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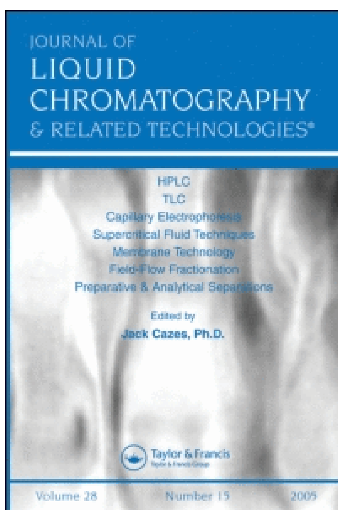

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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Steroid Analysis by High-Performance Liquid Chromatography

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To cite this Article Heftmann, Erich and Lin, Jiann-Tsyh(1982) 'Steroid Analysis by High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 5: 12, 121 — 173
To link to this Article: DOI: 10.1080/01483918208082218
URL: http://dx.doi.org/10.1080/01483918208082218

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JOURNAL OF LIQUID CHROMATOGRAPHY, 5(Suppl. 1), 121-173 (1982)

Steroid Analysis by High-Performance Liquid Chromatography

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1. INTRODUCTION

Liquid-column chromatography (LCC), which was the earliest chromatographic technique to be applied to steroids, has become the most recent and most promising addition to the armamentarium of the steroid analyst in the form of high-performance liquid chromatography (HPLC). Conventional column chromatography (CC) has survived the competition with planar and gas chromatography (GC) in the steroid field, because it offers greater capacity and a greater variety of experimental conditions. Both of these advantages are important in the analysis of steroids, because in nature steroids occur as complex mixtures of disparate concentrations and polarites. By virtue of their relatively large capacity, columns can be loaded with enough crude mixture to allow the detection of trace amounts of certain components. Steroids of diverse polarities can be fractionated by a wide range of sorbents and eluents, e.g., by gradient elution. Highly polar

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Presented at the International Symposium on H.P.L.C. and its Application in Endocrinology, London Hospital Medical College, London, England, December 1, 1981.

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compounds, such as conjugated steroid hormones, bile acids, and plant glycosides, can be chromatographed without hydrolysis or derivatization, and sensitive compounds are protected from light, air, and heat. Also, the column effluent can be collected and further analyzed by any appropriate chemical, physical, or biological method.

If LCC has not been used more extensively in the past, it was because conventional CC was less efficient and slower than planar and GC and because monitoring of steroids in the effluent was more laborious than in GC. The efficiency of liquid chromatography (LC) was greatly increased by the introduction of microparticulate sorbents (especially, spherical beads), which cause a minimum of eddy diffusion, and of surface-coated packing materials (especially, bonded stationary phases), which permit rapid exchange with the mobile phases. To increase the flow rate through finely powdered sorbents, high pressures are needed. Instrumentation for sample injection and automation for the analysis of column effluents were patterned after GC apparatus and, as a result, LCC is beginning to rival GC in convenience. At the same time, the rising cost of HPLC equipment has delayed its acceptance by poorly endowed laboratories. Another obstacle to the wider application of HPLC in the steroid field is that the choice of detectors is very limited, the refractive index (RI) and variable-ultraviolet (UV) detectors being practically the only ones suitable for most analyses.

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Before discussing the HPLC of specific classes of steroids, we will review publications on HPLC of steroids in general. The world literature on the chromatography of steroids was surveyed in 1976 [1] and 1982 [2], and HPLC of steroids was reviewed in 1979 [3]. Since 1979, the number of publications on the analysis of steroids by HPLC has more than doubled. Kautsky's book [4] contains a series of authoritative reviews on HPLC of steroids, which will be cited in the appropriate sections. One of them deals with the separation of steroid epimers by HPLC [5, cf. also 6] and another one with the study of enzyme-catalyzed steroid reactions by HPLC [7]. HPLC of steroids was also reviewed in connection with pharmaceutical

[8,9] and clinical [10-14] analysis and with the analysis of various natural products [12,15], including the lipids of insects and algae [16].

A number of publications have dealt with preparative HPLC of steroids [17-28]. As a rule of thumb, the weight of sorbent should be at least 1000 times the sample weight. The literature on sorbents also contains experiments with steroids, which are desirable test substances [29]. The water content of the eluent changes the activity of adsorbents [30] and the mechanism of sorption [31]. A change from adsorption to partition is obtained by a gradual increase in the water content of dichloromethane [32], as it elutes steroids from a silica column. When silver nitrate is incorporated in the eluent, a column of bonded octadecylsilane (ODS) serves for argentation chromatography [33]. The selectivity toward steroids of ODS-coated beads, which are the most common column packing materials now used [34], depends on the manufacturing process as well as on the method of operation [35]. They are often referred to as reversed-phase (R)) packing, although they may act both as lipophilic adsorbents and as carriers of the less polar phase in partition systems [36].

There have been considerable efforts to make the choice of mobile phases more rational [37-47]. A gradient elution scheme has been devised for the separation of 43 natural steroids on an ODS column [48]. Not only the composition and flow rate, but also the temperature may be varied to improve resolution [49]. Correlations of steroid structure with retention behavior in adsorption and partition HPLC have been attempted by Hara and Hayashi [50]. The complexity of mobile/stationary phase interactions with compounds having numerous possible isomers and epimers makes predictions very hazardous [6].

Derivatization of steroids may improve their chromatographic behavior or facilitate their detection. Thus, UV absorption is increased by converting hydroxylated steroids to benzoates or p-nitrobenzoates [51], ketonic steroids to 2,4-dinitrophenylhydrazones [52,53], and carboxylic acids to phenacyl esters [54] or p-nitrobenzyl esters [55,56]. The

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fluorometric detection of ketosteroids in the form of dansylhydrazones [57-60] increases the sensitivity of the analysis. For electrochemical detection, ketosteroids have been converted to p-nitrophenylhydrazones [61] and carboxylic acids to p-hydroxyanilides [62]. Derivatives are usually formed prior to injection, but it is also possible to derivatize the column effluent. For instance, the post-column conversion of Δ^4-3-ketones to isonicotinylhydrazones permits their analysis in the picomole range by fluorometry [63-65]. Other post-column reactions will be discussed in the appropriate sections of this review. In addition to the detectors mentioned, the moving-wire flame-ionization detector is also applicable to steroids [66,67], and a heated thin-layer plate, moving slowly past the column outlet, may serve as a poor man's transport detector [68]. Other, more rarely used detection methods for steroids include polarography [69], field desorption [70] and atmospheric pressure ionization [71] mass spectrometry [72], and nephelometry [73,74]. Automatic HPLC analytical systems can handle large series of routine analyses [75-77].

2. STEROLS

The sterols and their triterpene analogs are the least polar steroids, containing only 1-2 oxygen atoms per 26-30 carbon atoms. Isomerism may occur at positions 3,5, and 24. Except for sterols containing conjugated double bonds, they are not detectable with fixed-wavelength UV detectors. Thus, in the absence of a far-UV detector, a RI detector or UV-absorbing derivatives may be resorted to. Separation according to the number of double bonds was achieved by argentation chromatography of steryl benzoates on a silica column, containing 5% $AgNO_3$, and gradient elution with CH_2Cl_2 in petroleum ether [78]. Steryl acetates, differing not only in the number but also in the location of double bonds, were separated on an Al_2O_3-$AgNO_3$ (5:1) column, eluted with C_6H_{14} - PhMe mixtures at 100 psi [79].

Rees et al. [80] have chromatographed free sterols as well as steryl benzoates and acetates on an ODS column with mixtures of MeOH, $CHCl_3$, and H_2O. This method has been adapted to the analysis of the sterols in marine

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invertebrates [81], cholestenone in rat adrenals [82], sitosterol and sitosteryl glucoside in whiskey [83], and cholesterol in foods [84]. RP partition HPLC with MeCN in the mobile phase has been applied to free sterols by several investigators [85-87]. It forms the basis for the analysis of free and esterified cholesterol in blood serum [88].

More recently, four groups have independently reported the separation of individual cholesteryl esters on ODS columns. Swaczyna and Montag [89] separated eight esters with 2-PrOH-MeCN (11:9), and Perkins _et al._ [90] found that MeCN-$CHCl_3$-MeOH (1:1:1) elutes the saturated esters in the order of increasing chain length and the unsaturated ones in the order of decreasing number of double bonds, the _cis_-isomers being eluted ahead of the _trans_-isomers. Helmich _et al._ [91] analyzed complex mixtures of tritiated cholesteryl esters in picomole quantities with MeOH-Me_2CO (17:3) as eluent, and Carroll and Rudel [92] were able to determine the individual cholesteryl esters in blood quantitatively by their absorption at 213nm following elution with a linear gradient of H_2O (3 $\rightarrow$ 0%) in MeCN-THF (13:7).

Having found that double bond isomers and certain other sterols are not resolved by RP partition HPLC, Hansbury and Scallen [93,94] acetylated fractions from the RP chromatogram and subjected them to adsorption HPLC on a silica column. Thowsen and Schroepfer [95] have demonstrated earlier that double bond isomers of steryl acetates are eluted from a Porasil column by C_6H_{14}-C_6H_6 (9:1) in the order: Δ^0, Δ^5, $\Delta^{8(14)}$, Δ^8, Δ^7, $\Delta^{5,7}$, $\Delta^{8,14}$, $\Delta^{7,14}$.

Our approach has been to chromatograph free sterols, rather than their derivatives, on a Bondapak C_{18}-Porasil B column [96]. When 0.5% 2-PrOH in _n_-C_6H_{14} was used as an eluent, the separation was based on hydrophobic adsorption rather than RP partition chromatography and the column effluent could be monitored at 205nm. Such a system not only separates the Δ^5-, Δ^7-, and $\Delta^{5,7}$-C_{27}-sterols, but also sterols differing from each other by the number of carbon atoms and double bonds (Fig. 1). Aside from permitting the separation of underivatized sterols, this

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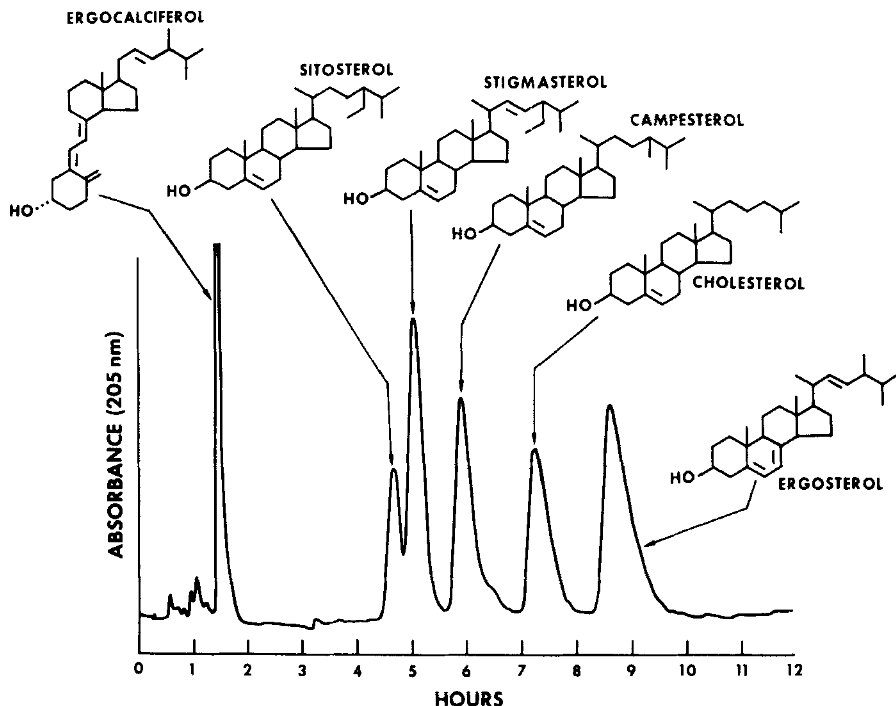

Fig. 1. HPLC of Sterols [96]. A column (16 ft x 1/8 in. ID) of Bondapak-Porasil B (37-75μm) was eluted with 0.5% 2-PrOH in n-C_6H_{14} at 0.4ml/min. The sterols, ranging in amounts from 99 to 373μg, were detected by their absorption at 205nm.

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system has the advantage of a relatively high load capacity. It allowed us, e.g., to isolate 18 μg of cholesterol in a 10-mg sample of commercial sitosterol. Colin et al. [97] have evaluated various approaches to the HPLC of free sterols and decided to use columns packed with pyrocarbon-coated silica gel. Such columns are capable of separating homologous sterols and geometric isomers.

The ketone analogs of the sterols absorb UV radiation at 254 and 280 nm. We have studied their chromatographic behavior with two UV detectors in tandem by eluting a silica column with CH_2Cl_2-n-C_6H_{14}-EtOAc (94:5:1) [98].

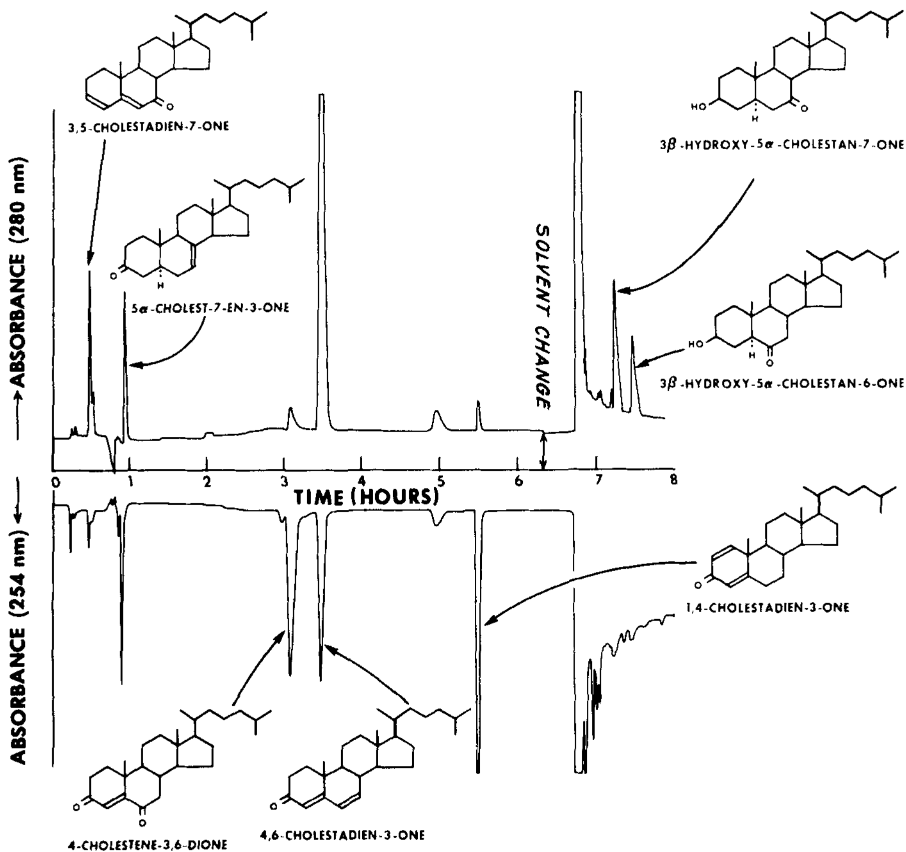

Fig. 2. HPLC of Keto Analogs of Cholesterol [98]. A column (2 ft x 1/4 in. OD) of LiChrosorb 60 (10μm) was eluted with CH_2Cl_2-EtOAc (99:1 and, after 6 hr 20 min, 3:1) at 0.5ml/min. The ketones, ranging in amounts from 6.5 to 4750μg, were detected by their absorption at 254 and 280nm.

As expected [6], 5α-cholestan-3-one was more strongly adsorbed than its 5β-epimer, and this pair was followed by 5-cholesten-3-one, which was less strongly adsorbed than its double-bond isomer 4-cholesten-3-one. The chromatogram in Fig. 2 was obtained by changing the eluent (arrow) from 1% to 25% EtOAc in CH_2Cl_2. HPLC on silica gel is being used extensively for sterol separations. It has enabled Redel and Capillon [99,100] to

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separate 25α(R), 26-hydroxycholesterol from its slightly less polar 25β(S)-epimer by recycling 2.5% Et_2O in C_6H_{14} through a Microporasil column, 60 cm in length. It was also used for the determination of the sterol composition of vegetable oils [101,102]. Ansari and Smith [103] have used both a silica column, eluted with n-C_6H_{14}-2-PrOH (24:1) or with gradually increasing concentrations of 2-PrOH, and an ODS column, eluted with 10 or 20% aq. MeCN to separate cholesterol autoxidation products, which were detected at 212nm. They succeeded in resolving the isomeric 5,6-epoxides and the 5α- and 7α-hydroperoxides. Oddly, the 3β-benzoate of the 5β, 6β-epoxide was the more mobile geometric isomer in both adsorption and RP partition chromatography.

