O. Nozaki / J. Chromatogr.9 – Anthroylnitrile derivatization of cortico– steroids Corticosteroids are 270 generally difficult compounds for derivatization because of its no active functional groups.Free form Steroids in free forms are under negative feedback regulation by pituitary (adrenocorticotropic hormone; ACTH, follicle stimulating hormone; FSH, lutenizing hormone; LH) and hypothalamic hormones (cortico– trophin–releasing hormone; CRH, LH releasing hor– mone; LH–RH, FSH–releasing hormone; FSH–RH), and show circadian rhythms of excretion.Sulfuric acid–ethanol fluorescence derivati– zation Cortisol, corticosterone and testosterone, which are non–fluorescent, were converted to fluorophores (excitation 365 nm, emission 520 nm) by incubation with a mixture of concentrated sulfuric acid and ethanol for a few minutes without heating.This sulfuric acid – ethanol derivatization method was relatively specific to cortisol, corticosterone, cor– tisone, dexamethasone and testosterone, and used originally for determination of 11–hydroxycorticoste– roids (11–OHCS) in serum or urine [28,29] (Table 1).A 935 (2001) 267–278 tion enzymes (delta 4–hydrogenase, steroid dehydro– genase, hydroxylase, etc.), hence conjugated steroids are targets of direct analysis by high–performance liquid chromatography (HPLC)–MS which is de– scribed in more detail later.Therefore, enrichment of free urinary steroids are carried out by extraction of urine specimens with octadecyl silica (ODS, C18) or BSA–ODS (bovine serum albumin bound ODS) gel packed minicolumns prior to the assay of free steroids [9,10].Rapid assay Separation assay by GC, HPLC or capillary elec– trophoresis (CE) takes a long time (e.g., 40–60 min) per specimen, and preparation procedures for speci– mens such as extraction or derivatization add more time to the assay.Albumin bound form Free steroids bind to albumin in serum [15], and steroids bound to albumin have no biological activi– ty. The binding force of steroids to albumin is weak, and albumin bound steroids are easily released from albumin with organic solvents (e.g., dichlorome– thane, ethyl acetate, and diethyl ether) and C 1 8 gels.The fluorescent meth– ods for steroids analysis reported were native fluores– cence, photochemical derivatization, sulfuric acid – ethanol fluorescence, and fluorescent derivatization with labeling reagents.Although the derivatization and its clean up pro– cedure have problems of time consumption for clinical assay, some inventions to solve the problem were developed (e.g., on–line photochemical deri– vatization of estrogens, simple sulfuric – ethanol de– rivatization of corticosteroids).Photochemical derivatization Estrogens were converted to fluorophores by on– line UV radiation post–column, and assayed by fluorescence detection (excitation 280 nm, emission 312 or 410 nm) [27].Recent progresses in separation analysis of steroids Steroid analysis has been carried out for medical diagnosis of stress, hypertension, Cushing syndrome, adrenogenital syndrome, amenorrhea, and infertility, etc.Native fluorescence of estrogens Only estrogens among steroids emit native fluores– cence (emission 310 nm), and this allows rapid and relatively specific analysis of estrogens [8].Sex steroid hormones (androgens, estrogens and progestins) bind to globulin (sex hormone binding globulin, SBG) [12] and corticosteroid hormones also bind to globulin (corticosteroid binding globulin, CBG) [13].Then the sulfuric acid–ethanol derivatization method was applied to the assay of cortisol and corticosterone by HPLC with fluorescence detection [30–32] (Fig.However, determina– tion of steroids in hair has advantages of easy conservation of hair specimens, no circadian rhythm of steroid excretion, and retrospective measure of steroid excretion.Smaller and shorter columns packed with smaller sized gels brought more rapid analysis times within 5 min per specimen with no loss of resolution capacity

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[26].Practical considera- tions are listed as follows.3.2.1.2.3.2.2.3.2.2.1.3.2.2.2.3.2.2.3.3.2.3.3.2.4.4.4.1.4.1.1.4.2.4.2.1.4.2.1.1.4.2.1.3.1).4.2.1.4.4.2.1.5.A 935 (2001) 267–278 271

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