International Journal of Agriculture Innovations and Research
Volume 4, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 08/06/2016 | Accepted on : 15/06/2016 | Published : 18/06/2016

Analysis Types of Steroids (Cholesterol-Estradiol-Cholecalciferol) by Thin-Layer Chromatographic

Kamal-Alahmad
School of Food Science and Technology, Jiangnan University, Wuxi, China
Laboratory of Food Enzymology and Food chemistry, Jiangnan University, Wuxi, China
Department of Food Science and Technology, Faculty of Agriculture, University of Alfurat, Deir Ezzor, Syria
Kamalalani85@yahoo.com (Kamal-Alahmad)

Sameh A. Korma
School of Food Science and Technology, Jiangnan University, Wuxi, China
State Key Laboratory of Food Science and Technology , Synergetic Innovation Centers of Food Safety and Nutrition, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu,China
Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Abdalbasit A. Gasmalla
School of Food Science and Technology, Jiangnan University, Wuxi, China
Laboratory of Food Enzymology and Food chemistry, Jiangnan University, Wuxi, China

Omar Mukama
Key laboratory of carbohydrate Chemistry and Biotechnology, School of biotechnology, Jiangnan University, Wuxi, 214122, China

***Abstract* – Thin layer chromatography has been used for the analysis of natural and synthetic steroids in various environmental materials. In this work I focused mainlyon steroid analysis in environmental materials suchas pharmaceuticals, plant products and other biological specimens. An overview of structural complexities and biological/nutritional aspects including hypocholesterolemic activities of phytosterols is provided in this workshop. This review also covers specific analyses of natural/synthetic standard mixtures to shed light on potential applicability in plant sample assays. Examples of combined chromatographic techniques (GC-TLC) characteristics of sterol components are discussed in the context of analyte substituent effects, structural factors and stationary /mobile phase considerations.**

***Keywords* – Steroids, Classification, Modifications, Analysis, (TLC) Technique, Detection, Columns, Stationary Phase, Mobile Phase, Pharmaceutical Compounds.**

I. INTRODUCTION

Steroids are terpenoid lipids characterized by the sterane or steroid nucleus: a carbon skeleton with four fused rings, generally arranged in a 6-6-6-5 fashion. Steroids varyby the functional groups attached to these rings and the oxidation state of the rings. The specificity of their different biological actions is due to the various groups attached to a common nucleus. When alcohol groups (OH) are attached, steroids should properly be called sterols (e.g., cortisol), whereas ketone groups (C=O) make them sterones (e.g. aldosterone).

Steroids comprise a large group of substances that mediate a very varied set of biological responses. The most widespread in the body is cholesterol, an essential component of cell membranes and the starting point for the synthesis of other steroids - sex hormones, adrenal cortical hormones (Mariani and Venturini, 1997) and the bile salts. Steroids (e..g glucocorticoids, mineralocorticoids and rogens, estrogens and progestagens) have major responsibilities as hormones controlling metabolism, salt balance, and the development and function of the sexual organs as well as other biological differences between the sexes. Steroids (naturally occurring or synthetic) such as, hydrocortisone, glucocortisteroids, corticosteroids, oestrogens, androgens, are also used for the treatment of various diseases such as allergic reactions,arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production. In addition, synthetic steroids (e.g., mifepristone) that mimic the action of progesterone are widely used as oral contraceptive agents. Other synthetic steroids (e.g., oxandrolone) are designed to mimic the stimulation of protein synthesis and muscle-building action of naturally occurring androgens. Steroids, such as nandrolone dromostanolone, stanozolol, are often used illegally to increase the performance of competitive athletes of almost all age groups They are banned in most sports competitions such as the Olympics (Mariani, Bellan and Morchio, 1999).

II. Types and Classification of Steroids:

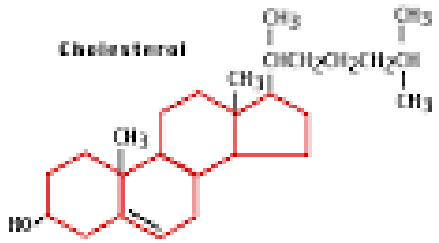
Different steroid compounds differ based on varying structural differences centered on the primary four ring steroid structure, which may involve variations in the functional groups attached to the central four rings, or other smaller modifications (such as the change in a single hydrogen, carbon, or oxygen atom).