Okazaki et al. [104-108] have developed a highly specific and sensitive method for the analysis of cholesterol in human serum lipoproteins. It requires only 10-20μl of serum and is based on protein fractionation by high-performance gel permeation chromatography. Cholesterol in each fraction is automatically determined by an enzymatic reaction performed on the column effluent after it leaves the UV detector for protein analysis (280 nm). A post-column enzyme reactor, containing cholesterol oxidase immobilized on controlled-pore glass, was also designed for the determination of cholesterol and its autoxidation products [109]. HPLC on a magnesium hydroxide column has been suggested as a method for separating wax esters from steryl esters in skin lipids [110].

The UV detector may show some specificity, e.g., when it is used to detect fungal contamination of grains [111]. Set at 282nm, it can indicate the presence of ergosterol in a mixture of sterols having no interfering absorption. A nephelometric detector has been proposed by Smith et al. [73,74] for cholesterol and other lipids fractionated by HPLC, and a chemical ionization mass spectrometer, interfaced with the liquid chromatograph by a moving-wire transport system, has also been used for their analysis [112].

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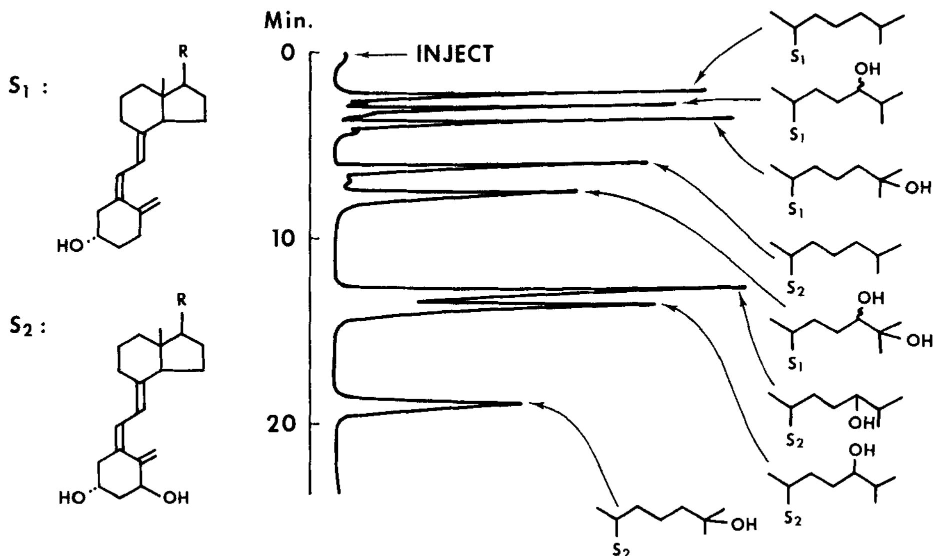

Fig. 3. HPLC of Vitamin D_3 Analogs [118]. A column (250 x 2.1mm ID) of Zorbax SIL (6μm) was eluted with 2% MeOH in CH_2Cl_2 at 0.4ml/min. The vitamins (10μg of each) were detected by their absorption at 254nm.

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3. VITAMINS D

The Vanhaelens [113] have contributed a superb review of the analysis of the vitamins D (D), and detailed descriptions of such methods have been published in Methods in Enzymology [114-116] and in the Symposium Proceedings of the Association of Vitamin Chemists [117]. It is not surprising that more work has been done on this particular application than on any other application of HPLC in the steroid field. The vitamins D are labile substances with characteristic UV absorption and, thus, ideal objects for this analytical method. Adsorption chromatography is very effective in resolving the isomeric hydroxylated metabolites, but inadequate for separating D_2 from D_3.

Fig. 3 [118] indicates some of the possibilities of adsorption HPLC on a silica column. The polarity of the D_3 analogs, which depends on the

number and position of the hydroxyl groups, increases in the order D_3 < 24 < 25 < 1α < 24,25 < 1,24 R < 1,24S < 1,25. Thus, the less polar 24-epimers are not resolved, while the 1α, 24-dihydroxy-D_3 epimers are separated from each other. Similar results have been reported by several other research groups [119-124]. Jones [125], who made a study of ternary solvent mixtures for silica HPLC, recommended C_6H_{14}-2-PrOH-MeOH (87:10:3) for samples of high lipid content and C_6H_{14}-EtOH-$CHCl_3$ (8:1:1) for differentiating D_2 and D_3 analogs. The sensitivity of detection by UV absorption can be doubled by converting the D metabolites to isotachysterol derivatives prior to HPLC and setting the detector to 290nm [126].

RP partition HPLC separates D_2 completely from D_3 if 90% aq. MeOH [127] or MeCN-CH_2Cl_2 (7:3) containing 0.5-2 ml MeOH/l [128] are used as mobile phases. The hydroxylated derivatives of D_3 were separated by RP HPLC with a linear gradient of aq. MeOH from 30 to 80% [129].

The analysis of biological products understandably requires some preliminary purification, depending on the nature of the other lipids present. In the analysis of blood, Sephadex LH-20 [130-135] low-pressure [136-143] or high-pressure [144-147] silica, and Celite [137,148] CC, affinity chromatography [149], or RP partition HPLC [150,151] are used for preliminary purification of the extract. The analysis is then carried out in a RP HPLC system if normal-phase HPLC was used for the preliminary fractionation and vice versa. Similar methods are applicable to skin samples [152,153]. HPLC was also used to prepare 25-hydroxy-D_2 from animal blood [154], to determine the configuration at C-24 of 24,25-dihydroxy-D_3 in human blood [155], and to prepare [^{3}H]-labeled D_3 from kidney tissue homogenates [156]. Of course, there is also a great deal of interest in the use of HPLC for the analysis of D in vitamin concentrate preparation [77,157-169], in milk [170-174] and milk products [175,176], in eggs [177], fish [178], margarine [179], and in livestock feed [180-184].

4. PREGNANE DERIVATIVES

The hormones in the pregnane series have a Δ^4-3-keto group, which allows their detection at 254nm. Because they are so important in medi-

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cine and so easy to chromatograph, there has been a multitude of publications on HPLC of pregnane derivatives. Several investigators have used slight variations of the method of Hesse and Hövermann [37, cf. Fig. 9] for the quantitative analysis, based on UV absorption, of individual corticosteroids in biolgical extracts by partition chromatography [185-195]. Gradient elution not only speeds up elution, but also sharpens the later peaks [196-200].

RP partition tends to give sharp and symmetrical peaks, and it has the additional advantage that polar impurities in the extracts are quickly eliminated from the column. The column material preferred by most analysts is bonded ODS [201-207]. For the greatest efficiency, adequate coverage of the silica by ODS groups seems to be essential [200]. Schöneshöfer and Dulce [198] have compared various bonded stationary phases and have opted for the polar Diol columns [197,200,208,209]. The more rarely used bonded residues include cyanopropylsilane [210], nitro [211], and phenyl [212-214] groups. Chromatography at elevated temperature is also rarely used [215].

Assay methods include, in addition to UV absorption, the RI for saturated C_{21} steroids [209,216] and the acid-induced fluorescence in the case of cortisol [217]. A high degree of specificity is obtained by combining HPLC with radioimmunoassay methods [218-220]. HPLC methods are now available for progesterone and its metabolites [209,216,221,222] (see Fig. 4), the 18-hydroxylated pregnane derivatives [200], 6β-hydroxycortisol [223], and the cortoic acids [224]. Even the tautomeric forms of aldosterone can be at least partially resolved by HPLC [225].

HPLC has been used for the assay of pharmaceutical preparations of deoxycorticosterone acetate [226], hydrocortisone phosphate [227], cypionate [228], and acetate [229,230], and other corticoid acetates [231]. Numerous semisynthetic analogs of C_{21} steroids have been analyzed by HPLC [232-236], e.g.: prednisone and prednisolone [237-246], methylprednisolone and its hemisuccinate [247-249], flucortolone [250], fludrocortisone acetate [251,252], dexamethasone [238,253,254] and betamethasone [255-257] and their esters,

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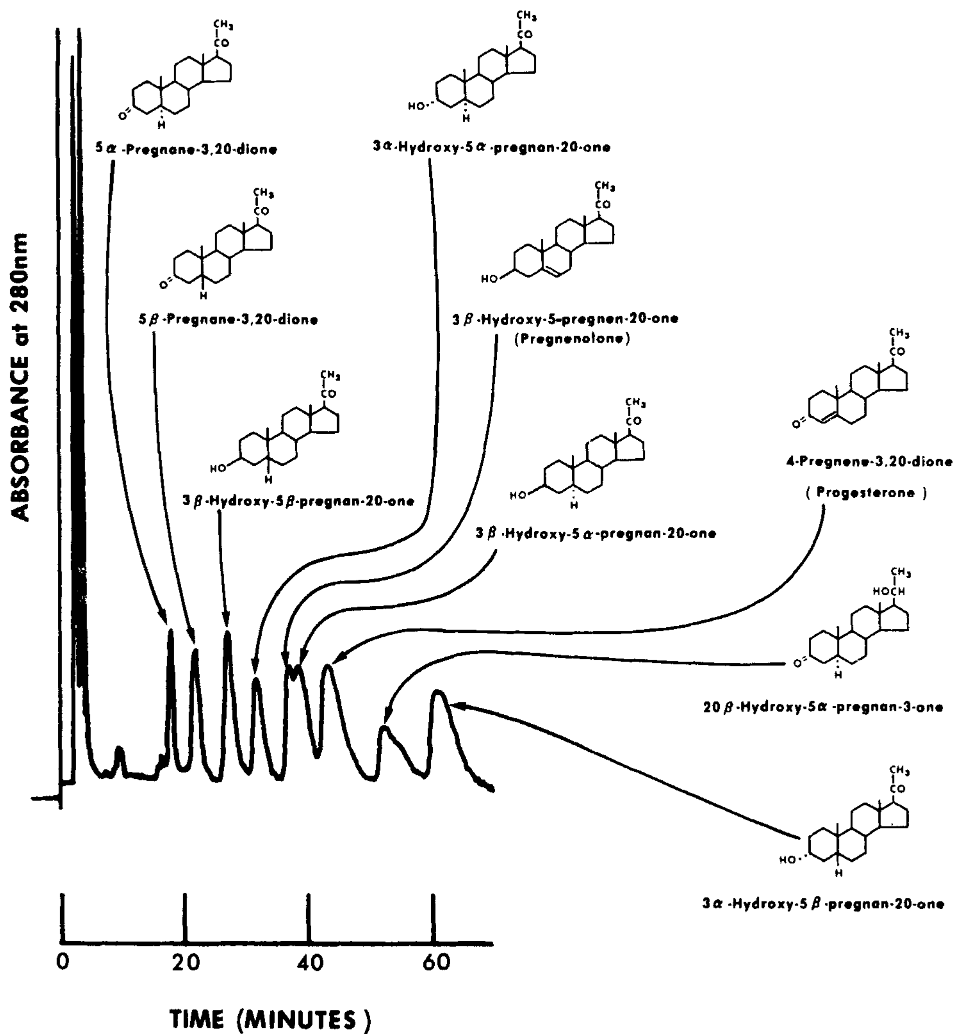

Fig. 4. HPLC of Progesterone Metabolites [216]. A column (600 x 4mm ID) of Zorbax SIL (6μm) was eluted with 0.25% EtOH in CH_2Cl_2 at 1ml/min. The metabolites, ranging in amounts from 17 to 135μg, were detected by their absorption at 280nm.

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halcinonide [258], triamcinolone acetonide [259,260], canrenone [261], the budesonide epimers [206,262-264], medrogestrone [265], and alphadione [266]. In the RP HPLC of the decomposition products of prednisolone, sulfite was added to the mobile phase [267], and the decomposition products of hydrocortisone was separated with a mobile phase containing an ion-pairing reagent [268].

5. ANDROSTANE DERIVATIVES

Numerous $C_{19}O_2$ and $C_{19}O_3$ compounds are known, which may be isomeric at positions 3, 5, and 17. Lafosse et al. [269], who made the first systematic study of free and conjugated 17-ketosteroids by RP HPLC, used a RI detector. In our work, both absorption [270,271] and RP partition [271] systems were used with UV as well as RI detectors. Fig. 5 [270] shows the separation of 11 androstane derivatives by HPLC on a silica column, eluted with CH_2Cl_2-MeCN-2-PrOH (179:20:1). Two UV detectors were connected in series, one set at 280nm and the other one at 254 nm. The order of elution of 17-ketosteroids was: 3β,5β> 3β, Δ^5> 3β, 5α >3α,5α> 3α,5β. Contrary to expectation, 5α-androstanedione was eluted before 5β-androstanedione and the 17β-hydroxysteroid, testosterone, was eluted before its 17α-epimer, epitesterone. Upon reinvestigation of the adsorption HPLC of androstane derivatives with 3% EtOH in C_6H_{14} as the eluent [271], we found that this solvent mixture elutes 5β-androstenedione before 5α-androstenedione, and epitestosterone before testosterone, while the order of the other 17-ketosteroids was unchanged. Fig. 6 [271] shows a RP chromatogram of $C_{19}O_3$ compounds obtained by use of a RI detector.

One of the important applications of HPLC in the androgen field is the determination of testosterone and its metabolites in biological extracts [272-275]. Pharmaceutical preparations of testosterone esters are readily assayed by UV absorption following HPLC [276]. Specific methods have been devised for norgestrel [277], methandienone [278-280], norethandrolone [281], norethindrone [282], and mibolerone [283], for trenbolone residues [284,285], and for various other 19-norsteroids [286], for testolactone [287],

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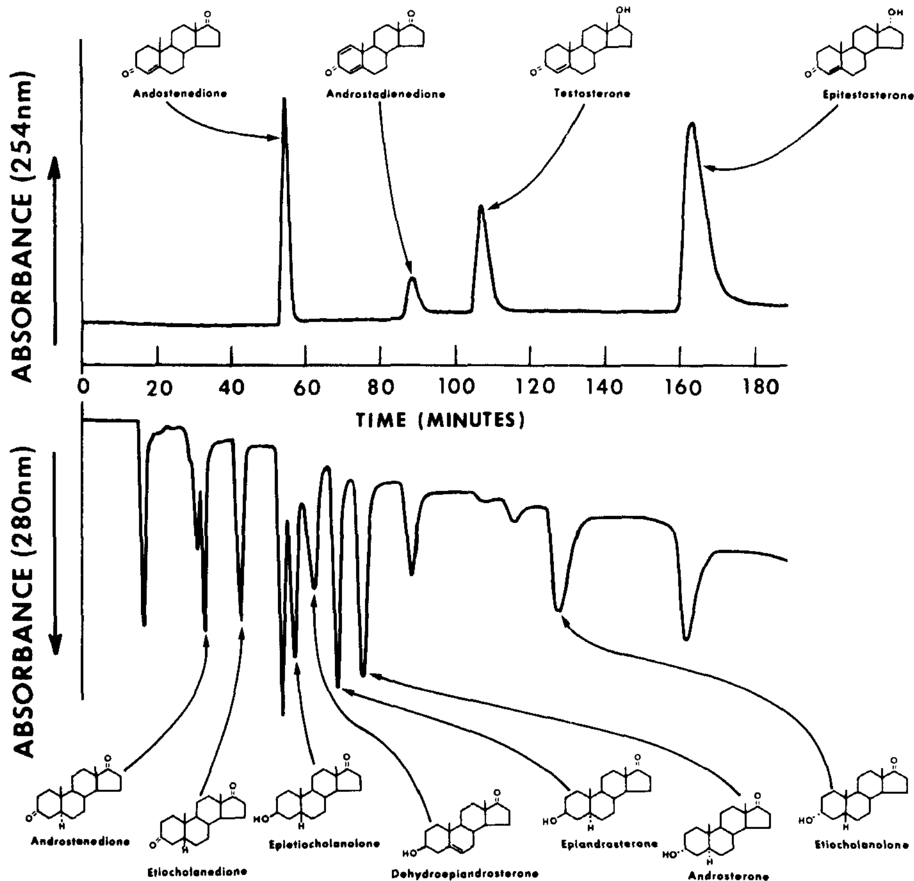

Fig. 5. Adsorption HPLC of Androstane Derivatives [270]. A column (600 x 4.6mm ID) of Partisil 5 (5μm) was eluted with CH_2Cl_2-MeCN-2-PrOH (179:20:1) at 0.4ml/min. The androstane derivatives, ranging in amounts from 6 to 297μg, were detected by their absorption at 280 and 254nm.

and for the myoneural blocking agent Org NC 45 [288]. The epimers of the ethynodiol diacetate were resolved by HPLC on an ODS column, the α-epimer being eluted before β-epimer by 80% aq. MeOH [289]. The _syn_- and _anti_-isomers of 17α-ethynyl-17β-acetoxy-19-nor-4-androsten-3-one oxime were resolved by adsorption HPLC on a silica column (290].

