APPLICATION OF SUBAMBIENT AND ELEVATED TEMPERATURES FOR TLC SEPARATION AND DETECTION PROTOCOLS

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This communication is focusing on the influence of temperature on planar chromatographic separation and detection, mainly on the author and co-workers approach. Particularly, advantage of planar over column separation of selected steroids at elevated and sub-ambient temperatures will be demonstrated. Moreover, ability of thin-layer chromatography for fast separation and efficient detection of analytes like calixarenes, cyclodextrins, macrocyclic antibiotics, prostaglandins, bile acids, estrogens, ergosterol, cholesterol and related steroids will be reported **[1-10]**. Furthermore, following topics from practical point of view will be discussed:

a) Construction, modification and application of vertical and horizontal chambers for non-forced planar chromatography in different temperatures.
b) Problem of pseudo non-linear van't Hoff plots.
c) Thermodynamic studies of the retention behaviour of selected analytes based on TLC retention data.
d) Optimalization of phosphomolybdic acid staining protocol for robust quantification of selected steroidal endocrine disrupters.
e) Capability of temperature-controlled micro 2D TLC system for separation of complex mixtures.

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PLANAR CHROMATOGRAPHY IN EUROPEAN PHARMACOPOEIA

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Thin layer chromatography is frequently applied for pre-purification, separation and sensitive detection of UV transparent compounds from pharmaceutical formulations using number of simple staining reagents including iodine, vanillin, ninhydrin, thymol, dichlorofluorescein, antimony chloride, ferric chloride, dinitrophenylhydrazine, anisaldehyde, potassium iodobismuthate, ferricyanide, dichromate, permanganate or hydroxide as well as phosphomolybdic, nitric or sulphuric acid. The main advantage of such approach is that the bands or spots developed can be easily and robustly inspected under visible and/or UV light and simply digitalized via office scanners or photographed using digital cameras.

It is noteworthy that almost 50% of the separation protocols that are listed in the general monographs of the European Pharmacopoeia are based on thin-layer chromatography (TLC 46%; HPLC 41%; GC 12%; electrophoresis 1%). The main goal of this presentation is to discuss the contribution of TLC separation and detection methods for quantification, purity confirmation and substance identification procedures described in the European Pharmacopoeia monographs **[1]**. Moreover, the comparison of TLC and HPLC detection for particular class of compounds as well as critical review of the visualisation protocols based on *e.g.* phosphomolybdic acid staining methodology will be discussed **[2,3]**.

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SEPARATION AND DETECTION OF ERGOSTEROL AND RELATED STEROIDS USING TEMPERATURE-CONTROLLED PLANAR CHROMATOGRAPHY

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The aims of this research were to study the effect of temperature on retention of selected steroids using normal and reversed phase chromatographic plates as well as optimization of the detection protocol based on phosphomolybdic acid (PMA) derivatization. As the components of interest ergosterol and number of related steroids that may be considered as the environmental biomarkers of the endocrine disrupting phenomenon including stigmasterol, cholesterol, dihydrocholesterol, 4-cholesten-3-one, 7-dehydrocholesterol and cholesterol acetate were selected. Moreover, retention of 7,8-dimethoxyflavone was also studied due to broad application of such substance as the internal standard frequently used for quantitative analysis of steroids.

Components of interest were separated on K60WF_{254}S and RP18W plates developed using mobile phases composed of methanol-dichloromethane and methanol-water mixtures. Commercially available horizontal chamber (Chromdes DS-L; Lublin, Poland) was thermostated in the air-circulating oven for temperatures ranging from 5 to 55^{o}C. In contrary to the retention data obtained previously for bile acids [1], the results of present study revealed relatively low temperature-retention response of the components of interest, particularly, for separation performed on the silica plates and methanol-dichloromethane mobile phase.

The best conditions for detection was determined using 3D-maps generated form the raw experimental data points that were obtained from the developed plates sprayed with 10% (w/v) PMA in methanol and heated at different temperatures (from 40 to 120^{o}C) and times (2-40 minutes). It has been found that similarly to bile acids **[2]** a quantitative effect of PMA dyeing is strongly time/temperature dependent and best conditions for robust detection can be expected if the plates are heated in the temperature between 60 and 80^{o}C for time more than 10 minutes.

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