YC (1998) Nitro-polycyclic aromatic hydrocarbon contents of fumes from heated cooking oils and prevention of mutagenicity by catechin. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 403(1-2):29-34