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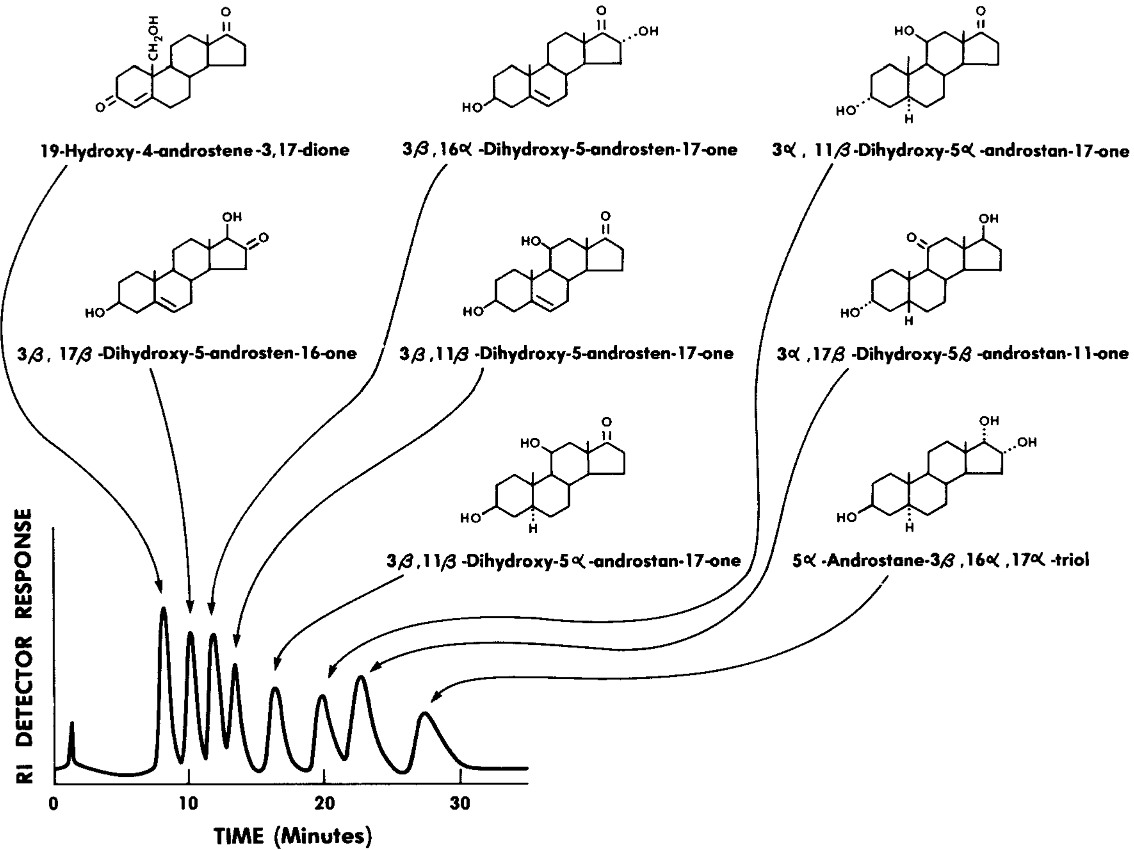

Fig. 6. RP HPLC of Androstane Derivatives [271]. A column (250 x 4.6mm ID) of Zorbax BP-ODS (8μm) was eluted with 55% aq. MeOH at 2ml/min. The androstane derivatives (100μg of each) were detected by RI.

6. ESTROGENS

The HPLC of estrogens was aptly reviewed by Schmidt [291]. Steroidal estrogens, having aromatic character, are easily detected by their UV absorption at 280 nm. Their polarity is determined in general by the number of hydroxyl groups and double bonds in the molecule. Adsorption HPLC on permaphase ETH [292,293] and silica [294-297] columns as well as RP partition HPLC on ODS [297-300] and bonded pyrrolidone [301] columns have been used for estrogen separations earlier. We have observed [302] that the two chromatographic principles are complementary. While the retention on a silica adsorption column is less influenced by keto groups and double bonds than it is in RP systems, epimeric estrogens are generally better resolved by adsorption than by partition. Fig. 7 [302] illustrates this in the separation of epimeric estriols on a silica column with n-C_6H_{14}-EtOH (9:1) and Fig. 8 [302] in the resolution of the equine estrogens on a RP column with 35% aq. MeCN.

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To increase the sensitivity of detection for biological applications, Schmidt et al. [303] and Roos [304,305] chromatographed the estrogens in the form of their dansyl derivatives. This permits as little as 400pg of estrogen to be detected by fluorometry, but poor resolution requires the borohydride reduction of the 17-ketoestrogens. Taylor et al. [306] have taken advantage of the natural fluorescence of estrogens when excited by UV radiation at 220nm, which allows the detection of as little as 100pg. Another approach to increased sensitivity is the electrochemical oxidation of the phenyl group that characterizes the estrogens. The minimum quantity of estrogen detectable by an electrochemical detector, which can be built for about $75 [307] is 10ng for urinary estriol [308] and may be as low as 0.5pg for estrogens in meat [309,310]. Shimada et al. [311,312] have separated catechol estrogens by HPLC on an ODS column, and were able to determine them at the manogram level with an electrochemical detector.

The separation of estrogen conjugates by HPLC was pioneered by Van der Wal and Huber [313,314], who found that although cellulose and poly-

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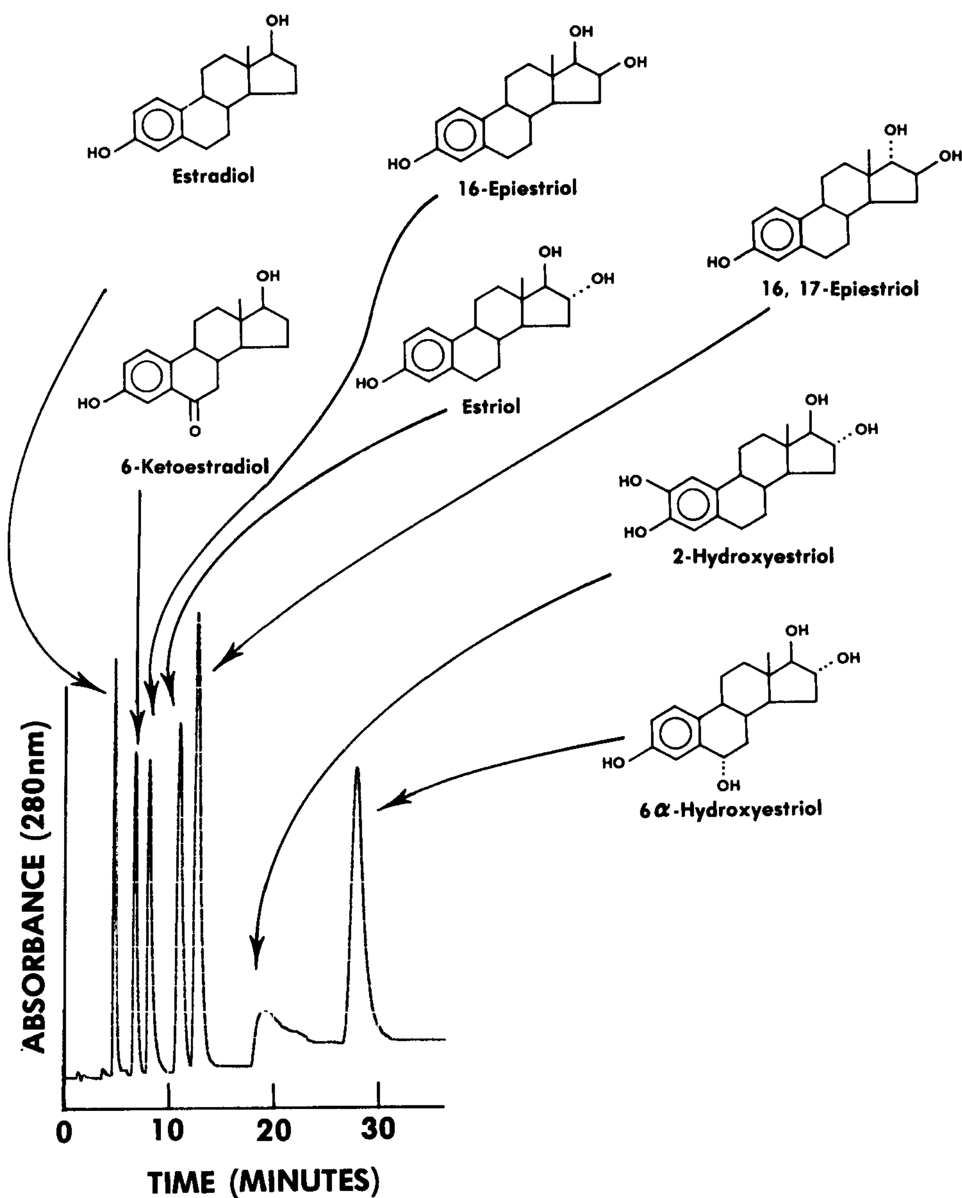

Fig. 7. Adsorption HPLC of Estrogens [302]. A column (250 x 4.6mm ID) or Zorbax BP-SIL (8μm) was eluted with n-C_6H_{14}-EtOH (9:1) at 2ml/min. The estrogens, ranging in amounts from 1.5 to 10μg, were detected by their absorption at 280nm.

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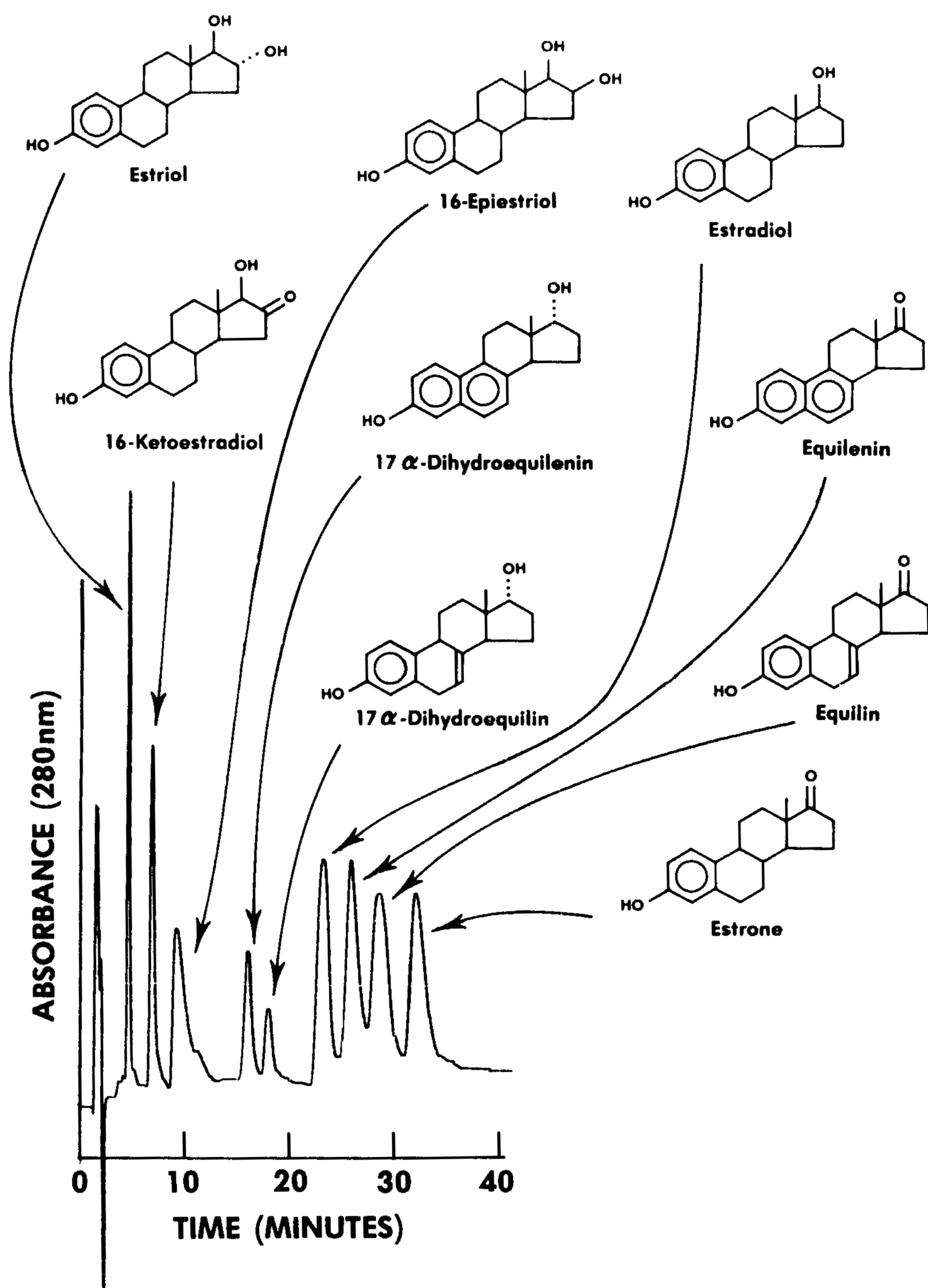

Fig. 8. RP HPLC of Estrogens [302]. A column (250 x 4.6mm ID) of Zorbax BP-ODS (8μm) was eluted with 35% aq. MeCN at 2ml/min. The estrogens, ranging in amounts from 1 to 3μg, were detected by their absorption at 280nm.

styrene anion exchangers have the greatest selectivity for the site of conjugation, ODS, coated with a liquid anion exchanger, will also serve [315]. Musey et al. [316] made similar observations. Fransson et al. [317] devised a system for separating estrogen sulfates and glucuronides, based on liquid-liquid ion-pair HPLC, but later work with similar systems by Hermansson [318,319] indicates that the underlying mechanism may be adsorption. The determination of estriol 16α-glucosiduronate in urine is based on RP partition [320]. By sequential chromatography in adsorption and RP systems Slikker et al. [321] have succeeded in resolving as many as 25 conjugated and free estrogens.

Among the steroidal estrogen analogs for which HPLC methods are now available are ethynylestradiol [322], quinestrol [323], and estramustine [324]. Williams and Goldzieher [325] have devised a scheme of following the metabolsim of ethynylestrogens by means of HPLC.

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7. MISCELLANEOUS STEROID HORMONES

In this section we review literature dealing with more than one class of steroid hormones. HPLC of steroid hormones has previously been reviewed by Fitzpatrick [326] and HPLC of sex hormones by Douglas [327]. O'Hare and Nice [328] have summarized the HPLC of steroid hormones in adrenal and testicular cells and tissues, and Jaglan and Krzeminski [329] have contributed a review on HPLC of natural and synthetic hormones in food and feed.

Fig. 9, adopted from one of the earliest publications on HPLC of steroid hormones [37], illustrates the kind of results obtainable by simple elution of a silica column with a selected solvent mixture and UV detection. Kautsky et al. [330,331] have used HPLC for the purification of ovarian hormones, Schöneshöfer and Dulce [198] have compared various HPLC systems for the purification of adrenal and gonadal steroids prior to radioimmunoassay, and Cochran and Ewing [332] have devised a method of isolating 14 testicular steroids by HPLC following Celite CC. In a study of pregnenolone metabolism, paper chromatography was used prior to HPLC [333]. TLC served Okumura [334] as a guide in the selection of chromatographic systems

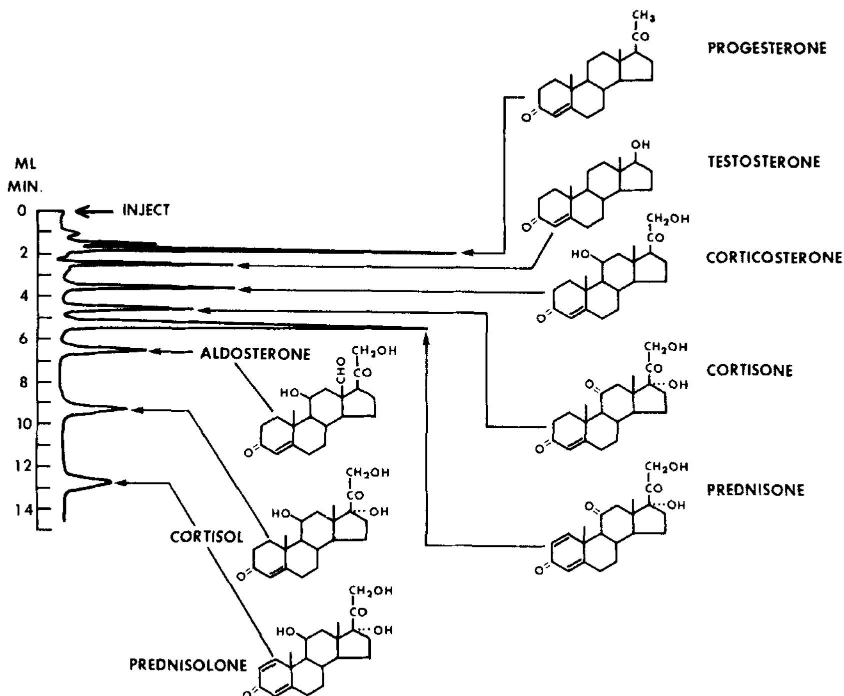

Fig. 9. HPLC of Steroid Hormones [37]. A column (300 x 3mm ID) of Spherosil XOA-400 (4-8μm) was eluted with CH_2Cl_2-EtOH-H_2O (936:47:17) at 1.14ml/min. The hormones were detected at 240nm.

