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The identification of related substances in 9α-fluoroprednisolone-21 acetate by means of high-performance liquid chromatography with diode array detector and mass spectrometry

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Abstract: A method for the analysis and identification of the principal related substances in 9α-fluoroprednisolone acetate is described. This compound has been chosen as a model for the investigation of related substances which can be originated in the general procedure for introducing a fluorine substituent at position 9 of a corticosteroid molecule. HPLC procedures, both in reversed and in normal phase were used; a rapid scanning UV detector which allows direct spectrophotometric data to be obtained on chromatographic peaks, proved to be a tool of great importance. Thus, after reversed-phase chromatographic separation and observation of the UV spectra and their respective second derivatives, it was possible to characterize some of the principal effective and potential related substances such as 9α-fluorohydrocortisone acetate, 9α-bromoprednisolone acetate, 9β, 11β-epoxyprednisolone acetate and 9(11)-dehydro-prednisolone acetate, emerging as chromatographic peaks. Identification of 9α-bromoprednisolone acetate and of 9α-fluorohydrocortisone acetate which proved to be the most significant impurities, was confirmed by means of an exhaustive study of the mass spectra of these substances conveniently isolated by normal-phase HPLC. The chromatographic, spectrophotometric and mass-spectrometric characteristics of the studied compounds are reported.

Keywords: *9α-Fluoroprednisolone acetate; related substances; HPLC; diode array detector; UV spectra; second derivative; mass spectrometry.*

Introduction

9α-Fluorohydrocortisone acetate (**I**) and the analogous compound 9α-fluoroprednisolone acetate (**II**) were synthesized by Fried and Sabo [1] and by Hirschmann *et al.* [2], respectively, in the context of a series of fruitful studies of the structure and physiological activity of steroid compounds derived from hydrocortisone acetate. This research resulted in the preparation of compounds such as (**II**) which are up to 25 times as active as the hydrocortisone acetate in the hepatic glycogen test in the rat and in the test of the systemic inhibition of the rat granuloma (glycocortical and anti-inflammatory activity). In the substitution of the 9α-hydrogen atom it was observed that the increase in activity was inversely proportional to the dimensions of the substituent halogen atom. Despite the successive verification of the disadvantage of increased sodium retention in these compounds [3], their value remains intact, not only for topical uses but also, indirectly, as a model for an important and positive modification of the steroid molecule. This model is exploited in other similarly modified steroid structures (e.g. triamcinolone, dexamethasone and betamethasone) which have largely maintained the advantages and eliminated the disadvantages. Therefore, it was of interest to study the impurities which are likely to accompany 9α-fluoroprednisolone acetate (and the analogous compound not acetylated at position 21). Certain synthetic intermediates of this steroid (and the analogous compound without the double bond at 1,2) are common to the synthesis of other similar corticosteroids which are derived from them by methylation or hydroxylation at position 16, forming other important classes of corticosteroid derivatives.

Consequently, an understanding of the pattern of formation of the potential impurities of 9α-fluoroprednisolone would be useful in

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the identification of similar patterns for the other, above-mentioned classes of corticosteroids.

Experimental

Materials

Samples of 9α-fluoroprednisolone and of the 21-acetate (samples 0450S for the former and 01212M and V-68-S for the latter) were examined. The liquid chromatographic solvents used were: acetonitrile for HPLC (Merck), methanol for HPLC (Carlo Erba), chloroform for HPLC (Carlo Erba, stabilized with amylene), tetrahydrofuran for HPLC (Merck) and water which had previously been purified by passage through a Millipore 'Milli-Q' apparatus. All mobile phases were filtered through 0.45 μm pore filters.

Chromatographic procedures

Equipment. HPLC analyses were performed using: (1) a liquid chromatograph composed of a programmer (Waters Automated Gradient Controller equipped with two Waters Pumps, model M-6000A and M-45), a Hewlett–Packard 1040A (DAD spectrophotometer) equipped with an HP-9000-300 computer, a colour monitor, an HP-9153 disk drive, an HP-Think Jet printer, an HP-Color Pro Plotter and a Rheodyne 7125 (20 μl loop) injection valve for the reversed-phase trials; and (2) a liquid chromatograph composed of a Perkin–Elmer Series 410 Four-Solvent Pump System, a Perkin–Elmer LC-95 variable wavelength detector, a Hewlett–Packard 3390-A recorder–integrator and a Rheodyne 7125 (20 μl loop) injection valve for normal phase trials and preparative chromatography. The mass spectra were obtained with a Hewlett–Packard HP-5988A mass spectrometer equipped with an HP-59970C MS ChemStation.