There are many different types of steroids, the majority of which do not have anything what so ever to do with muscle growth, strength, or athletic performance. Many steroids, in fact, destroy and break down muscle tissue these are known as corticosteroids (Zdena, 2001). There exist hundreds of different types of steroids and steroidal compounds in nature, and they exist in plants, animals, insects, and even fungi. Naturally occurring steroids are synthesized in cells of the different aforementioned organisms.

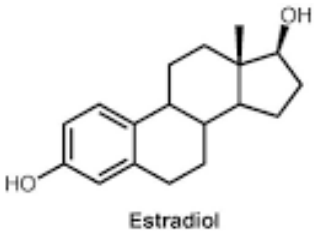
There exist many different types of steroids in the human body, such as: Cholesterol, Cholecalciferol (Vitamin D), Estrogen, Testosterone, and Cortisol

(among many others) (Klaus, Fischer and Hauck, 2004)Vitamin D in particular is what is known as a secosteroid, which is a steroidal molecule but contains a broken ring.

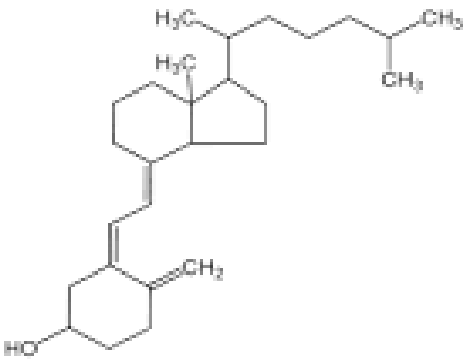
In the human body, Cholesterol is the precursor to the development of all other steroids manufactured in the human body. This is to say that Cholesterol is the base steroidal compound that the body's cells use to synthesize all other steroids (Estrogen, Testosterone, Vitamin D, etc.).



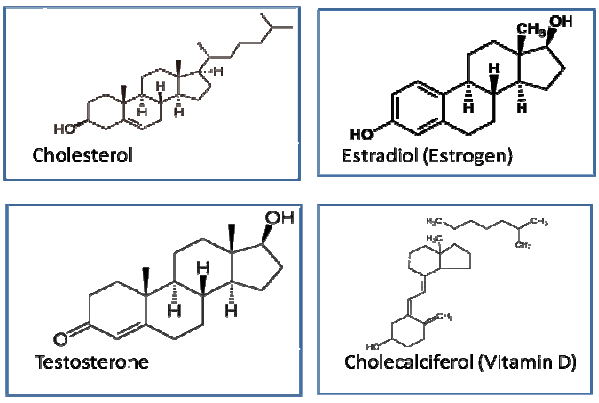
(Cholesterol)



(Estradiol)



(Cholecalciferol)



Types of steroides

Steroids have been classified into a number of groups based on their functions as follows: (i) sterols and steroid alcohols, usually with double bonds; (ii) sex hormones - steroids produced mainly in the testis (androgens) or ovary (estrogens); (iii adrenocortical hormones - steroids produced in the cortex of the adrenal gland; (iv) bile acids - steroids usually bonded to taurine o glycine and functioning as emulsion stabilizing agents in the intestine, sapogenins - plant products with a steroid bonded to carbohydrates; (vi) cardiac glycosides - plant products similar to sapogenins and used as heart stimulants and (vii) vitamin D (Sasaki, Wakabayashi and Yamaguchi, 2007).

III. Thin-layer Chromatography (TLC) Technique

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (Hakpour et al., 2005). This layer of adsorbent is known as the stationary phase.

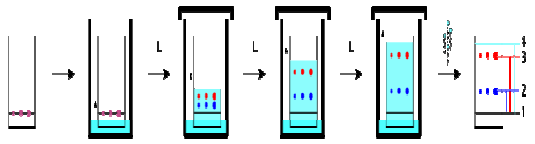
After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary. Because different analyses ascend the TLC plate at different rates, separation is achieved.

