SESSION IV

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CHAIRPERSONS:

Ágnes Móricz and Gertrud Morlock

16.

Liquid chromatography coupled with bioactivity tests. HPLC or HPTLC?

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There is a great demand for new, easy-to-obtain, bioactive agents in the fight against various human, animal and plant diseases. This problem necessitates the interminable production and isolation/detection of new, effective chemicals in medicine, like antimicrobials against pathogens, antioxidants to treat cardiovascular diseases, estrogens to balance the hormonal and homeostatic systems and enzyme inhibitors or inducers to prevent e.g. Alzheimer's disease or diabetes. As the plant kingdom is rich in unexplored compounds having the most diverse chemical structures it could be expected that drug industry effuses a lot of new drugs sourced from plants. Chemical analyses of plant constituents is strongly linked to the use of liquid chromatographic systems with column or planar arrangements, which enable the efficient separation and isolation of plant ingredients.

Effect-directed analysis gave a new impetus for the discovery of new potential drug compounds from natural sources. Liquid chromatographic hyphenations were introduced and generally used for simultaneous or parallel detection and characterization of separated bioactive substances. The open planar layer chromatographic systems provide implicitly the performance of direct assays in situ in the adsorbent bed after development and drying of the chromatoplate. Column liquid chromatography generally gives more efficient separation, however its compatibility with the biological systems is often problematic.

In this lecture I would like to summarize and compare the opportunities provided by planar layer (TLC/HPTLC) and column liquid (HPLC) chromatographic systems in the effect-directed discovery of bioactive compounds.

References

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G. Morlock, W. Schwack Coupling of planar chromatography to mass spectrometry. Trends Anal. Chem. 29 (2010) 1157–1171.

17.

Latest research in hyphenated HPTLC

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Latest progress is reported in the combination of HPTLC with bioassays [1-3], open source-based multivariate data analysis [4], mass spectrometry and structure elucidating techniques [5, 6]. Complementary to commonly used target analysis that focuses on known bioactive compounds, a planar chromatographic bioprofiling can provide comprehensive effect-directed answers and can easily discover unknown active compounds in complex samples. For example, estrogen-effective compounds are discovered in wine, beer, spices, nutraceuticals, river water and waste water. A similar outcome is shown for antibiotics; especially, the discovery of natural antibiotics, their quantitation as well as equivalency calculation is outlined. Mostly, these discovered, active compounds are unknown; hence, an option for a fast structure elucidation is demonstrated with the planar separation as a start.
Some advantages of this strategy: Only effective compounds are focused and characterized in a complex sample that might consist of up to 4000 different single compounds. At one go, 20 samples on a plate are analyzed in parallel, which makes effect-directed analysis (EDA) efficient, taking between 5 and 15 min per sample depending on the bioassay. This way, attention is drawn to important active components in complex samples in a highly streamlined workflow, making HPTLC-EDA an important cost-efficient tool in the analytical toolbox of experts.

References:
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18.

Thin-layer chromatography-direct bioautography as a semi-quantitative method

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Thin-layer chromatography–direct bioautography (TLC-DB) is a hyphenation of TLC with a bioassay which is performed directly on a developed and dried (HP)TLC plate. TLC-DB, followed by analytical and/or spectroscopic methods, belongs to the effect directed analysis (EDA) which enables detection and identification of compounds responsible for biological effect in a given sample [1,2]. Planar chromatography is a very convenient separation technique to be used in EDA because of limited purification steps, possibility of analyzing many samples in parallel and evaporation of mobile phase that could influence test organisms used in bioassays. In case of TLC-DB, mostly antimicrobial properties of separated compounds are measured. The developed and dried plate is dipped in a suspension of microorganisms growing in a proper broth and then incubated under optimized conditions. Microorganisms grow directly on a plate surface excluding places where antimicrobial agents are located. Also other effects can be measured using TLC-DB, e.g. antioxidant, antimutagenic, enzyme inhibition or estrogenic activities. Zones of inhibition/activity can be visible directly or after treating with specific detection reagents.

TLC-DB is a perfect method for biological screening. Biological fingerprints compared with UV chromatograms and those obtained after chemical derivatization deliver qualitative information. However, there are few examples for using this method for quantitative (or at least semi-quantitative) measurements [2,3]. This problem will be discussed in detail basing on our own results as well as those described in the literature. The focus will be done on TLC-DB with microbiological detection but also other tests (DPPH, planar yeast estrogen screen (p-YES), enzymatic) will be discussed. Various types of calibration curves (linear, exponential, sigmoidal) will be presented.

References

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