Chromatographic conditions. For the reversed-phase chromatography a Spherisorb ODS 5 μm column (250 × 4.6 mm i.d.) was used. The mobile phases for the isocratic elution were: (a) tetrahydrofuran–water (35:65, v/v); and (b) acetonitrile–water (36:64, v/v). All measurements were taken at room temperature (25°C), the flow rate was 1 ml min^{-1} and the injection volume was 20 μl (fixed loop) for all trials; the monitoring wavelength used with the DAD was 240 nm; working with the DAD, the UV spectra recorded in correspondence with the various peaks ranged from 210 to 410 nm.

For the normal-phase chromatography a Lichrosorb Si-100, 5 μm column (250 × 4.6 mm i.d.) was used. The mobile phase for isocratic elution was: chloroform (stabilized with amylene)–methanol–water (978.6:20:1.4, v/v/v). All measurements were taken at room temperature (25°C), the flow rate was 1 ml min^{-1}, the wavelength of the detector was 254 nm. The injection volume was 20 μl (fixed loop) for all trials.

Preparation of sample solutions.

(1) *Quantitative evaluation of related substances using reversed- and normal-phase HPLC.* This evaluation was performed using 50-μg samples and following the procedure described in one of our previous reports [4]. The methanolic solution was injected as such in the reversed-phase chromatography. For the normal-phase HPLC, 5 ml of methanolic solution were evaporated to dryness and then dissolved in 5 ml of mobile phase.

(2) *Normal- and reversed-phase preparative chromatography.* Both normal- and reversed-phase techniques were used in preparative chromatography for the isolation of the principal impurities in microgram quantities to be used for the mass spectrometric identification. In the case of normal-phase chromatography the principal impurity peak was observed to have an r.r.t. of 0.72, i.e. preceding the peak of 9α-fluoroprednisolone acetate. This characteristic allowed maximal reduction of interference due to residual traces of the main compound. The chromatographic column and conditions employed were those described in the paragraph headed 'Chromatographic conditions' for analytical chromatography. Ten injections of 250 μg/20 μl of sample material (1.25% solution) were performed in order to obtain a total of approximately 23 μg of the substance corresponding to the peak at r.r.t. 0.72. The residue was evaporated to dryness at room temperature and then dissolved in 20 μl of anhydrous ethanol immediately prior to mass spectrometric analysis. Reversed-phase preparative chromatography, using eluent (b) (acetonitrile–water 36:64, v/v) was used: firstly, as a technique to verify the composition of and to separate the two components present in the above-mentioned fraction (r.r.t. 0.72) which was obtained using direct-phase chro-

Table 1
Relative retention times of the peaks obtained in the liquid chromatographic analysis of sample 01212M with the eluents (a) and (b). For comparison, in the right side of the table, r.r.t. of the reference compounds are reported

Related substance No.	r.r.t. in eluent (a) (THF–water)	Per cent content	Related substance No.	r.r.t. in eluent (b) (ACN–water)	Per cent content	Reference compound	r.r.t. in eluent (a) (THF–water)	r.r.t. in eluent (b) (ACN–water)
1	0.49	0.21	1	0.37	0.13	9α-Fluoroprednisolone	0.48	0.40
2	0.75	0.02	2	0.67	0.02	17α,21-Dihydroxy-pregna-1,4,(9–11)-triene-3,20-dione	0.45	0.61
3	0.89	0.27	3	0.87	0.15	9β,11β-Epoxy-1,4-pregnadiene-17, 21-diol-3,20-dione	0.45	0.48
Main	1.00	—	Main	1.00	—	9α-Fluorohydrocortisone	0.53	0.49
4	1.10	0.05	5	1.12	0.30	9α-Fluorohydrocortisone acetate	1.16	1.12
5	1.16	0.37	4	1.22	0.04	9α-Bromoprednisolone acetate	1.28	1.34
6	1.24	0.58	6	1.35	0.60	9β,11β-Epoxy-1,4-pregnadiene-17α, 21-diol-3,20-dione,21-acetate	0.96	1.47
7	1.63	(Traces)	7	1.57	0.07			
8	1.80	0.04	8	2.04	0.04	17α,21-Dihydroxy-pregna-1,4,(9–11)-triene3,20 dione-21 acetate	1.13	2.04
		∑1.54%			∑1.35%	Prednisolone acetate	0.89	1.03