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include (Jadhav et al., 2007)analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, as saying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents (Sharma and Sharma, 1991).

The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products (Kotiyan and Vavia, 2000).

Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal-phase silica gel is used as the stationary phase, it can be considered polar. Given two compounds that differ in polarity, the more polar compound has a stronger interaction with the silica and is, therefore, more capable to dispel the mobile phase from the binding places (Djakovic, Stojanovic and Penov, 2004). As a consequence, the less polar compound moves higher up the plate (resulting in a higher Rf value). [6] If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more

capable of dispelling solutes from the silica binding places, and all compounds on the TLC plate will move higher up the plate. It is commonly said that "strong" solvents (eluents) push the analyzed compounds up the plate, whereas "weak" eluents barely move them. The order of strength/weakness depends on the coating (stationary phase) of the TLC plate (Klaus, Fischer and Hauck, 2004).



(Development of a TLC plate, a purple spot separates into a red and blue spot)

IV. Columns and Detection Techniques

The polarity and molecular volatility of a steroidalcompound play a pivotal role in the GC separation.During the chromatographic separation processes,GC partition between sterol solutes and the stationary liquid phase is influenced by the polarity of bothinteracting partners.

Component resolution of astructurally similar sterol mixture can be dramatically improved by increasing the polarity of the stationary phase used in capillary GC at the expense ofincreases in solute retention times (Baran, Wlodarczyk and Bartoszuk, 2007).

V. Thin-Layer Chromatography of Steroids

Most authors wrote a lot of information on the TLC of steroids, and it included information on sample preparation as well as stationary-phase and mobile-phase systems useful for the separation of steroidal pharmaceuticals (Meliha et al., 2007). The authors also provided detailed methods of detection and quantification of steroids, and later on, updated their review to include coverage through 1994 have also reviewed the application of TLC to steroids in pharmaceutical analysis while Jain has provided some information on the analysis of steroid hormones in his review on TLC in clinical chemistry (Yka, 2009).

TLC continues to be an important method for the determination of steroids because of its advantages. Many samples can be analyzed simultaneously and quickly.

Table * shows several thin-layer chromatographic systems designed for the analysis of steroids (Claudia et al., 2006)

Analyte	Stationary Phase	Mobile Phase	Remarks
Cholesterol Allylestrenol Pregnandiol	**RP-HPTLC plates**	**Acetronitrile/methanol Acetronitrile/methanol&water**	**Mixture of 10gr copper And 5 ml-o-phosphoric acid,dissolved in 95ml/methanol.**
Androgens And gestagens	**silica**	**Chloroform/acetone(9:1) Hexane/dichloromethane/ Acetronitrile(4:3:2)**	**HPTLC separation of anabolic androgens.**
Steroids	**silica**	**Chloroform/ethanol/water (188:12:1)**	**Detection under UV by radioimmunoassay.**
Oxo-steroids	**silica gel**	**Chloroform/methanol(97:3)**	**Measurement of 17 oxo-steroids in boil fluids with TLC and fluorometric scanning detection.**

The 5 androstane isomers were analyzed by thin-layer chromatography using optimum mobile phases. The choice of proper mobile phase and the optimization of the mobilephase composition are very important for the analysis of androstane isomers by thin-layer chromatography (TLC) (Vegh, 2003). In the 1st step, the proper solvent system was found to be themixture of chloroform, acetone, and petroleum ether chosen from 7 elution systems. In the second step, the composition of the mobile phase was optimized by"simplex" and “prism” methods. The optimum TLC system can be applied for the separation of androstane isomers from real samples such as drug formulation, biological and natural resources. Separation of a large number of ecdysteroids was investigated with eleven mobile phases and three stationary phases. Only the use of four mobile phases on three stationary phases enabled the separation of all the ecdysteroids from each other in at least one system (euske and Schonbeiter, 2001). The TLC behavior of ecdysteroids containing different numbers of hydroxyl groups, side chain variations, and extra double bonds, and of positional isomers and stereoisomer, was reported and interpreted.

