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Steroids

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I. INTRODUCTION

The purpose of this chapter is to present advances in the techniques and applications of steroid thin-layer chromatography (TLC) starting with late 1993, the last year covered in the chapters on this topic in the first and second editions of this Handbook (1,2). Readers are referred to these earlier chapters for detailed discussions and extensive tabular data on sample preparation, layers, mobile phases, detection, identification, quantification, and applications of steroid TLC based on papers published up to 1994. The references given in this chapter are not comprehensive but were chosen selectively to update the material presented in the earlier editions.

Steroids are natural and synthetic compounds with a cyanopentanoperhydrophenanthrene skeleton and include bile acids, androgens, estrogens, corticosteroids, ecdysteroids, sterols (cholesterol), and vitamin D. The latter two are given only relatively minor coverage in this chapter because their TLC analysis is described further in the chapters on lipids (Chap. 22) and lipophilic vitamins (Chap. 23), respectively.

Thin-layer chromatography continues to be an important method for the determination of steroids because of its inherent advantages: Many samples can be analyzed simultaneously and quickly at relatively low cost, minimal cleanup of samples is required because layers are not reused, and multiple separation techniques and detection procedures can be applied. Detection limits are often in the low nanogram range, and quantitative densitometric methods are accurate and precise. Preparative layer chromatography (PLC) on thick layers allows up to several grams of steroidal substances to be isolated.

An encyclopedia chapter on steroid analysis by TLC was published recently (3). A review on chromatographic procedures, including TLC, for phytoecdysteroids included lists of numerous mobile phases and detection spray reagents (4). A database was presented (5) that contains information on TLC methods for steroids published in pharmacopeias of various countries, including the United States, United Kingdom, and China.

II. SAMPLE PREPARATION

Steroids and their metabolites are analyzed by TLC in a variety of samples such as biological (clinical) samples, plants, pharmaceutical formulations, and raw materials and synthesis and biotransformation products of importance in steroid production. Classical liquid–liquid extraction techniques and solvent partitioning and column chromatography cleanup methods have been used most often to prepare test samples to be applied on TLC plates. In some analyses, steroidal conjugates in samples or extracts are cleaved by enzymatic and/or nonenzymatic hydrolysis methods before or after TLC.

Heating under vacuum was found to accelerate the removal of extractives from plant biological matrices without significant modification in a study of the separation and characterization of