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suitable for HPLC of various steroid hormones. HPLC has been used for the separation of C_{18} and C_{19} steroids [335]. Mixtures of mestranol and norethindrone [336] and of ethynylestradiol and norethindrone [337] have been assayed by HPLC.

8. BILE ACIDS

HPLC of bile acids has been reviewed by Nambara and Goto [338-340] and by Elliott and Shaw [341]. Because the carboxylic or sulfonic acids are very polar and because most bile acids lack UV chromophores, derivatization was used initially. The phenacyl [342], 4-nitrobenzyl [55,56,343-345], 1-naphthylmethyl [346], and 7-methoxy-4-methylenecoumaryl [347] esters not

only have desirable chromatographic properties but also absorb strongly in the near UV or exhibit a fluorescence. Solvent systems for HPLC have been derived from TLC data [343], and gradient elution has been employed occasionally [347,348].

Although satisfactory separations have been accomplished with silica columns [56,342,349], with ion exchangers [350,351], and by liquid-liquid ion-pair HPLC [352], the overwhelming majority of current analytical work is performed on underivatized bile acids with RP columns. Most analysts now exploit the UV absorption of bile acids at 210 nm or even shorter wavelengths [353-357] instead of relying on the RI detector. An attempt has been made to introduce the polarographic detector into bile acid analysis [358], and post-column derivatization in a fixed-enzyme reactor has recently been accomplished. The latter involves passing the effluent through a column, containing immobilized 3α-hydroxysteroid dehydrogenase, and then determining the reduced nicotinamide-adenine dinucleotide (NADH) formed by fluorometry [359-362]. As little as 10ng of bile acids can be detected by this method.

Fig. 10, adapted from Shaw and Elliott [349], illustrates the separation of conjugated bile acids obtainable by RP partition chromatography. As can be seen, not only are the glyco conjugates well separated from the corresponding tauro conjugates, but position isomers are also resolved. Shaw *et al*. [363] have calculated "relative capacity factors" for α-hydroxy bile acids, but their correlations between these factors and bile acid structure break down when the hydroxyl groups are spaced too closely or when other functional groups are also present. RP HPLC has been applied to the free [364] and sulfated [357,365,366] bile acids and to the free and conjugated bile acids in human bile [350,355,367-377] and serum [356,373,376-380].

9. CARDIAC GENINS AND GLYCOSIDES

HPLC of cardiac glycosides has been reviewed by Seiber *et al*. [381]. The unsaturated lactone ring in the cardenolides and bufadienolides allows their detection at 220nm. Both normal and RP partition HPLC are appli-

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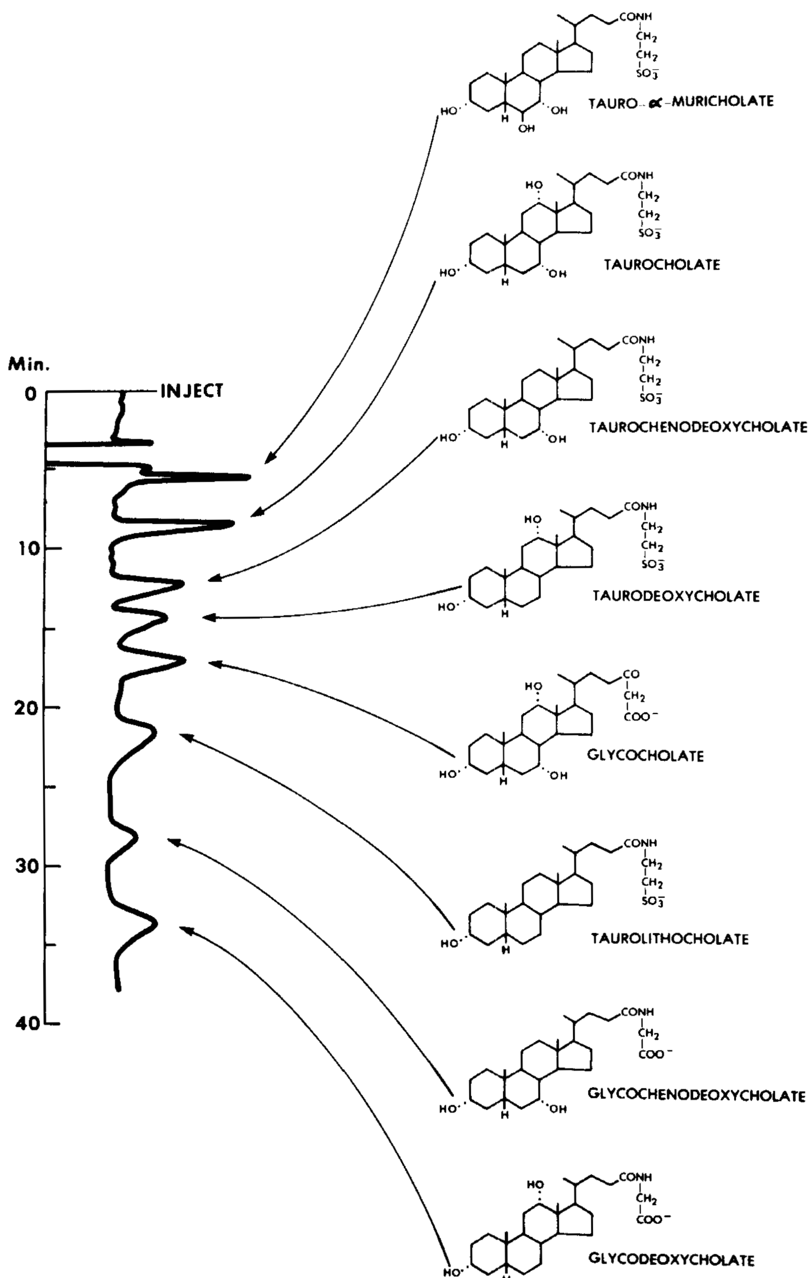

Fig. 10. HPLC of Conjugated Bile Acids [349]. A Fatty Acid Analysis Column (Waters) (300 x 4mm ID) was eluted with 8.8mM phosphate buffer (pH 2.5) -2-propanol (17:8) at 1ml/min. The bile acids were detected by RI.

cable to their fractionation. An example of the former is the separation of cardiac genins and glycosides on a silica column with n- pentanol-MeCN-isooctane-H_2O (35:12:124:2) is shown in Fig. 11 [382]. Conversion to the 4-nitrobenzoates made these compounds detectable at a concentration of 20ng/ml without changing their order of elution [383,384]. Post-column detection of cardiac glycosides with hydrochloric acid, which produces an intense fluorescence, has been proposed [385].

Other silica HPLC methods are in the literature [386-388], but comparison of normal and RP partition systems has indicated that the latter are superior [389]. By now, RP HPLC has been applied to the cadiac glycosides by several investigators [390-395], and the method has been used to trace tritiated digoxin in patients [396-398]. A sensitive and specific procedure for the analysis of metabolites isolated by HPLC is the radioimmunoassay [399-402].

The bufadienolides are also best chromatographed in RP systems. Both the half esters and the genins occurring in toads [403-405] and the glycosides occurring in plants [406] could be separated in this manner.

10. MISCELLANEOUS PLANT STEROIDS

Ecdysteroids, like other steroid hormones, are easily detected by their UV absorption. In contrast to insects, plants contain a large variety of ecdysteroids, which differ mainly in the number and location of hydroxyl groups. They are generally quite polar and easy to separate by partition chromatography. Hashimoto [407] and Lafont *et al.* [408] have reviewed the HPLC of ecdysteroids. Fig. 12 [409] shows the first application of HPLC to steroids. Amberlite XAD-2 for the 1.5-m long column was pulverized and sifted in the laboratory, and gradient elution with aq. EtOH took 12 hrs, but several ecdysteroids could be isolated by this method. Ecdysteroids have subsequently been separated by HPLC on silica [410-413] and on RP [411-414] columns with equally satisfactory results. These methods have been applied to plants [414] as well as insects [415-417].

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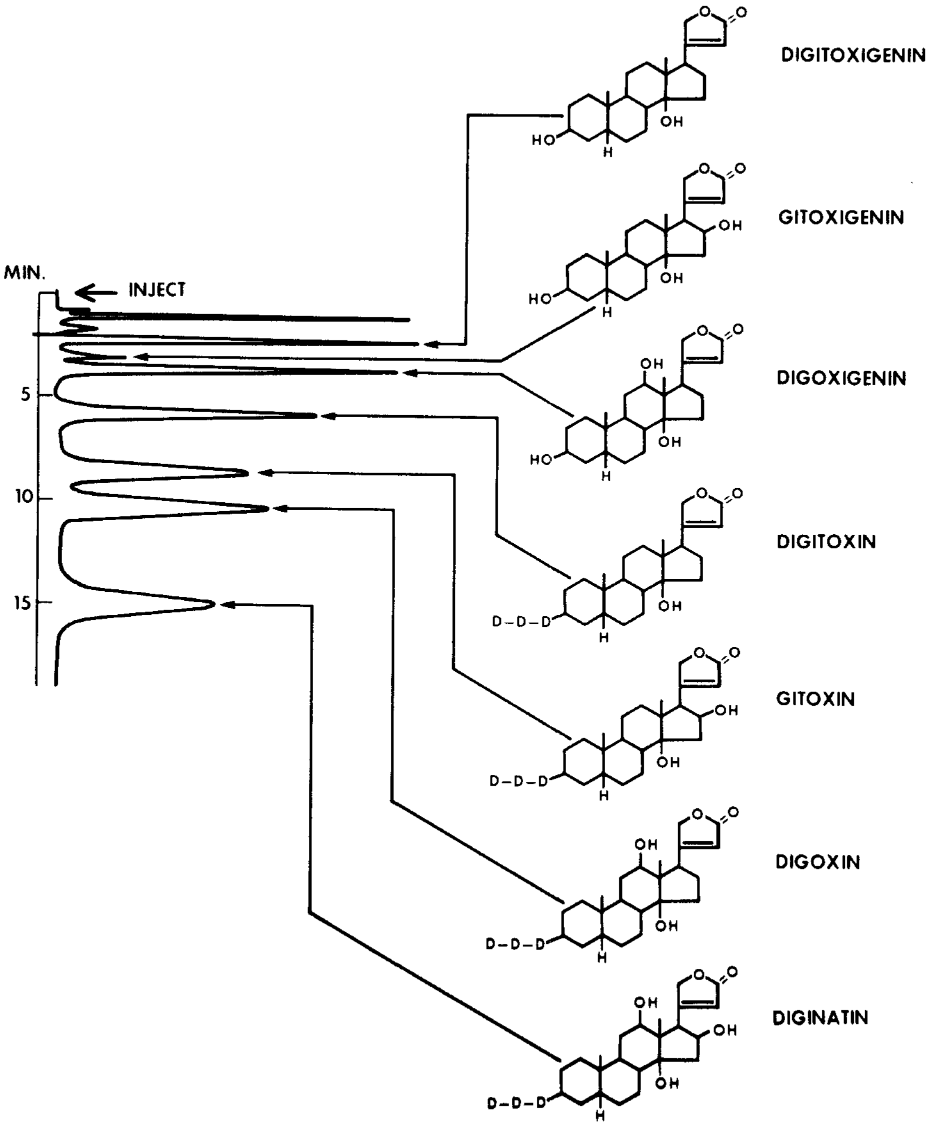

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Fig. 11. HPLC of Cardiac Genins and Glycosides [382]. A column (250 x 3mm ID) of LiChrosorb SI-60 (10 μm) was eluted with n-pentanol-MeCN-isooctane-H_2O (35:12:124:2) at 1.3ml/min. The cardiac genins and glycosides were detected at 220nm.

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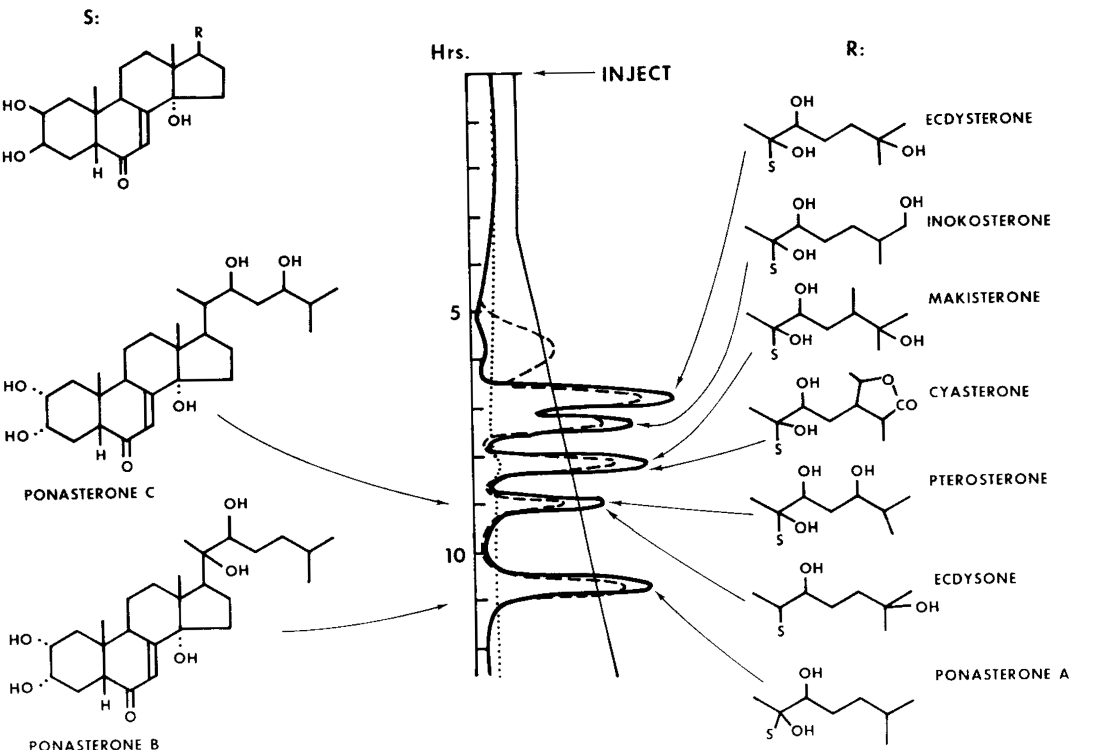

Fig. 12. HPLC of Ecdysteroids [409]. A column (1.5m x 9mm ID) of Amberlite XAD-2 (200 to 400-mesh) was eluted with a linear gradient of 20% → 70% EtOH in H_2O. The ecdysteroids, ranging in amounts from 100 to 500μg were detected by continuous monitoring at 230 (---), 250 (—), and 300 (....) nm.

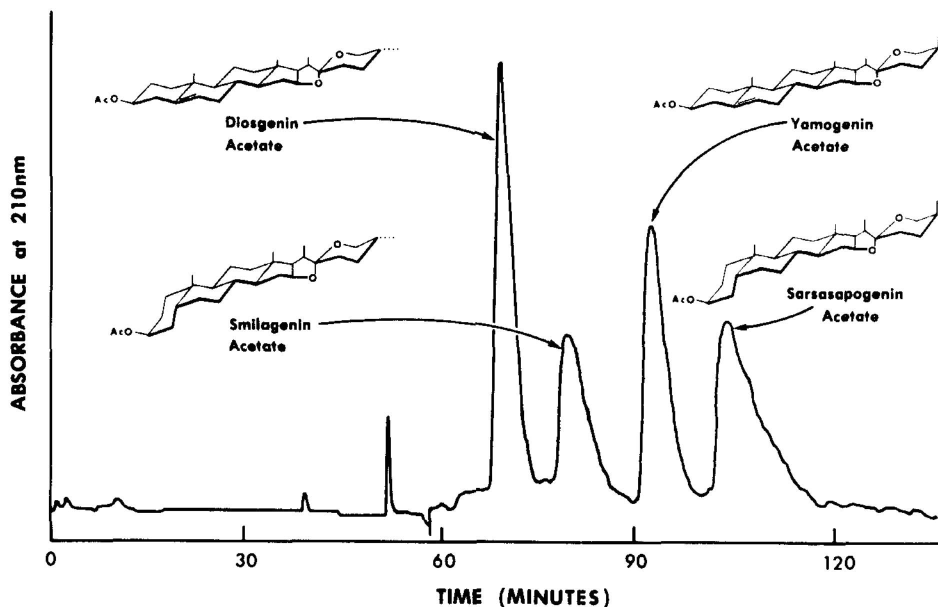

Fig. 13. HPLC of Sapogenin Acetates [418]. A column (100 x 4.6mm ID) of Zorbax SIL (6μm) was eluted with 1% Me_2CO in C_6H_{14} at 2ml/min. The sapogenin acetates, ranging in amounts from 28 to 1153μg, were detected by their absorption at 210nm.

