APPLICATION OF THERMOSTATED MICRO-TIN-LAYER CHROMATOGRAPHY FOR PHARMACEUTICAL ANALYSIS AND PHYSICOCHEMICAL INVESTIGATIONS

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Thin-layer chromatography (TLC) is still commonly used as a simple and efficient tool for separation and quantification of several analytes, which are present in complex pharmaceutical, biological, and environmental samples [1-3]. The main advantage of TLC is separation protocol simplicity. Principally, chromatographic process driven by capillary forces can be performed in small glass, metal or PTFE containers, without any additional and expensive equipment [4]. Liquid samples can be accurately transferred into the plate in form of small spots or thin bands via homemade sprayers [5]. Moreover, separated spots can be easily visualized by use of wide range of detection reagents and inspected under visible or UV light conditions and then digitalized using simple office scanners [6]. In analytical practice, a modern high-performance-thin-layer chromatography (HPTLC) is particularly suitable for efficient separation and sensitive visualization of active compounds from complex mixtures, including pharmaceutical formulations [7].

This contribution summarizes the author’s research, which was recently focused on qualitative and quantitative determination of low molecular mass compounds using thermostated micro-thin-layer chromatography [4-10]. Particularly, the result of studies on separation of methyltestosterone, testosterone and its derivatives under NP and RP chromatographic systems conditions and wide range of temperature shows high capacity of micro-TLC method for efficient isocratic separation of analytes characterized by wide range of polarities. It has been proofed that micro-TLC can be successfully applied for fast, accurate and robust quantification of testosterone residues in testosterone enanthate samples. Our experimental data have revealed that micro-TLC plate working under 2D development protocol is capable of separation of more than 240 spots. High separation throughput of one and two dimensional chromatographic processes involving micro-plates was demonstrated by separation of complex samples including the Azucalen herbs extract as well as water and organic liquids extracts of the Spirulina maxima dyes derived from pharmaceutical formulations. The raw quantitative data obtained from microchromatograms acquired under visible light conditions were explored using cluster analysis and principal components analysis. Chemometric investigations revealed that the best extraction liquids for isolation of dye mixtures from Spirulina samples were methanol, ethanol, tetrahydrofuran, and dichloromethane. Moreover, it was found that the liquids’ parachor values could be used for estimation of the dye extraction
efficiency from complex samples. It has been also demonstrated that micro-TLC method may be useful for fast fingerprinting of complex biological mixtures as well as convenient tool for optimization of binary mobile phase composition for solid-phase extraction (SPE) procedures. Our research has revealed that based on micro-TLC retention data, the steroids SPE elution volumes may be predicted beyond the experimental data range that was available for solid-phase extraction experiment, particularly for mobile phases contained high level of water.

References