matography; and secondly, as a technique of primary separation of the two principal impurities (Nos 4 and 5) following the protocol described in 'Chromatographic conditions' in (c) for analytical chromatography. In this second case injections of 200–250 μg/20 μl of sample were performed and the two fractions corresponding to impurities Nos 5 and 6 were collected separately. These eluates were then extracted 3 times with a half volume of dichloromethane. The extracts obtained were evaporated to dryness and the residues dissolved in methanol for the verification of chromatographic purity before mass spectrometric analyses. Unfortunately, although this second technique is theoretically more selective, it produced fractions containing appreciable quantities not only of the principal compound but also of an unknown compound which appeared to be a degradation product of impurity No. 6 due to the extraction and consequent concentration process. Consequently, as further illustrated in the description of the mass spectrometry trials, the technique utilized was that by normal-phase chromatography.

(3) *Identification of related substances using HPLC.* Methanol solutions (1 mg ml^{-1}) were prepared with the reference substances of the compounds listed in Table 1 (possible impurities). Methanol dilutions (1:20, v/v) of these solutions were injected individually in order to study their chromatographic and spectrophotometric behaviour. To confirm the presence of such impurities in 9α-fluoroprednisolone acetate, sample No. 01212M, solutions of this sample were prepared (50 μg/20 μl) and 0.5 μg (1.0%) of the possible impurities were added individually to each sample solution; the increase in the corresponding peaks was observed.

Results and Discussion

Detection and quantitative evaluation of related substances

Normal-phase chromatography. The analyses of 9α-fluoroprednisolone acetate, sample No. 01212M, showed six peaks corresponding to impurities with r.r.t. and per cent of total content (in brackets) as follows: 0.45 (0.16%); 0.50 (0.22%); 0.65 (0.16%); 0.72 (0.95%) (principal impurity); 1.27 (0.23%); and 1.37 (0.07%). The total impurity content was 1.79%. The profile of the sample is shown in Fig. 1.

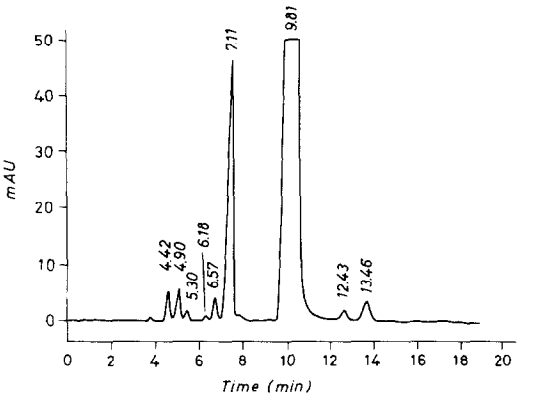

Figure 1
Normal-phase chromatography of sample 01212M, 50 μg. Column: Lichrosorb Si-100, 5 μm, 250 × 4.6 mm i.d. Mobile phase: chloroform (stabilized with amylene)–methanol–water, 978.6:20:1.4 (v/v/v). Flow rate: 1 ml min^{-1}. Detector wavelength: 254 nm. Temperature: 25°C approx.

Reversed-phase chromatography. Results of analyses of sample No. 01212M with eluents (a) and (b) are reported in Table 1; Figs 2 and 3 show the respective chromatographic profiles. The profiles of sample No. V.68-S are analogous qualitatively and quantitatively to those described for sample No. 01212M.