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Steroidal sapogenins and alkaloids occur in plants in the form of glycosides, the saponins and glycoalkaloids, respectively. The sapogenins exhibit isomerism at C-5 and C-25, and additional isomerism occurs at C-22 in the alkaloids. The genins are relatively nonpolar, and the saturated analogs are difficult to detect by UV absorption without derivatization. For the HPLC of sapogenins, we have therefore chosen to use the acetates, which are somewhat easier to detect [418]. Fig. 13 illustrates the resolution of two pairs of isomers by adsorption HPLC. Tigogenin and neotigogenin, which have conformations similar to diosgenin and yamogenin, respectively, are not separable from the latter under these conditions. To accomplish such a separation, hydrophobic adsorption HPLC on an ODS column with MeCN-

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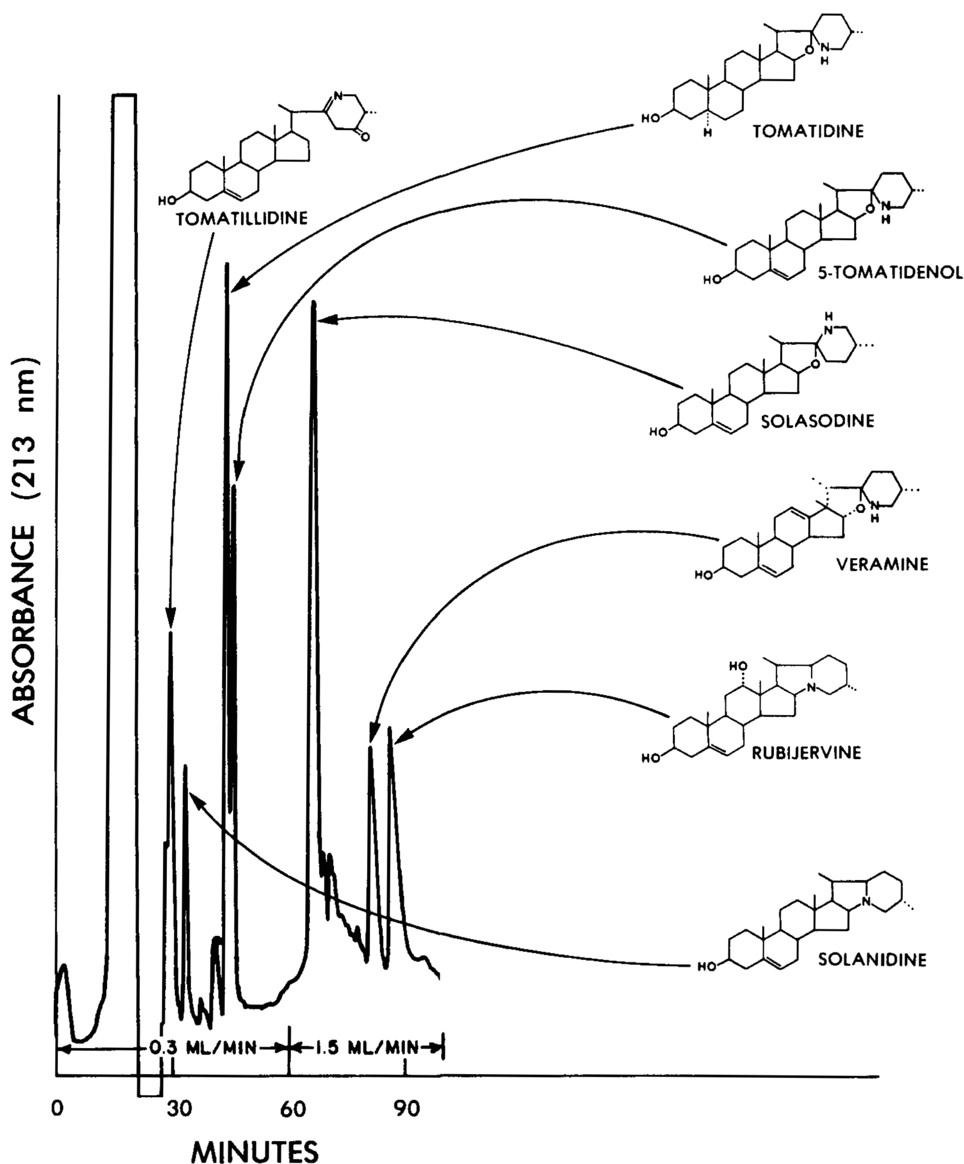

Fig. 14. HPLC of Steroidal Alkaloids [422]. A column (500 x 4.6mm ID) of Zorbax SIL (6μm) was eluted with n-C_6H_{14}-MeOH-Me_2CO (18:1:1) at 0.3 and then at 1.5ml/min. The alkaloids, ranging in amounts from 6.3 to 210μg, were detected by their absorption at 213nm.

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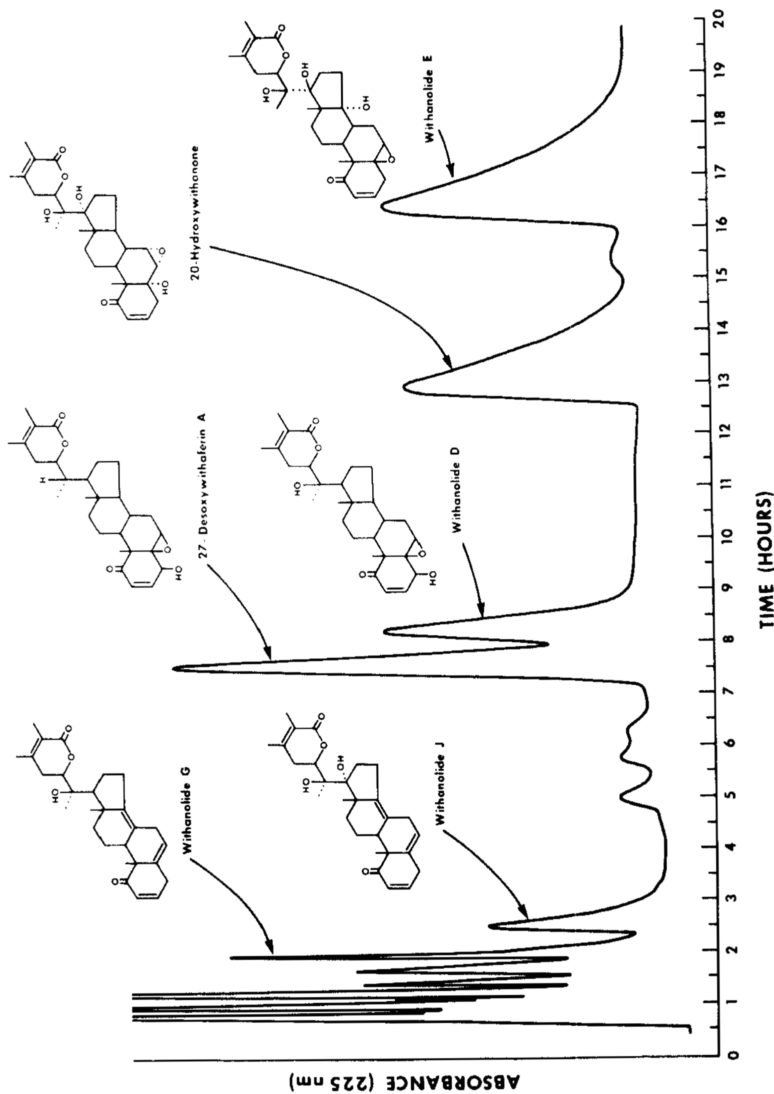

Fig. 15. HPLC of Withanolides [427]. A coiled column (12 ft x 1/8 in. OD) of Porasil A (37-75μm) was eluted with n-C_6H_{14}-2-PrOH (9:1) at 0.2ml/min. The withanolides, ranging in amounts from 0.75 to 25μg, were detected by their absorption at 225nm.

C_6H_{14}-tetrahydrofuran (17:2:1) was used. In each case, the Δ^5-analog was eluted ahead of the 5α-analog. Such methods have been found applicable to plant extracts [419-421].

Fig. 14 [422] shows the separation of steroidal alkaloids by adsorption HPLC on a silica column by increasing the flowrate of the mobile phase from 0.3 to 1.5 ml/min after 1 hr. Quantitative assays, based on HPLC [423] and a combination of centrifugal chromatography and HPLC [424] have been devised. The Bushways [425,426] have used HPLC to analyze potatoes for glycoalkaloids.

The withanolides have both an α,β-unsaturated δ-lactone group and an α,β-unsaturated carbonyl group in the molecule and are therefore easily detected by their UV absorption. They differ from each other mainly in the position and number of double bonds and oxygen functions. Fig. 15 [427] is an example of the type of separation obtained by HPLC on silica. The method was found to be applicable to plant extracts [427-429]. The physalins, in analogy to the withanolides, are also easily detected by their absorption at 254nm, due to their α,β-unsaturated carbonyl group. Sen et al. [430] have separated several physalins by RP partition HPLC on an ODS column with MeOH-H_2O (3:2) as the eluent.

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12. BIBLIOGRAPHY

1. Heftmann, E., "Chromatography of Steroids," J. Chromatogr. Library Ser., Vol. 8, Elsevier, Amsterdam, 1976.

2. Heftmann, E., in "Chromatography," J. Chromatogr. Library Ser., Vol. Heftmann, E. (ed.), Elsevier, Amsterdam, 1982.

3. Heftmann, E.; Hunter, I. R., J. Chromatogr. 165,283-99 (1979).

4. Kautsky, M. P., ed. "Steroid Analysis by HLPC. Recent Applications," Chromatogr. Sci. Ser., Vol. 16, Cazes, J. (ed.), Dekker, New York 1981.

5. Redel, J. J.; Capillon, J., in "Steroid Analysis by HLPC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 343-58.

6. Heftmann, E., J. Liquid Chromatogr. 2, 1137-55 (1979).

7. Studebaker, J. F., in "Steroid Analysis by HLPC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 323-42.

8. Michaelis, A. F.; Cornish, D. W.; Vivilecchia, R., J. Pharm. Sci. 62, 1399-1416 (1973).

9. Shroff, A. P., Amer. Lab. 8, 13-32 (1976).

10. Dixon, P. F.; Stoll, M. S.; Lim, C. K., Ann. Clin. Biochem. 13,409-32 (1976).

11. Onishi, S., Kagaku no Ryoiki Zokan 109, 219-29 (1976).

12. Pryde, A.; Gilbert, M. T., "Applications of High-Performance Liquid Chromatography," Chapman & Hall, London, 1979.

13. Hawk, G. H. (ed.), "Biological/Biomedical Applications of Liquid Chromatography II, "Chromatogr. Sci. Ser., Vol 12, Cazes, J. (ed.), Dekker, New York, 1979.

14. Tsuji, A.; Kawasaki, T., Rinsho Kagaku 9, 134-44 (1980).

15. Kingston, D. G. I., J. Nat. Prod. 42, 237-60 (1979).

16. Thompson, M. J.; Patterson, G. W.; Dutky, S. R.; Svoboda, J. A., Kaplanis, J. N., Lipids 15, 719-33 (1980).

17. Baker, D. R.; Henry, R. A.; Williams, R. C.; Hudson, D.R., J. Chromatogr. 83, 233-43 (1973).

18. Pei; P.; Ramachandran, S.; Henly, R. S., Amer. Lab. 7, 37-40 (1975).

19. Hunter, I. R.; Walden, M. K.; Wagner, J. R.; Heftmann, E., J. Chromatogr. 119, 223-6 (1976).

20. Hara, S.; Nakahata, M., Yakugaku Zasshi 97, 823-5 (1977).

21. Hara, S., J. Chromatogr. 137, 41-52 (1977).

22. Hara, S.; Nakahata, M., J. Liquid Chromatogr. 1, 43-54 (1978).

23. Godbille, E.; Devaux, P., J. Chromatogr. 122, 317-29 (1976).

24. Zinecker, K., GIT Fachzeit. Lab. 20, 821-2 (1976).

25. Beck, W.; Halasz, I., Fresenius' Z. Anal. Chem. 291, 312-18 (1978).

26. Shaw, R.; Elliott, W. H., J. Chromatogr. 177, 289-95 (1979).

27. Gasparrini, F., Cacchi, S.; Caglioti, L.; Misiti, D.; Giovannoli, M. J. Chromatogr. 194, 239-44 (1980).

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28. Artaud, J.; Iatrides, M. C.; Tisse, C.; Zahra, J. P.; Estienne, J., Analusis 8, 277-86 (1980).

29. Davydov, V. Ya.; Gonzalez, M. E.; Kiselev, A. V.; Lenda, K., Chromatographia 14, 13-18 (1981).

30. Engelhardt, H.; Wiedemann, H., Anal. Chem. 45, 1641-6 (1973).

31. Van den Berg, J. H. M.; Milley, J.; Vonk, N.; Deelder, R. S., J. Chromatogr. 132, 421-7 (1977).

32. Berry, L. V.; Engelhardt, H., J. Chromatogr. 95, 27-38 (1974).

33. Tscherne, R. J.; Capitano, G., J. Chromatogr. 136, 337-41 (1977).

34. Cooke, N. H. C.; Olsen, K., J. Chromatogr. Sci. 18, 512-24 (1980).

35. Nice, E. C.; O'Hare, M. J., J. Chromatogr. 166, 263-7 (1978).

36. Siggia, S.; Dishman, R. A., Anal. Chem. 42, 1223-9 (1970).

37. Hesse, C.; Hovermann, W., Chromatographia 6, 345-8 (1973).

38. Hara, S., Kagaku no Ryoiki 31, 13-21 (1977).

39. Hara, S., Kagaku no Ryoiki 31, 199-207 (1977).

40. Hara, S.; Hirasawa, M.; Itokawa, H., Yakugaku Zasshi 98, 401-4 (1978).

41. Hara, S.; Fuji, Y.; Hirasawa, M.; Miyamoto, S., J. Chromatogr. 149 143-59 (1978).

42. Jandera, P.; Janderová, M.; Churáček, J.; J. Chromatogr. 148 79-97 (1978).

43. Hara, S.; Hirasawa, M.; Miyamoto, S.; Ohsawa, A., J. Chromatogr. 169, 117-27 (1979).

44. Hara, S.; Ohsawa, A., J. Chromatogr. 200, 85-94 (1980).

45. Oka, K.; Hara, S., J. Chromatogr. 202, 187-94 (1980).

46. Stewart, J. G.; Williams, P. A., J. Chromatogr. 198, 489-94 (1980).

47. Lee, G.J.-L.; Carlson, R. M. K.; Kushinsky S., J. Chromatogr. 212, 108-14 (1981).

48. O'Hare, M. J.; Nice, E. C.; Magee-Brown, R.; Bullman, H., J. Chromatogr. 125, 357-67 (1976).

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49. Yost, R. W.; MacLean, W. M.; Poile, A. F., Perkin - Elmer Liq. Chromatogr. Appl. Study No. 57.