A simple thin-layer chromatography immunostaining method using monoclonal antibody against solamargine was developed for the determination of solasodine glycosides. In this method, the solasodine glycosides separated by silica gel TLC were transferred to a polyvinylidene, difluoride membrane (Szepesi and Gazdag, 1996). The membrane was treated with sodium periodate solution and the with bovine serum albumin (BSA), resulting in a solasodine glycoside-BSA

conjugate. Individual spots were stained by monoclonal antibody against solamargine. The newly established immunostaining method can be extended to the analysis of the distribution of solasodine glycosides in the plant extract. Folin-Ciocalteu's reagent along with three new solvent systems was used for the study of 9 anabolic steroids prohibited in sports, namely testosterone undecanoate, methyl-testosterone methandienone, testosterone, testosterone propionate, nandrolone ethylsterenol, oxygmetholone, and stanozolol (Fenske, 2000).

A rapid, selective and precise stability indicating high performance thin layer chromatography method was developed and validated for the estimation of estradiol(ESD) in bulk and pharmaceutical dosage forms. ESD is widely used in postclimacteric replacement therapy. The developed method employed silica gel 60Fas the stationary phase and chloroform– acetone–isopropyl alcohol–glacial acetic acid (9:1:0.4:0.1) as mobile phase (Woodbury et al., 1995). The dense and compact spot of the drug occurred at an Rf value of 0.40 ± 0.02. Spectrodensitometric scanning-integration was performed on a Camag system at a wavelength of 286 nm. The polynomial regression data for thecalibration plots exhibited good linear relationship.

VI. Quantitative Analysis of Steroides by Thin-Layer Chromatographic (TLC)

GC–FID is most commonly used to quantitativesterols in various sample matrices by virtue of alarge linear mass range of response of the FIDsystem. For quantitative measurement of sterols, it isimperative to determine the linear range of responsefor each GC–FID system to a sterol standard (Markowski, 2003). Allquantitation requires statistical method validation inthe context of reproducible retention times, precision,recovery studies with spike samples and absoluteresponse factors. Losses of sterol analysesduring isolation and separation must be corrected forthe final results of analyze quantification by usinginternal standardization with standards not present insamples or radioisotopes.As compared to GC, quantitativeanalysis of sterols by HPLC is somewhat limited.Using dose–response calibration curves, HPLC–PDA is useful for quantitation of specific sterols (Rezanka, 2002).

VII. Analysis Sterols and Steryl Esters in Vegetable Oils by (TLC)

Apparently, the sterols occurring in vegetable oils are mainly desmethylsterols. In other words, the edible oils contain much smaller number of 4-methylsterols and 4.49-diemthylsterols in relatively low abundance. The most abundant sterol components present in the sterol fractions of commodity vegetable oils (i.e. coconut, canola, cocoa butter,corn, cottonseed, linseed, olive, palm, peanut, rice bran, safflower, sesame, soybean, sunflower oils).

As sterol esters are sensitive to saponification with strongly basic reagents, these compounds along with free sterols or by themselves are commonly isolated from lipid extracts or vegetable oils by CC, CC/TLC (Mariani et al,. 2009).

VIII. Conclusion

Thin layer chromatography (TLC) is a globally accepted practical solution to characterizeraw herbs, active constituent-enriched extracts and their formulations. Standardized TLC procedures can be used effectively for screening analysis as well as quality evaluation of a plant or its derived herbal products. Owing to the simplicity and efficiency of TLC, specific and rapid determination of various steroids in humans and various other animals can be carried out. The procedure can be employed for the routine analysis of steroids in pharmaceutical formulations and in bulk drug preparations as well as for the quality assurance of related extracts and market samples. Interest in TLC has increased with improvements in TLC instrumentation and methods, and especially in the last few years, with the development of new MS methods for detection. If standard compounds are not available, identification ofunknowns has to be done with more specific techniques, such as infra-red spectroscopy and MS detection.