Identification of the impurities

The procedures described in the literature for the synthesis of 9α-fluoroprednisolone acetate are those generally described for the introduction of a halogen at position 9 of the corticosteroid structure as according to refs 5–8. Consequently 17α,21-dihydroxy pregna-1,4,(9–11)-triene-3,20-dione-21 acetate (9(11)dehydroprednisolone acetate), 9α-bromoprednisolone acetate and 9β,11β-epoxyprednisolone acetate are possible impurities derived from the synthesis of 9α-fluoroprednisolone acetate and as such should be studied and identified. One must also consider prednisolone acetate and 9α-fluorohydrocortisone acetate since, in some synthetic procedures, the introduction of the double bond at position 1 could be a final step or the prednisolone acetate could be contaminated by hydrocortisone acetate from its synthesis. To be prudent, one should also consider the two free steroids 9α-fluoroprednisolone and 9α-fluorohydrocortisone.

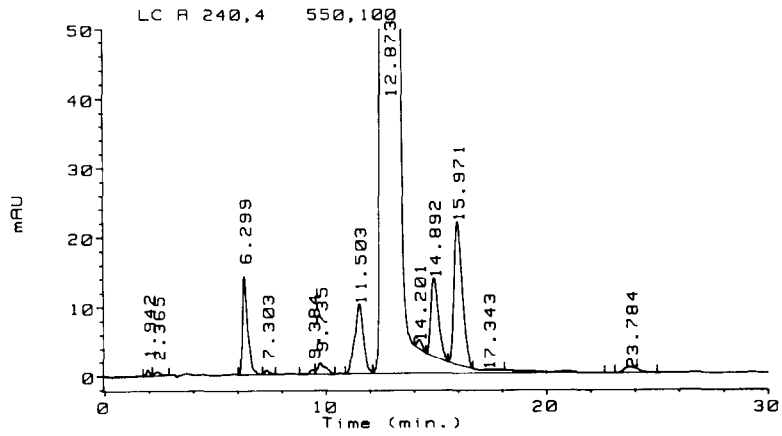

Figure 2
Reversed-phase chromatography of sample 01212M, 50 μg. Column: Spherisorb ODS-2, 5 μm, 250 × 4.6 mm i.d. Mobile phase: tetrahydrofuran–water, 35:65 (v/v). Flow rate: 1 ml min^{-1}. Detector wavelength: 240 nm. Temperature: 25°C approx.

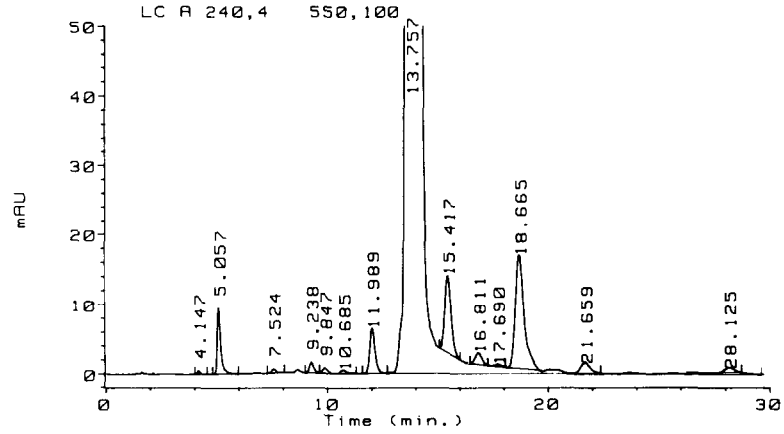

Figure 3
Reversed-phase chromatography of sample 01212M, 50 μg. Column: Spherisorb ODS-2, 5 μm, 250 × 4.6 mm i.d. Mobile phase: acetonitrile–water, 36:64 (v/v). Flow rate: 1 ml min^{-1}. Detector wavelength: 240 nm. Temperature: 25°C approx.

Following our investigation scheme, the principal impurities observed in the high resolution chromatograms of sample 01212M of 9α-fluoroprednisolone acetate were studied using the diode array detector. Using normal-phase chromatography a single, fairly evident impurity peak was observed as were five other less significant peaks. The more evident peak (r.r.t. 0.72, 0.95%) was collected and re-examined by reversed-phase chromatography using mobile phases (a) and (b). This peak was demonstrated to consist of the two principal impurities (Nos 5 and 6) using eluent (a) or (b) with practically no other contaminant. The quantitative analyses are coherent with the data from the reversed-phase chromatography.