50. Hara, S.; Hayashi, S., J. Chromatogr. 142, 689-703 (1977).

51. Fitzpatrick, F. A.; Siggia, S., Anal Chem. 45, 2310-14 (1973).

52. Henry, R. A.; Schmit, J. A.; Dieckman, J. F., J. Chromatogr. Sci. 9, 513-20 (1971).

53. Fitzpatrick, F. A.; Siggia, S.; Dingman, J., Anal Chem. 44, 2211-16 (1972).

54. Stellaard, F.; Hachey, D. L.; Klein, P. D., Anal. Biochem. 87, 359-66 (1978).

55. Okuyama, S.; Uemura, D.; Hirata, Y., Chem. Lett. 1976, 679-82.

56. Shaikh, B.; Pontzer, N. J.; Molina, J. E.; Kelsey, M.I., Anal. Biochem. 85, 47-55 (1978).

57. Kawasaki, T.; Maeda, M.; Tsuji, A., J. Chromatogr. 163, 143-50 (1979).

58. Kawasaki, T.; Maeda, M.; Tsuji, A., Proc. Symp. Chem Physiol. Pathol. 19, 117-22 (1979).

59. Kawasaki, T.; Maeda, M.; Tsuji, A., Yakugaku Zasshi 100, 925-32 (1980).

60. Goehl, T. J.; Sundaresan, G. M.; Prasad, V. K.; J. Pharm. Sci. 68, 1374-6 (1979).

61. Shimada, K.; Tanaka, M.; Nambara, T., Anal. Lett. 13, 1129-36 (1980).

62. Ikenoya, S.; Hiroshima, O.; Ohmae, M., Kawabe, K., Chem. Pharm. Bull. 28, 2941-7 (1980).

63. Horikawa, R.; Tanimura, T.; Tamura, Z., J. Chromatogr. 168, 526-9 (1979).

64. Schwedt, G.; Reh, E., Chromatographia 13, 779-84 (1980).

65. Reh, E.; Schwedt, G., Fresenius' Z, Anal. Chem. 303, 117-20 (1980).

66. Kiuchi, K.; Ohta, T.; Ebine, H., J. Chromatogr. Sci. 13, 461-6 (1975).

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67. Aitzetmüller, K.; Koch, J., J. Chromatogr. 145, 195-202 (1978).

68. Boshoff, P. R.; Hopkins, B. J.; Pretorius, V., J. Chromatogr. 126, 35-41 (1976).

69. Kutner, W.; Debowski, J.; Kemula, W., J. Chromatogr. 191, 47-60 (1980).

70. Schulten, H.-R.; Beckey, H. D., J. Chromatogr. 83, 315-20 (1973).

71. Horning, E. C.; Carroll, D. I.; Dzidic, I.; Haegele, K. D.; Horning, M. G.; Stillwell, R. N., J. Chromatogr. 99, 13-21 (1974).

72. Yamauchi, E.; Mizuno, T.; Azuma, K., Shitsuryo Bunseki 28, 227-34 (1980).

73. Jorgenson, J. W.; Smith, S. L.; Novotny, M., J. Chromatogr. 142. 233-40 (1977).

74. Smith, S. L.; Jorgenson, J. W.; Novotny, M., NBS Spec. Publ. Trace Org. Anal. 519, 429-36 (1979).

75. Beyer, W. F.; Gleason, D. D., J. Pharm. Sci. 64, 1557-60 (1975).

76. Kern, H.; Imhof, K., Amer. Lab. 10, 131-40 (1978).

77. Abdou, H. M.; Russo-Alesi, F. M.; Fernandez, V., Pharm. Technol. 5, 42-5 (1981).

78. Smith, W. B.; Hogle, L., Rev. Latinoamer. Quím. 7, 20-2 (1976).

79. Pascal, R. A., Jr.; Farris, C. L.; Schroepfer, G. J., Jr., Anal. Biochem. 101, 15-22 (1980).

80. Rees, H. H.; Donnahey, P. L.; Goodwin, T. W., J. Chromatogr. 116, 281-91 (1976).

81. Popov, S.; Carlson, R. M.; Wegmann, A.; Djerassi, C., Steroids 28, 699-732 (1976).

82. Tallová, J.; Hakl, J., J. Chromatogr. 202, 487-90 (1980).

83. Byrne, K. J.; Reazin, G. H.; Andreasen, A. A., J. Assoc. Off. Anal. Chem. 64, 181-5 (1981).

84. Newkirk, D. R.; Sheppard, A. J., J. Assoc. Off. Anal. Chem. 64, 54-7 (1981).

Downloaded At: 17:54 24 January 2011

85. Trocha, P. J.; Jasne, S. J.; Sprinson, D. B., Biochemistry 16, 4721-6 (1977).

86. Hansbury, E.; Scallen, T. J., J. Lipid Res. 19, 742-6 (1978).

87. Kikuchi, N., Miki, T., Mikrochim. Acta 1, 89-96 (1978).

88. Duncan, I. W.; Culbreth, P. H.; Burtis, C. A., J. Chromatogr. 162, 281-92 (1979).

89. Swaczyna, H.; Montag, A., Lebensmittelchem. Gerichtl. Chem. 35, 20-1 (1981).

90. Perkins, E. G., Hendren, D. J.; Bauer, J. E.; El-Hamid, A. H., Lipids 16, 609-13 (1981).

91. Helmich, O.; Špaček, P.; Hradec. J., J. Chromatogr. 213, 105-11 (1981).

92. Carroll, R. M.; Rudel, L. L., J. Lipid Res. 22, 359-63 (1981).

93. Hansbury, E.; Scallen, T. J., J. Lipid Res. 21, 921-9 (1980).

94. Hansbury, E.; Scallen, T. J., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 253-76.

95. Thowsen, J. R.; Schroepfer, G. J., Jr., J. Lipid Res. 20, 681-5 (1979).

96. Hunter, I. R.; Walden, M. K.; Heftmann, E., J. Chromatogr. 153, 57-61 (1978).

97. Colin, H.; Guiochon, G.; Stouffi, A., Anal. Chem. 51, 1661-6 (1979).

98. Hunter, I. R.; Walden, M. K.; Bailey, G. F.; Heftmann, E., Lipids 14, 687-90 (1979).

99. Redel, J.; Capillon, J., J. Chromatogr. 151, 418-20 (1978).

100. Redel, J., J. Chromatogr. 168, 273-6 (1979).

101. Cortesi, N.; Fedeli, E.; Tiscornia, E., Riv. Ital. Sostanze Grasse 55, 168-75 (1978).

102. Artaud, J., Iatrides, M. C.; Estienne, J., Ann. Falsif. Expert. Chim. 72, 153-7 (1979).

103. Ansari, G. A. S.; Smith, L. L., J. Chromatogr. 175, 307-15 (1979).

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104. Okazaki, M.; Ohno, Y.; Hara I., J. Chromatogr. 221, 257-64 (1980).

105. Hara, I.; Okazaki, M.; Ohno, Y., J. Biochem. 87, 1863-5 (1980).

106. Okazaki, M.; Hara, I., J. Biochem. 88, 1215-18 (1980).

107. Okazaki, M.; Ohno, Y.; Hara, I., J. Biochem. 89, 879-87 (1981).

108. Okazaki, M.; Shiraishi, K.; Ohno Y.; Hara, I., J. Chromatogr. 223, 285-93 (1981).

109. Ögren, L.; Csiky, I.; Risinger, L.; Nilsson, L. G.; Johansson, G., Anal. Chim. Acta 117, 71-9 (1980).

110. Stewart, M. E.; Downing, D. T., Lipids 16, 355-9 (1981).

111. Seitz, L. M.; Mohr, H. E.; Burroughs, R.; Sauer, D. B., Cereal Chem. 54, 1207-17 (1977).

112. Privett, O. S.; Erdahl, W. L., Chem. Phys. Lipids 21, 361-88 (1978).

113. Vanhaelen-Fastré, R.; Vanhaelen, M., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 173-251.

114. Koshy, K. T., in "Methods in Enzymology," Vol. 67, McCormick, D. B.; Wright, L. D. (eds.), Academic Press, New York, 1980, pp. 357-70.

115. Tanaka, Y.; DeLuca, H. F.; Ikekawa, N., in "Methods in Enzymology," Vol. 67, McCormick, D. B.; Wright, L. D. (eds.), Academic Press, New York, 1980, pp. 370-85.

116. Shepard, R. M.; DeLuca, H. F., in "Methods in Enzymology," Vol. 67, McCormick, D. B.; Wright, L. D. (eds.), Academic Press, New York, 1980, pp. 393-413.

117. Association of Vitamin Chemists, "Symposium Proceedings. Applications of High-Pressure Liquid Chromatographic Methods for Determination of Fat-Soluble Vitamins A, D, E, and K in Foods and Pharmaceuticals," Feb. 9, 1978, Chicago , IL, and May 19, 1978, San Francisco, CA.

118. Ikekawa, N.; Koizumi, N., J. Chromatogr. 119, 227-32 (1976).

119. Jones, G.; DeLuca, H. F., J. Lipid Res. 16, 448-53 (1975).

Downloaded At: 17:54 24 January 2011

120. Tartivita, K. A.; Sciarello, J. P.; Rudy, B. C., J. Pharm. Sci. 65, 1024-7 (1976).

121. Tsukida, K.; Kodama, A.; Saiki, K., J. Nutr. Sci. Vitaminol. 22, 15-20 (1976).

122. Hofsass, H.; Grant, A.; Alicino, N. J., Greenbaum, S. B., J. Assoc. Off. Anal. Chem. 59, 251-60 (1976).

123. Antalick, J. P., Debruyne, H.; Faugere, J. G., Ann. Falsif. Expert. Chim. 70, 497-502 (1977).

124. Vanhaelen-Fastré, R.; Vanhaelen, M., J. Chromatogr. 153, 219-26 (1978).

125. Jones, G., J. Chromatogr. 221, 27-37 (1980).

126. Seamark, D. A., Trafford, D. J. H.; Hiscocks, P. G.; Makin, H. L. J., J. Chromatogr. 197,271-3 (1980).

127. Osadca, M.; Araujo, M., J. Assoc. Offic. Anal. Chem. 60, 993-7 (1977).

128. Landen, W. O., Jr., J. Chromatogr. 211, 155-9 (1981).

129. Matthews, E. W.; Byfield, P. G.; Colston, K. W.; Evans, I. M.; Galante, L. S.; MacIntyre, I., FEBS Lett. 48, 122-5 (1974).

130. Lambert, P. W.; Syverson, B. J.; Arnaud, C. D.; Spelsberg, T. C., J. Steroid Biochem. 8, 929-37 (1977).

131. Eisman, J. A.; Shepard, R. M.; DeLuca, H. F., Anal. Biochem. 80, 298-305 (1977).

132. Schaefer, P. C.; Goldsmith, R. S., J. Lab. Clin. Med. 91, 104-8 (1978).

133. Pelc, B.; Holmes, A. L., J. Chromatogr. 173, 403-7 (1979).

134. Lambert, P. W.; Lindmark, E. A.; Spelsberg, T. C., in "Biological/ Biomedical Applications of Liquid Chromatography I.," Chromatogr. Sci. Ser., Vol. 10, Cazes, J. (ed.), Dekker, New York, 1979, pp. 637-57.

Downloaded At: 17:54 24 January 2011

135. Parviainen, M. T.; Savolainen, K. E.; Alhava, E. M.; Maenpaa, P.H., Ann. Clin. Res. 13, 26-33 (1981).

136. Koshy, K. T.; VanDerSlik, A. L., Anal. Biochem. 74, 282-91 (1976).

137. Koshy, K. T.; VanDerSlik, A. L., Anal. Lett. 10, 523-37 (1977).

138. Koshy, K. T.; VanDerSilk, A. L., J. Agric. Food Chem. 25, 1246-9 (1977).

139. Jones, G., in "Vitamin D. Biochemical, Chemical, and Clinical Aspects Related to Calcium Metabolism," Norman, A. W.; Schaefer, K.; Coburn, J. W.; DeLuca, H. F.; Fraser, D.; Grigoleit, H. G.; Van Herrath, D. V. (eds.), De Gruyter, Berlin, 1977, pp. 491-500.

140. Gilbertson, T. J.; Stryd, R. P., Clin. Chem. 23, 1700-4 (1977).

141. Stryd, R. P.; Gilbertson, T. J., Clin. Chem. 24, 927-30 (1978).

142. Aksnes, L., Clin Chim. Acta 104, 133-46 (1980).

143. Koshy, K. T.; VanDerSlik, A. L., J. Agric. Food Chem. 28, 161-2 (1980).

144. Ray A. C.; Dwyer, J. N.; Reagor, J. C., J. Assoc. Off. Anal. Chem. 60, 1296-1301 (1977).

145. Jones, G., Clin Chem. 24, 287-98 (1978).

146. Matsuyama, N.; Okano, T.; Takada, K.; Takao, T.; Terao, Y., Hashimoto, N.; Kobayashi, T., J. Nutr. Sci. Vitaminol. 25, 469-78 (1979).

147. Dokoh, S.; Morita, R.; Fukunaga, M.; Yamamoto, I.; Miyaji, A.; Torizuka, K., in "Vitamin D. Basic Research and its Clinical Applications," Norman, A. W. et al. (eds.), De Gruyter, Berlin, 1979, pp. 239-42.

148. Koshy, K. T.; VanDerSlik, A. L., Anal. Biochem. 85, 283-6 (1978).

149. Nilsson, B.; Tejler, L.; Dymling, J.-F., in "Biological/Biomedical Applications of Liquid Chromatography II," Chromatogr. Sci. Ser., Vol. 12, Cazes, J. (ed.), Dekker, New York, 1979, pp. 349-58.

Downloaded At: 17:54 24 January 2011

150. Okano, T.; Mizuno, N.; Shida, S.; Takahashi, N.; Kobayashi, T.; Kuroda, E.; Kodama, S.; Matsuo, T., J. Nutr. Sci. Vitaminol. 27, 43-54 (1981).

151. Kuroda, E.; Okano, T.; Mizuno, N.; Kobayashi, T.; Kodama, S.; Ninomiya, M.; Morishita, Y.; Matsuo, T., J. Nutr. Sci. Vitaminol. 27, 55-65 (1981).

152. Takada, K.; Okano, T.; Tamura, Y., Matsui, S.; Kobayashi, T., J. Nutr. Sci. Vitaminol. 25, 385-98 (1980).

153. Kobayashi, T.; Okano, T.; Takada, K.; Matsuyama, N.; Takao, T.; Kuroda, E.; Kodama, S.; Matsuo, T., J. Pharmacobiodyn. 3, S-15, S-31 (1980).

154. Okano, T.; Matsuyama, N.; Kobayashi, T.; Kuroda, E.; Kodama, S.; Matsuo, T., J. Nutr. Sci. Vitaminol. 25, 479-94 (1979).

155. Redel, J.; Mawer, E. B.; Hann, J. T.; Bazely, N., FEBS Lett. 117, 134-6 (1980).

156. Clemens, T. L.; Fraher, L. J.; O'Riordan, J. L. H.; Little, C. J.; Dale, A., Chromatographia 13, 141-4 (1980).

157. Steuerle, H.; J. Chromatogr. 115, 447-53 (1975).

158. Rückemann, H.; Ranfft, K., Z. Lebensm.-Unters. Forsch. 164, 272-3 (1977).

159. Albertario, E.; Gnocchi, A., Cron. Chim. 55, 12-18, 32 (1978).

160. Hofsass, H.; Alicino, N. J.; Hirsch, A. L.; Ameika, L.; Smith, L. D., J. Assoc. Off. Anal. Chem. 61, 735-45 (1978).

161. Barnett, S. A.; Frick, L. W., Anal. Chem. 51, 641-5 (1979).

162. Vanhaelen-Fastré, R.; Vanhaelen, M., J. Chromatogr. 179, 131-42 (1979).

163. De Vries, E. J.; Zeeman, J.; Esser, R. J.; Borsje, B.; Mulder, F. J., J. Assoc. Off. Anal. Chem. 62, 129-35 (1979).

164. Mulder, F. J.; De Vries, E. J.; Borsje, B., J. Assoc. Off. Anal Chem. 62, 1031-40 (1979).

Downloaded At: 17:54 24 January 2011

165. Mulder, F. J., De Vries, E. J.; Borsje, B., J. Assoc Off. Anal. Chem. 64, 58-60 (1981).

166. De Vries, E. J.; Zeeman, J.; Esser, R. J. E., Borsje, B.; Mulder, F. J., J. Assoc. Off. Anal. Chem. 62, 1285-91 (1979).