References

[1] C. Mariani, S.Venturini, Riv. Ital. Sostanze Grasse 74 (1997).
[2] C. Mariani, G. Bellan, G. Morchio, A. Pellegrino, Riv. Ital. Sostanze Grasse 76 (1999) 59.
[3] Zdena T. Thin-layer chromatography of urinary 17-oxosteroids using dansylhydrazine as a prelabeling reagent. J. Chromatogr. Biomed. Appl., 2oo1; 570: 396-398.
[4] Klaus R, Fischer W, Hauck HE. Analysis and chromatographic separation of some steroid hormones on NH2layers.Chromatographia, 2004; 39: 97-102.
[5] Sasaki T, Wakabayashi M, Yamaguchi T, KasugaY,Nagatsuma M, Lida T, Nambara,2007.
[6] hakpour M, Jamshidi A, Entezami AA, Mirzadeh H.HPTLC procedure for determination of levonorgestrel in the drug-release media of an in-situ-forming delivery system. J. Planar Chromatogr.,2005.
[7] harma JD, Sharma AK. Thin layer chromatographic study of some anabolic sport steroids. Indian Journ. Forensic Sci., 1991; 5: 117-122.
[8] Kotiyan PN, Vavia PR. Stability indicating HPTLC method for the estimation of estradiol .J. Pharm. Biomed. Anal.2000; 22: 667-671.
[9] Jadhav AN, RumallaCS ,Avula B, Khan IA. HPTLCmethod for determination of 20-Hydroxyecdysone in sidarhombifolia L. and dietry supplements. Chromatographia, 2007; 66: 797-800.
[10] Djakovic-Sekulic T, Stojanovic S, Penov-Gasi K. Evaluation of the liphophilicity of some dehydroepiandrosterone derivatives using RP-18 HPTLC chromatography. Chromatographia, 2004; 60: S201-S205.
[11] Klaus R, Fischer W, Hauck HE. Analysis and chromatographic separation of some steroid hormones on NH2layers.Chromatographia, 2004; 39:97
[12] Baran M, Wlodarczyk E, Bartoszuk MA. Improved detection of ergosterol, stigmasterol and selected steroids on silica coated TLC plates using phosphomolybdic acid staining. J. Liq. Chromatogr. Relat. Technol., 2007; 30: 2629- 2634.

[13] Meliha L, Fehim K, Miroslav S, Aleksandra M. Planar chromatography of steroid hormones and anabolics. ActaChimicaSlovenica, 2007; 54: 88-91.
[14] Yka A. Spectrodensitometry application to analytical identification of estradiol, hydrocortisone, testosterone and cholesterol on Diol plates. J. Liq. Chromatogr.Rel. Technol., 2009; 32: 1084-1095.
[15] Claudia C, Anamaria H, Sorin H. Analysis ofsomesteroids by thin-layer chromatography using optimum mobile phases. J. Pharm. and Biomed. Anal., 2006; 41: 633-637.
[16] Vegh Z. True color photo documentation of UVirradiated thin layer chromatograms. J. Planar Chromatogr., 2003; 6: 341-345.
[17] euske M, Schonbeiter H. Thin-layer chromatography on silica coated aluminium sheet as an adjunct to radioimmunoassay of steroids. J. Chromatogr., 2001; 563: 178- 183.
[18] Szepesi G, Gazdag M. Steroids. In Handbook of Thin Layer Chromatography. Sherma J, Fried B (Eds.) Marcel Dekker, New York, 1996, p. 971.
[19] Fenske M. Determination of cortisol and cortisone in human morning and overnight urine by thin layer chromatography and fluorescence derivatization with isonicotinic acid hydrazide. Chromatographia, 2000.
[20] S.E. Woodbury, R.P. Evershed, J.B. Rossell, R.E. Griffith, P.Farnell, Anal. Chem. 67 (1995) 2685.
[21] W. Markowski, J. Chromatogr. A 635 (2003) 283.
[22] T. Rezanka, J. Chromatogr. 598 (2002) 219.
[23] C. Mariani, G. Bellan, G. Morchio, A. Pellegrino, Riv. Ital Sostanze Grasse 76 (2009) 59.

AUTHORS' PROFILES

First Author:
Kamal Alahmad, Master student, school of food science and technology, Jiangnan University, Wuxi, China.

Second Author:
Sameh A. Korma, Master student, School of Food Science and technology, Jiangnan University, Wuxi, China.