

in daylight and then under 366 nm UV light after spraying with 20% ethanolic sulfuric acid and heating at 110°C] (92); ethinyl estradiol with methyltestosterone, norethisterone, progesterone, estradiol benzoate, testosterone propionate, and mestranol as reference substances [toluene–ethyl acetate (7:3), detection under 254 nm UV light and then under 366 nm UV light after detection with sulfuric acid reagent] (93); and betamethasone 17 α -valerate with prednisolone acetate, hydrocortisone acetate, and dexamethasone acetate as reference substances [methyl ethyl ketone–toluene (2:3), detection under 254 nm UV light and then under 366 nm UV light after detection with sulfuric acid reagent] (94). The latter three procedures are approved for testing of pharmaceutical identity and quality.

A semiquantitative OPLC purity test was described for a steroid bulk drug substance used as the active ingredient in contraceptive products (95). The automatic Personal OPLC BS-50 instrument (see Chap. 7) was used with HPTLC silica gel sheets presealed for OPLC and cyclohexane–ethyl acetate–chloroform (3:1:1) as the mobile phase. The R_f values of the monitored compounds follow: 6-hydroxy derivative, 0.05; starting material, 0.16; 6-dehydro derivative, 0.36; main component, 0.40; 5-methoxy derivative, 0.59; ketal derivative, 0.80. The intermediate precision (RSD) of the R_f values measured on different plates at different times in two laboratories ranged from 3.01% to 4.17% ($n = 13–32$).

B. Metabolism Studies

Steroid metabolites were identified by silica gel TLC or HPTLC in the published studies cited in this section. Readers should consult the individual references for details of the sample preparation, mobile phases, layers, and detection/quantification methods: androgen and estrogen metabolism during sex differentiation in single-sex populations of the Nile tilapia (*Oreochromis niloticus*) (96); progesterone and the oligodendroglial lineage: stage-dependent biosynthesis and metabolism (97); progesterone metabolism in human endometrial and gland cells in culture (98); in vitro effects of γ -hexachlorocyclohexane on in vitro biosynthesis and metabolism of steroids in goldfish (*Carassius auratus*) (99); in vivo steroid metabolism in embryonic and newly hatched steelhead trout (*Oncorhynchus mykiss*) (100); metabolism of testosterone by dermal papilla cells cultured from human pubic and axillary hair follicles and the relation to hair growth in 5- α -reductase deficiency (101); steroid metabolism in theca externa cells from preovulatory follicles of the domestic hen (*Gallus domesticus*) (102); gonadal in vitro androstenedione metabolism and changes in some plasma and gonadal steroidal hormones during sex inversion of the protandrous sea bass (*Lates calcarifer*) (103); androstenedione metabolism in the indifferent stage of bovine gonad development (104); factors influencing testosterone metabolism by anuran larvae (105); progesterone metabolism by the filamentous fungus *Cochliobolus lunatus* (106); and corticosteroid metabolism in human immortalized keratinocytes (107).

C. Additional Miscellaneous Applications

Thin-layer chromatography was among the analytical methods covered in a review of the roles of natural sex steroids and their xenobiotic analogs in animal production (108).

Qualitative and/or quantitative analysis of steroids by TLC or HPTLC was applied in the following biological, biochemical, biotechnological, and biomedical studies: election of *Mycobacterium* sp. strains with the capacity to biotransform high concentrations of β -sitosterol based on TLC analysis of biotransformation products after extraction from bacterial cultures with methanol and ethyl acetate (109); steroid profiles in gonad and blood tissue samples from cultured jundia [*Rhamdia quelen* (Quoy and Gaimard)] (110); gonadotropin-induced steroidogenic shift toward maturation-inducing hormone in Japanese yellowtail during final oocyte maturation (111); steroidogenic pathways to 17,20- β -dihydroxy-4-pregnen-3-one and 17,30- β -21-trihydroxy-4-pregnen-3-one in the ovarian follicles of bambooleaf wrasse (*Pseudolabrus sieboldi*) (112); model structure and substrate specificity of 17- β -hydroxysteroid dehydrogenase from *Cochliobolus lunatus* (113); in vivo evidences of early neurosteroid synthesis in the developing rat central nervous system and placenta (114); effects of cytochrome P450 inhibitors and of steroidal hormones on the formation of 7-hydroxylated metabolites of pregnenolone in mouse brain micro-