167. Walker, G. A.; Carpenter, B. E.; Tueschler, D. L.; J. Pharm. Sci. 69, 846-9 (1980).

168. Lofty, P. A.; Jordi, H. C.; Bruno, J. V., J. Liquid Chromatogr. 4, 155-64 (1981).

169. De Vries, E. J.; Mulder, F. J.; Borsje, B., J. Assoc. Off. Anal. Chem. 64, 61-70 (1981).

170. Thompson, J. N.; Maxwell, W. B.; L'Abbé, M., J. Assoc. Off. Anal. Chem. 60, 998-1002 (1977).

171. Henderson, S. K.; Wickroski, A. F., J. Assoc. Off. Anal. Chem. 61, 1130-4 (1978).

172. Furuta, K.; Adachi, A.; Kobayashi, T., Bitamin 52, 409-15 (1978).

173. Adachi, A.; Kobayashi, T., J. Nutr. Sci. Vitaminol. 25, 67-78 (1979).

174. Koshy, K. T.; VanDerSlik, A. L., J. Agric. Food Chem. 27, 650-2 (1979).

175. Barnett, S. A.; Frick, L. W.; Baine, H. M., Anal. Chem. 52, 610-14 (1980).

176. Cohen, H.; Wakeford, B., J. Assoc. Off. Anal. Chem. 63, 1163-7 (1980).

177. Koshy, K. T.; VanDerSlik, A. L., J. Agric. Food Chem. 27, 180-3 (1979).

178. Iguchi, M.; Kikutani, N.; Tomomatsu, T.; Doguchi, M., Tokyo-Toritsu Eisei Kenkyusho Kenkyu Nempo 1980, 204-8.

179. Van Niekerk, P. J.; Smit, S. C., J. Amer. Oil Chem. Soc. 57, 417-21 (1980).

180. Knapstein, H.; Püchel, P., Landwirtsch. Forsch. 32, 41-6 (1979).

181. Cohen, H.; Lapointe, M., J. Agric. Food Chem. 26, 1210-13 (1978).

182. Cohen, H.; Lapointe, M., J. Chromatogr. Sci. 17, 510-13 (1979).

Downloaded At: 17:54 24 January 2011

183. Lein, D. G.; Campbell, H. M.; Cohen, H., J. Assoc. Off. Anal. Chem. 63, 1149-52 (1980).

184. Cohen, H.; Lapointe, M., J. Assoc. Off. Anal. Chem. 63, 1158-62 (1980).

185. Hesse, C.; Pietrizik, K.; Hotzel, D., Z. Klin. Chem. Klin. Biochem. 12, 193-7 (1974).

186. Gaetani, E.; Laureri, C. F., Farmaco, Ed. Prat. 29, 110-18 (1974).

187. Trefz, F. K.; Byrd, D. J.; Kochen, W., J. Chromatogr. 107, 181-9 (1979).

188. Matsunaga, T.; Nagoya Shiritsu Daigaku Igakkai Zasshi 26, 330-47 (1975).

189. Schwedt, G.; Bussemas, H. H.; Lippmann, C., J. Chromatogr. 143, 259-66 (1977).

190. De Vries, C. P.; Popp-Snijders, C.; De Kieviet, W.; Akkerman-Faber, A. C., J. Chromatogr. 143, 624-7 (1977).

191. Matsuzawa, T.; Kato, M.; Sekiguchi, M.; Ishiguro, I., Rinsho Kagaku 5, 239-42 (1977).

192 Ishii, D.; Hibi, K.; Asai, K.; Nagaya, M.; Mochizuki, K., Mochida, Y., J. Chromatogr. 156, 173-80 (1978).

193. Frey, F. J.; Frey, B. M.; Benet, L. Z., Clin. Chem. 25, 1944-7 (1979).

194. Saito, Z., Hihumi, S.; Mimo, N.; Hashiba, T.; Takeda, R., Proc. Symp. Chem. Physiol. Pathol. 19, 123-7 (1979).

195. De Vries, C. P.; Lomecky-Janousek, M.; Popp-Snijders, C., J. Chromatogr. 183, 87-91 (1980).

196. Saito, Z.; Hashiba, T.; Miyamoto, M.; Takeda, R., Nippon Naibumpi Gakkai Zasshi 53, 765-75 (1977).

197. Cavina, G.; Moretti, G.; Alimenti, R.; Gallinella, B., J. Chromatogr. 175, 125-40 (1979).

198. Schoneshofer, M.; Dulce, H. J., J. Chromatogr. 164, 17-28 (1979).

199. Ballerini, R.; Chinol, M., Ghelardoni, M., J. Chromatogr. 193, 413-20 (1980).

Downloaded At: 17:54 24 January 2011

200. O'Hare, M. J.; Nice, E. C.; Capp, M.; J. Chromatogr. 198, 23-39 (1980).

201. Garg, D. C.; Ayres, J. W.; Wagner, J. G., Res. Commun. Chem. Pathol. Pharmacol. 18, 137-46 (1977).

202. Gallant, S.; Bruckheimer, S. M.; Brownie, A. C., Anal. Biochem. 89, 196-202 (1978).

203. Scott, N. R.; Dixon, P. F., J. Chromatogr. 164, 29-34 (1979).

204. Canalis, E.; Caldarella, A.; Reardon, G. E., Clin. Chem. 25, 1700-3 (1979).

205. Reardon, G. E., Caldarella, A. M., Canalis, E., Clin. Chem. 25, 122-6 (1979).

206. Ryrfeldt, A.; Tonnesson, M.; Nilsson, E.; Wickby, A., J. Steroid Biochem. 10, 317-24 (1979).

207. Culbreth, P. H.; Sampson, E. J., J. Chromatogr. 212, 221-8 (1981).

208. Schöneshöfer, M.; Skobolo, R.; Dulce, H. J., J. Chromatogr. 222, 478-81 (1981).

209. Purdy, R. H.; Durocher, C. K.; Moore, P. H.,Jr.; Rao, P. N., in "Steroid Analysis by HLPC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 81-104.

210. Das Gupta, V.; Ghanekar, A. G., J. Pharm. Sci. 67, 889-91 (1978).

211. Van den Berg, J. H.; Mol, C. R.; Deelder, R. S.; Thijssen, J. H., Clin. Chim. Acta 78, 165-72 (1977).

212. Tymes, N. W., J. Chromatogr. Sci. 15, 151-5 (1977).

213. Chan, T. H., Moreland, M.; Hum, W. T.; Birmingham, M. K., J. Steroid Biochem. 8, 243-5 (1977).

214. Gordon, G.; Wood, P. R., Proc. Anal. Div. Chem. Soc. 14, 30-2 (1977).

215. Burgess, C., J. Chromatogr. 149, 233-40 (1978).

216. Lin, J.-T.; Heftmann, E., Hunter, I. R., J. Chromatogr. 190, 169-74 (1980).

217. Gotelli, G. R.; Wall, J. H.; Kabra, P. M.; Marton, L. J., Clin. Chem. 27, 441-3 (1981).

Downloaded At: 17:54 24 January 2011

218. Schöneshöfer, M., Fenner, A.; Altinok, G.; Dulce, H. J., Clin. Chem. Acta 106, 63-74 (1980).

219. Schöneshöfer, M.; Jaster, H. J.; Fenner, A., Fresenius' Z. Anal. Chem. 301, 130-1 (1980).

220. Schöneshöfer, M.; Maxeiner, J.; Fenner, A., J. Chromatogr. 224, 229-37 (1981).

221. Tylor, R. B.; Kendle, K. E., Acta Endocrinol. 88, 157-63 (1978).

222. Purdy, R. H.; Durocher, C. K.; Moore, P. H., Jr.; Rao, P. N., J. Steroid Biochem. 13, 1307-16 (1980).

223. Roots, I., Holbe, R.; Hövermann, W.; Nigam, S.; Heinemeyer, G,; Hildebrandt, A. G., Eur. J. Clin. Pharmacol. 16, 63-71 (1979).

224. Farhi, R. L.; Monder, C., Anal. Biochem. 90, 58-68 (1978).

225. De Vries, C. P.; De Jong, A. P. J. M., J. Steroid Biochem. 13, 387-94 (1980).

226. Smith, E., J. Assoc. Off. Anal. Chem. 62, 812-17 (1979).

227. Korpi, J.; Wittmer, D. P.; Sandmann, B. J.; Haney, W. G., J. Pharm. Sci. 65, 1087-9 (1976).

228. Munson, J. W.; Wilson, T. D., J. Pharm. Sci. 70, 177-81 (1981).

229. Lea, A. R.; Kennedy, J. M.; Low, G. K.-C., J. Chromatogr. 198, 41-7 (1980).

230. Gagne, D.; Lauriault, G.; Lebelle, M. J.; Lodge, B. A.; Wilson, W. L., Can. J. Pharm. Sci. 15, 12-14 (1980).

231. Van Dame, H. C., J. Assoc. Off. Anal. Chem. 63, 1184-8 (1980).

232. Smith, M. D., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 105-44.

233. Saito, A.; Amatsu, E.; Ono, T.; Hihumi, S.; Mimou, T.; Hashiba, T.; Sakato, S.; Miyamoto, M.; Takeda, R., Folia Endocrinol. Japon. 55, 1296-1306 (1979).

234. Cavina, G.; Moretti, G.; Gallinella, B.; Alimenti, R.; Barchiesi, R., Boll. Chim Farm. 117, 534-44 (1978).

Downloaded At: 17:54 24 January 2011

235. Amin, M.; Schneider, P. W., Analyst 103, 1076-9 (1978).

236. Baker, J. K.; Fifer, E. K., J. Pharm. Sci. 69, 590-2 (1980).

237. Loo, J. C. K.; Butterfield, A. G.; Moffatt, J.; Jordan, N., J. Chromatogr. 143, 275-80 (1977).

238. Loo, J. C. K.; Jordan, N., J. Chromatogr. 143, 314-16 (1977).

239. Wong, D.; Chung, P.; Lai, J., Tai-wan Yao Hsueh Tsa Chih 28, 11-15 (1977).

240. Müller, K. H.; Stuber, B., Pharm. Acta Helv. 53, 124 (1978).

241. Bunch, E. A., EDRO SARAP Res. Tech. Rep. 3, 117-18 (1978).

242. Frey, F. J.; Frey, B. M.; Benet, L. Z., Clin. Chem. 25, 1944-7 (1979).

243. Rose, J. Q.; Jusko, W. J., J. Chromatogr. 162, 273-80 (1979).

244. Scott, N. R.; Chakraborty, J.; Marks, V., Anal. Biochem. 108, 266-8 (1980).

245. Agabeyoglu, I. T.; Wagner, J. G.; Kay, D. R., Res. Commun. Chem. Pathol. Pharmacol. 28, 163-76 (1980).

246. Rocci, M. L., Jr., Jusko, W. J., J. Chromatogr. 224, 221-7 (1981).

247. Smith, M. D., J. Chromatogr. 164, 129-37 (1979).

248. Smith, M. D.; Hoffman, D. J., J. Chromatogr. 168, 163-9 (1979).

249. Smith, M. D., J. Pharm. Sci. 69, 960-4 (1980).

250. Butte, W., J. High Resolut. Chromatogr. Chromatogr. Commun. 2,74 (1979).

251. Abdou, H. M.; Ast, T. M.; Cioffi, F. J., J. Pharm. Sci. 67, 1397-9 (1978).

252. Ast, T. M.; Abdou, H. M., J. Pharm. Sci. 68, 421-3 (1979).

253. Tsuei, S. E.; Ashley, J. J.; Moore, R. G,; McBride, W. G., J. Chromatogr. 145, 213-20 (1978).

254. Belliardo, F.; Bertolino, A., J. Liquid Chromatogr. 4, 293-8 (1981).

255. Upton, L. M.; Townley, E. R.; Sancilio, F. D., J. Pharm. Sci. 67, 913-16 (1978).

256. Li Wan Po, A.; Irwin, W. J.; Yip, Y. W., J. Chromatogr. 176, 399-405, (1979).

Downloaded At: 17:54 24 January 2011

257. Peterson, M. C.; Nation, R. L.; Ashley, J. J., J. Chromatogr. 183, 131-9 (1980).

258. Kirschbaum, J.; Poet, R.; Bush, K.; Petrie, G., J. Chromatogr. 190, 481-5 (1980).

259. Higgins, J. W., Chromatogr. 115, 232-5 (1975).

260. Gordon, G.; Wood, P. R., Analyst 101, 876-82 (1976).

261. Neurath, G. B.; Ambrosius, D., J. Chromatogr. 163, 230-5 (1979).

262. Wickby, A.; Nilsson, L.; Hällsås, G., J. Chromatogr. 157, 51-64 (1978)

263. Wickby, A.; Thalén, A.; Oresten, G., J. Chromatogr. 157, 65-74 (1978).

264. Roth, G.; Wickby, A.; Nilsson, L.; Thalen, A., J. Pharm. Sci. 69, 766-70 (1980).

265. Robinson, W. T., Cosyns, L., Arzneim.-Forsch. 29, 882-5 (1979).

266. Dondi, F.; Betti, A.; Guerrieri, S.; Blo, G.; Marchi, M.; Guberti, A., Minerva Anestesiol. 46, 649-52 (1980).

267. Dekker, D.; Beijnen, J. H., J. Chromatogr. 193, 480-2 (1980).

268. Hansen, J.; Bundgaard, H., Arch. Pharm. Chem. 8, 91-9 (1980).

269. Lafosse, M.; Keravis, G.; Durand, M. H., J. Chromatogr. 118, 283-94 (1976).

270. Hunter, I. R.; Walden, M. K.; Heftmann, E., J. Chromatogr. 176, 485-7 (1979).

271. Lin, J.-T,; Heftmann, E., J. Chromatogr., in press.

272. Patricot, M. C; Revol, A., Bull. Trav. Soc. Pharm. Lyon 22, 31-6 (1978).

273. Cochran, R. C.; Darney, K. J., Jr.; Ewing, L. L.,J. Chromatogr. 173, 349-55 (1979).

274. Shaikh, B.; Hallmark, M. R.;Issaq, H. J.; Risser, N. H.; Kawalek, J. C., J. Liquid Chromatogr. 2, 943-56 (1979).

275. Van der Hoeven, T., J. Chromatogr. 196, 494-7 (1980).

276. Carignan, G.; Lodge, B. A.; Skakum, W., J. Pharm. Sci. 69, 1214-16 (1980).

277. Patthy, M.; Tomori, E., J. Chromatogr. 191, 145-54 (1980).

Downloaded At: 17:54 24 January 2011

278. Butterfield, A. G.; Lodge, B. A.; Pound, N. J.; Sears, R. W., J. Pharm. Sci. 64, 441-3 (1975).

279. Frischkorn, C. G. B.; Frischkorn, H. E., J. Chromatogr. 151, 331-8 (1978)

280. Frischkorn, C. G. B.; Dürbeck, H. W., Fresenius' Z. Anal. Chem. 290, 160-1 (1978).

281. Carignan, G.; Lodge, B. A., J. Chromatogr. 179, 184-6 (1979).

282. Loo, J. C. K.; Brien, R., J. Liquid Chromatogr. 4, 871-7 (1981).

283. Krzeminski, L. F.; Cox, B. L.; Dunn, G. H., III, J. Agric. Food Chem. 26, 891-3 (1978).

284. Stan, H.-J.; Hohls, F. W., Z. Lebensm.-Unters.-Forsch. 169, 266-270 (1979).

285. Holder, C. L.; Blakemore, W.M.; Bowman, M. C., J. Chromatogr. Sci. 17, 91-7 (1979).

286. Higgins, J. W., J. Chromatogr. 148, 335-42 (1978).

287. Carignan, G.; Lodge, B. A.; Skakum, W., J. Chromatogr. 206, 174-6 (1981).

288. Paanakker, J. E.; Van de Laar, G. L. M., J. Chromatogr. 183, 459-66 (1980).

289. Görög, S.; Herenyi, B., J. Chromatogr. 152, 240-2 (1978).

290. Huettemann, R. E.; Shroff, A. P., J. Chromatogr. Sci. 13, 357-60 (1975).

291. Schmidt, G. J., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 145-172.

292. Butterfield, A. G.; Lodge, B. A., Pound, N. J., J. Chromatogr. Sci. 11, 401-5 (1973).

293. Roos, R. W., J. Chromatogr. Sci. 14, 505-12 (1976).

294. Dolphin, R. J., J. Chromatogr. 83, 421-9 (1973).

295. Dolphin, R. J.; Pergande, P. J., J. Chromatogr. 143, 267-74 (1977).

296. Gotelli, G. R.; Wall, J. H.; Kabra, P. M.; Marton, L. J., Clin. Chem. 24, 2132-4 (1978).

Downloaded At: 17:54 24 January 2011

297. Roos, R. W., J. Assoc. Off. Anal. Chem. 63, 80-7 (1980).

298. Keravis, G.; Lafosse, M.; Durand, M. H., Chromatographia 10, 678-81 (1977).

299. Fukuchi, H.; Tsukiai, S.; Inoue, M., Yakuzaigaku 38, 102-8 (1978).

300. Capitano, G.; Tscherne, R., J. Pharm. Sci. 68, 311-14 (1979).

301. Mourey, T. H.; Siggia, S., Anal. Chem. 52, 881-5 (1980).

302. Lin, J.-T.; Heftmann, E., J. Chromatogr. 212, 239-44 (1981).

303. Schmidt, G. J.; Vandemark, F. L.; Slavin, W., Anal. Biochem. 91, 636-45 (1978).

304. Roos, R. W., J. Pharm. Sci. 67, 1735-9 (1978).

305. Roos, R. W.; Medwick, T., J.Chromatogr. Sci. 18, 626-30 (1980).

306. Taylor, J. T.; Knotts, J. G.; Schmidt, G. J., Clin. Chem. 26, 130-2 (1980).

307. Shihabi, Z. K.; Scaro, J.; Thomas, B. F., J. Chromatogr. 224, 99-104 (1981).

308. Hiroshima, O.; Ikenoya, S., Ohmae, M., Kawabe, K., Chem. Pharm. Bull. 28, 2512-14 (1980).

309. Frischkorn, C. G.; Smyth, M. R.; Frischkorn, H. E., Golimowski, J., Fresenius' Z. Anal. Chem. 300, 407-12 (1980).

310. Smyth, M. R.; Frischkorn, C. G., Fresenius' Z. Anal. Chem. 301, 220-3 (1980).

311. Shimada, K.; Tanaka, T.; Nambara, T., J. Chromatogr. 178, 350-4 (1979).

312. Shimada, K.; Tanaka, T.; Nambara, T., J. Chromatogr. 223, 33-9 (1981).

313. Van der Wal, S.; Huber, J. F. K., J. Chromatogr. 102, 353-74 (1974).

314. Van der Wal, S.; Huber, J. F. K., J. Chromatogr. 135, 305-21 (1977).

315. Van der Wal, S.; Huber, J. F. K., J. Chromatogr. 149, 431-53 (1978).

316. Musey, P. I.; Collins, D. C.; Preedy, J. R., Steroids 31, 583-92 (1978).

Downloaded At: 17:54 24 January 2011

317. Fransson, B.; Wahlund, K.-G.; Johansson, I. M.; Schill, G., J. Chromatogr. 125, 327-44 (1976).

318. Hermansson, J., J. Chromatogr. 152, 437-45 (1978).

319. Hermansson, J., J. Chromatogr. 194, 80-4 (1980).

320. Keravis, G.; Durand, M. H., Pathol. Biol. 28, 351-2 (1980).

321. Slikker, W., Jr.; Lipe, G. W.; Newport, G. D., J. Chromatogr. 224, 205-19 (1981).

322. Bagon, K. R.; Hammond, E. W., Analyst 103, 156-61 (1978).

323. Tsilifonis, D. C.; Chafetz, L., J. Pharm. Sci. 69, 1461-3 (1980).

324. Brooks, M. A., Dixon, R., J. Chromatogr. 182, 387-94 (1980).

325. Williams, M. C., Goldzieher, J. W., Steroids 36, 255-82 (1980).

326. Fitzpatrick, F. A., in "Advances in Chromatography," Vol. 16, Giddings, J. C.; Cazes, J.; Grushka, E.; Brown, P. R. (eds.), Dekker, New York, 1978, pp. 37-73.