In reversed-phase chromatography good results were obtained using mobile phases (a) tetrahydrofuran–water and (b) acetonitrile–water. Using these mobile phases the verification of the r.r.t. of the reference compounds allowed a comparison with the r.r.t. of the principal impurities observed, thereby supplying a preliminary indication of the identities of the latter (data shown in Table 1). The elution sequence is approximately parallel for the compounds which elute with retention times less than that of the principal peak (impurities Nos 1–3), while this sequence changes for the pair of compounds which immediately follow the principal peak. The succession of impurities 4–6 in tetrahydrofuran–water [mobile

phase (a)] shows r.r.t. 1.10, 1.16 and 1.24, while in acetonitrile–water [mobile phase (b)] this succession differs in that the peak No. 5 in mobile phase (a) here becomes the fourth, and the succession presents r.r.t. 1.12, 1.22, 1.35 with a generally improved resolution. In any case, the peak No. 6 represents the principal impurity (approximately 0.6% content); the second impurity by per cent content (approximately 0.3%) is better separated from the principal impurity in this acetonitrile–water eluent.

From the data reported in Table 1 we see that 9α-fluorohydrocortisone acetate shows r.r.t. approximately 1.16 and 1.12, respectively in the two mobile phase systems (a) tetrahydrofuran–water and (b) acetonitrile–water. These values are closely proximate to those shown for impurity No. 5 in the two mobile phase systems. Furthermore, we see that 9α-bromoprednisolone acetate shows r.r.t. 1.28 and 1.34, respectively in mobile phases (a) and (b). These values are very similar to those shown for impurity No. 6 (r.r.t. 1.24 and 1.35). Therefore, for the two principal impurities of the examined sample, the coincidence of their r.r.t. with those of the reference compounds is verified.

Another element of identification is the examination of the UV spectra obtained at the selected peaks using the diode array detector and, in particular, of the second derivatives of the spectra which are more clearly distinctive about the nature of the compounds, as demonstrated in two of our previous reports [9, 10].

Figure 4 shows the spectra taken at the top of the peak and the respective second derivatives for impurity peaks Nos 5 and 6 using mobile phase (b). The difference between the second derivatives is evident: that for No. 5 has a single minimum (hydrocortisone type, according to our previous definition [10]); and that for No. 6 has also a very evident second minimum, (prednisolone type profile, according to our previous definition [10]). In fact, as we have elsewhere demonstrated [10] and summarized here (Table 2), the presence of the bromine substituent at position 9 produces a consistent bathochromic effect which is evident not only at the principal maximum but also at the secondary one at 277.5 nm. This spectrum corresponds completely to the one previously observed by us for 9α-bromo desonide [10]. Likewise characteristic are the spectra of the 9β, 11β-epoxides which present a single

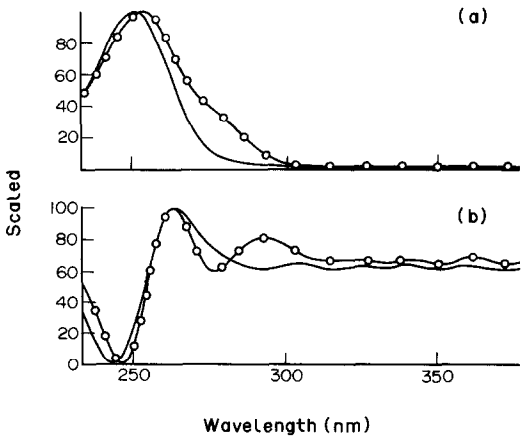

Figure 4
(a) UV spectrum of the related substance 9α-fluorohydrocortisone acetate taken at the apex of the peak obtained with the eluent (b) (continuous line) and of the related substance 9α-bromoprednisolone acetate, taken in the same chromatogram (circled line). (b) Second derivative profiles of the above described spectra.

minimum at 250 nm in their second derivative of the spectra. Since this type of spectrum was observed in none of the peaks considered, the presence of these compounds can be excluded.

Regarding the hypothesis of the presence of other impurities one can observe that 9α-fluoroprednisolone shows a r.r.t. similar to those of the impurity corresponding to peak No. 1 in both mobile phases. In mobile phase (a) this impurity does not separate from 17α,21 dihydroxy-pregna-1,4,(9–11)triene-3,20 dione.