327. Douglas, S. L., in "GLC and HPLC Determination of Therapeutic Agents," Chromatogr. Sci. Ser., Vol. 9. Pt. 2, Tsuji, K. (ed.), Dekker, New York, 1978, pp. 917-36.

328. O'Hare, M. J.; Nice, E. C., in "Steroid Analysis by HLPC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 277-322.

329. Jaglan, P. S.; Krzeminski, L. F., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 359-80.

330. Kautsky, M. P.; Thurman, G. W.; Hagerman, D. D., J. Chromatogr. 114, 473-5 (1975).

331. Kautsky, M. P.; Hagerman, D. D., in "Biological/Biomedical Applications of Liquid Chromatography," Chromatogr. Sci. Ser., Vol. 10, Hawk, G. L. (ed.), Dekker, New York, 1979, pp. 123-35.

332. Cochran, R. C., Ewing, L. L., J. Chromatogr. 173, 175-81 (1979).

333. Allenmark, S., Å:Son Berg, A.; Hammar, M.; Lindström, E., J. Chromatogr. 224, 399-405 (1981).

Downloaded At: 17:54 24 January 2011

334. Okumura, R., J. Liquid Chromatogr. 4, 1035-64 (1981).

335. Satyaswaroop, P. G., Lopez de la Osa, E.; Gurpide, E., Steroids 30, 139-45 (1977).

336. Helboe, P., Thomsen, M., Arch. Pharm. Chemi, Sci. Ed. 6, 397-402 (1978).

337. Swynnerton, N. F.; Fischer, J. B., J. Liquid Chromatogr. 3, 1195-1204 (1980).

338. Nambara, T., Bunseki 1980, 572-4.

339. Nambara, T,; Goto, J., Tokyo Tanabe Q. 31, 63-73 (1980).

340. Goto, J.; Nambara, T., Rinsho Kagaku 9, 126-33 (1980).

341. Elliott, W. H.; Shaw, R., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 1-40.

342. Shaw, R.; Elliott, W.H., Anal. Biochem. 74, 273-81 (1976).

343. Issaq, H. J.; Shaikh, B., Pontzer, N. J.; Barr, E. W., J. Liquid Chromatogr. 1, 133-49 (1978).

344. Okuyama, S.; Uemura, D.; Hirata, Y., Bull. Chem. Soc. Jpn. 52, 124-6 (1979).

345. Okuyama, S., Gastroenterol. Jpn. 14, 129-34 (1979).

346. Matthees, D. P.; Purdy, W. C., Anal. Chim. Acta 109, 161-4 (1979).

347. Okuyama, S.; Uemura, D.; Hirata, Y., Chem. Lett. 1979, 461-2.

348. Baker, R. W. R.; Ferrett, J.; Murphy, G. M., J. Chromatogr. 146, 137-42 (1978).

349. Shaw, R.; Elliott, W. H., Lipids 13, 971-5 (1978).

350. Goto, J.; Hasegawa, M.; Kato, H.; Nambara, T., Clin. Chim. Acta 87, 141-7 (1978).

351. Miyazaki, S.; Tanaka, H.; Horikawa, R.; Tsuchiya, H., Imai, K., J. Chromatogr. 181, 177-85 (1980).

352. Parris, N. A., Anal. Biochem. 100, 260-3 (1979).

353. Parris, N. A., J. Chromatogr. 133, 273-9 (1977).

Downloaded At: 17:54 24 January 2011

354. Sian, M. S.; Rains, A. J., Clin. Chim. Acta 98, 243-52 (1979).

355. Dupont, T.; Baylocq, D.; Guffroy, A.; Champault, G.; Beaugrand, M.; Hecht, Y.; Ferrier, J. P., Gastroenterol. Clin. Biol. 3, 349-54 (1979).

356. Guffroy, A.; Baylocq, S.; Pellerin, F.; Ferrier, J. P., C. R. Séances Acad. Sci. Sér. C. 290, 393-6 (1980).

357. Goto, J.; Kato, H.; Nambara, T., J. Liquid Chromatogr. 3, 645-55 (1980).

358. Kemula, W.; Kutner, W., J. Chromatogr. 204, 131-4 (1981).

359. Okuyama, S.; Kokubun, N.; Higashidate, S.; Uemura, D.; Hirata, Y., Chem. Lett. 1979, 1443-6.

360. Okuyama, S., Kanzo 20, 1194 (1979).

361. Arisue, K.; Ogawa, Z,; Hosotsubo, H.; Furukawa, I.; Koda, K.; Hayashi, C.; Miyai, K.; Ishida, Y., Proc. Symp. Chem. Physiol. Pathol. 19, 133-7 (1979).

362. Arisue, K.; Ogawa, Z.; Koda, K.; Hayashi, C.; Ishida, Y., Rinsho Kagaku 9, 104-10 (1980).

363. Shaw, R.; Rivetna, M.; Elliott, W. H., J. Chromatogr. 202, 347-61 (1980).

364. Gligorijević, M. L.; Kosanić, M. M.; Prekajać, J. S., Glas. Hem. Drus. Beograd 45, 523-9 (1980).

365. Goto, J.; Kato, H.; Nambara, T., Lipids 13, 908-9 (1978).

366. Goto, J.; Kato, H.; Nambara, T.; Kimura, H.; Sato, T., Proc. Symp. Chim. Physiol. Pathol. 19, 138-42 (1979).

367. Bloch, C. A.; Watkins, J. B., J. Lipid Res. 19, 510-13 (1978).

368. Laatikainen, T.; Lehtonen, P.; Hesso, A., Clin. Chim. Acta 85, 145-50 (1978).

369. Shimada, K.; Hasegawa, M.; Goto,J.; Nambara, T., J. Chromatogr. 152, 431-6 (1978).

370. Shaw, R., Smith, J. A.; Elliott, W. H., Anal. Biochem. 86 450-6 (1978).

Downloaded At: 17:54 24 January 2011

371. Baylocq, D.; Guffroy, A.; Pellerin, F.; Ferrier, J. P., C. R. Séances Acad. Sci. Sér. C 286, 71-3 (1978).

372. Bayloq, D.; Guffroy, A., Pellerin, F.; Ferrier, J. P., Feuill. Biol. 106, 151-3 (1979).

373. Nambara, T.; Goto, J.; Hasegawa, M.; Kato, H., in "Biological/ Biomedical Applications of Liquid Chromatography II, Chromatogr. Sci. Ser., Vol. 12, Hawk, G. L. (ed.), Dekker, New York, 1979, pp. 359-74.

374. Goto, J.; Kato, H.; Saruta, Y.; Nambara, T., J. Liquid Chromatogr. 3, 991-1003 (1980).

375. Maruyama, K.; Tanimura, H.; Hikasa, Y., Clin. Chim. Acta 100, 47-54 (1980).

376. Mingrone, G.; Greco, A. V.; Passi, S., J. Chromatogr. 183, 277-86 (1980).

377. Nakayama, F.; Nakagaki, M., J. Chromatogr. 183, 287-93 (1980).

378. Uenoyama, R.; Suminoe, K.; Takeda, H.; Hasegawa, S.; Baba, S.; Kameno, Y., Proc. Symp. Chem. Physiol Pathol. 19, 128-32 (1979).

379. Baba, S.; Uenoyama, R.; Suminoe, K.; Takeda, R.; Hasegawa, S.; Kameno, Y., Kobe J. Med. Sci. 26, 89-100 (1980).

380. Takeda, F.; Suminoe, K.; Hasegawa, O.; Uenoyama, R.; Baba, S.; Kameno, Y., Kanzo 21, 1406 (1980).

381. Seiber, J. N.; Nelson, C. J.; Benson, J. M., in "Steroid Analysis by HPLC. Recent Applications," Kautsky, M. P. (ed.), Dekker, New York, 1981, pp. 41-80.

382. Lindner, W.; Frei, R. W., J. Chromatogr. 117, 81-6 (1976).

383. Nachtmann, F.; Spitzy, H.; Frei, R. W., Anal. Chem. 48, 1576-9 (1976).

384. Nachtmann, R.; Spitzy, H.; Frei, R. W., J. Chromatogr. 122, 293-303 (1976).

Downloaded At: 17:54 24 January 2011

385. Gfeller, J. C.; Frey, G.; Frei, R. W., J. Chromatogr. 142, 271-81 (1977).

386. Evans, F. J., J. Chromatogr. 88, 411-12 (1974).

387. Cobb, P. H., Analyst 101, 768-76 (1976).

388. Tokunaga, H.; Kimura, T.; Kawamura, J., Iyakuhin Kenkyu 7, 10-16 (1976).

389. Erni, R.; Frei, R. W., J. Chromatogr. 130, 169-80 (1977).

390. Castle, M. C., J. Chromatogr. 115, 437-45 (1975).

391. Benson, J. M.; Seiber, J. N., J. Chromatogr. 148, 521-7 (1978).

392. Davydov, V. Ya.; Kiselev, A. V.; Mironova, I. V.; Sapoinikov, Y. M., Chromatographia 11, 591-5 (1978).

393. Fujii, Y.; Fukuda, H.; Saito, Y.; Yamazaki, M., J. Chromatogr. 202, 139-43 (1980).

394. Davydov, V. Ya.; Gonzalez, M. E.; Kiselev, A. V., J. Chromatogr. 204, 293-301 (1981).

395. Harkiss, K. J.; Linley, P. A.; Mesbah, M. K., J. High Resolut. Chromatogr. Chromatogr. Commun. 4, 37-8 (1981).

396. Gault, M. H.; Ahmed, M.; Tibbo, N.; Longerich, L.; Sugden, D., J. Chromatogr. 182, 465-72 (1980).

397. Gault, H.; Kalra, J.; Ahmed, M.; Kepkay, D.; Longerich, L.; Barrowman, J., Clin. Pharmacol. Ther. 29, 181-90 (1981).

398. Eriksson, B.-M.; Tekenbergs, L.; Magnusson, J.-O.; Molin, L., J. Chromatogr. 223, 401-8 (1981).

399. Nelson, H. A.; Lucas, S. V.; Gibson, T. P., J. Chromatogr. 163, 169-77 (1979).

400. Fletcher, S. M.; Lawson, G.; Law, B.; Moffat, A. C., J. Forensic Sci. Soc. 20, 203-9 (1980).

401. Morais, J. A.; Zlotecki, R. A.; Sakmar, E.; Stetson, P. L., Wagner, J. G., Res. Commun. Chem. Pathol. Pharmacol. 31, 285-98 (1981).

402. Loo, J. C. K.; McGilveray, I. J.; Jordan, N., J. Liquid Chromatogr. 4, 879-86 (1981).

Downloaded At: 17:54 24 January 2011

403. Shimada, K.; Hasegawa, M.; Hasebe, K.; Fujii, Y.; Nambara, T., J. Chromatogr. 124, 79-87 (1976).

404. Shimada, K.; Hasegawa, M.; Hasebe, K.; Fujii, Y.; Nambara, T., Chem. Pharm. Bull. 24, 2995-3000 (1976).

405. Verpoorte, R.; Phan-quốc-Kinh; Svendsen, A. B., J. Nat. Prod. 43, 347-52 (1980).

406. Tittel, G.; Wagner, H., Planta Med. 39, 125-34 (1980).

407. Hashimoto, M., Kagaku no Ryoiki, Zokan 109, 127-35 (1976).

408. Lafont, R.; Sommé-Martin, G.; Mauchamp, B.; Maume, B. F.; Delbecque, J. P., in "Progress in Ecdysone Research," Developments in Endocrinology, Vol. 7, Hoffmann, J. A. (ed.), Elsevier, Amsterdam, 1980, pp. 45-68.

409. Hori, M., Steroids 14, 33-46 (1969).

410. Nigg, H. N.; Thompson, M. J.; Kaplanis, J. N.; Svoboda, J. A., Robbins, W. E., Steroids 23, 507-16 (1974).

411. Gilgan, M. W., J. Chromatogr. 129, 447-50 (1976).

412. Lafont, R.; Martin-Sommé, G.; Chambet, J.-C., J. Chromatogr. 170, 185-94 (1979).

413. Dinan, L. N.; Donnahey, P. L.; Rees, H. H.; Goodwin, T. W., J. Chromatogr. 205, 139-45 (1981).

414. Ogawa, S.; Yoshida, A.; Kato, R., Chem. Pharm. Bull. 25, 904-8 (1977).

415. O'Neill, M. P.; Holman, G. M.; Wright, J. E., J. Insect Physiol. 23, 1243-4 (1977).

416. Holman, G. M.; Meola, R. W., Insect Biochem. 8, 275-8 (1978).

417. Lafont, R.; Beydon, P.; Sommé-Martin, G.; Blais, C., Steroids 36, 185-208 (1980).

418. Hunter, I. R.; Walden, M. K.; Bailey, G. F.; Heftmann, E., J. Nat. Prod. 44, 245-51 (1981).

419. Higgins, J. W., J. Chromatogr. 121, 329-34 (1976).

420. Anon., Fen Hsi Hua Hsueh 6, 103-6 (1978); C. A. 92, 135493.

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421. Mahato, S. B.; Sahu, N. P.; Roy, S. K., J. Chromatogr. 206, 169-73 (1981).

422. Hunter, I. R.; Walden, M. K.; Heftmann, E., J. Chromatogr. 198, 363-6 (1980).

423. Crabbe, P. G.; Fryer, C., J. Chromatogr. 187, 87-100 (1980).

424. Nes, W. D.; Heftmann, E.; Hunter, I. R.; Walden, M. K., J. Liquid Chromatogr. 3, 1687-96 (1980).

425. Bushway, R. J.; Barden, E. S.; Bushway, A. W.; Bushway, A. A., J. Chromatogr. 178, 533-41 (1979).

426. Bushway, R.J.; Barden, E. S.; Wilson, A. M.; Bushway, A. A., J. Food Sci. 45, 1088-9 (1980).

427. Hunter, I. R.; Walden, M. K.; Heftmann, E.; Glotter, E.; Kirson, I., J. Chromatogr. 170, 437-42 (1979).

428. Gustafson, M. E.; Nicholas, A. W.; Rosazza, J. P., J. Chromatogr. 137, 465-7 (1977).

429. Tishbee, A.; Kirson, I., J. Chromatogr. 195, 425-30 (1980).

430. Sen, G.; Mulchandani, N. B.; Patankar, A. V., J.Chromatogr. 198, 203-6 (1980).

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