Since this latter compound, in mobile phase (b), separates from free 9α-fluoroprednisolone (r.r.t. 0.61 and 0.41 respectively) impurity No. 1 can be considered as prevalently due to the free 9α-fluoroprednisolone and not to a mixture of this with the triene. Free triene (non-acetylated at position 21) with r.r.t. 0.61 in system (c) does not appear to be an impurity present in appreciable quantities in the examined sample. Regarding the sensitivity limits of the procedures to obtain spectra on the chromatographic peaks we refer to the preceding demonstrations [10].

The isolation of certain peaks using preparative chromatography and the successive mass spectrometric examination allowed confirmation of the identity of some components demonstrated to be impurities of this sample of 9α-fluoroprednisolone acetate.

The difficulties in obtaining the compounds corresponding to the two peaks No. 6 (principal impurity) and No. 4 (secondary impurity), which were detected in the sample using

Table 2
Spectrophotometric properties of 9α-fluoroprednisolone acetate and analogues

Compound	Substituent in 9	Wavelength of the absorption maximum in the UV spectrum (nm)	Minima in the second derivative of the absorption spectrum (nm)	Type of the second derivative profile
9α-Bromoprednisolone acetate	Br	245.5	245.5 ; 277.5	Prednisolone type
9α-Bromodesonide	Br	245.5	245.5 ; 277.5	Prednisolone type
Beclomethasone dipropionate	Cl	239.5	239.5 ; 269.5	Prednisolone type
9α-Fluoroprednisolone	F	241.5	239.5 ; 267.5	Prednisolone type
9α-Fluoroprednisolone acetate	F	241.5	239.5 ; 269.5	Prednisolone type
Betamethasone dipropionate	F	239.5	239.5 ; 267.5	Prednisolone type
17α,21-Dihydroxy pregna-1,4,(9–11)-triene-3,20-dione	None	241.5	241.5 ; 271.5	Prednisolone type
17α,21-Dihydroxy-pregna-1,4,(9–11)-triene-3,20-dione-21 acetate	None	241.5	241.5 ; 271.5	Prednisolone type
9β,11β-Epoxy-1,4-pregnadiene-17α,21-diol-3,20-dione	None	251.5	249.5 ; —	Hydrocortisone type
9β,11β-Epoxy-1,4-pregnadiene-17α,21-diol-3,20-dione,21 acetate	None	251.5	249.5 ; —	Hydrocortisone type
16β-Methyl-9β,11β-epoxy-1,4-pregnadiene-17α,21 diol-3,20-dione	None	251.5	251.5 ; —	Hydrocortisone type
16β-Methyl-9β,11β-epoxy-1,4-pregnadiene-17α,21 diol-3,20-dione	None	251.5	251.5 ; —	Hydrocortisone type
16β-Methyl-17α,21-dihydroxy-pregna-1, 4,9 (11) triene-3,20 dione	None	241.5	241.5 ; 271.5	Prednisolone type
16α-Methyl-17α,21-dihydroxy-pregna-1, 4,9 (11) triene-3,20 dione	None	241.5	241.5 ; 271.5	Prednisolone type

mobile phase (c) (see 'Chromatographic conditions', singularly and in a conveniently purified form, prompted us to try to examine the fraction obtained by direct-phase preparative chromatography. This fraction contains a mixture of these two impurities which is practically free from the principal peak and other components (see 'Chromatographic conditions'). In order to confirm these two impurities by mass spectrometry (MS), it was necessary to study in preliminary experiments the spectra of the compounds to be identified. These compounds were: 9α-fluorohydrocortisone acetate (**1**), 9α-fluoroprednisolone (**2**) and its acetate (**3**) and 9α-bromoprednisolone acetate (**4**).

The spectra for the first three compounds were obtained using the DIP (direct introduction probe) technique, electron impact (EI) ionisation at 70 eV and heating from room temperature to 250°C at a velocity of 45°C min^{-1}. The spectrum of compound **3** is reported in Fig. 5. An analysis of the data demonstrates a parallel behaviour for the acetates with a loss of: acetic acid at C_{21} (−60), fluoridric acid (−20), the entire lateral chain at C_{17} (−101) and, from the resultant masses, for further H_2O and HF, —HCO (−29) and $—CH_2CHO$ (−43) losses. The final resultant masses *m/z* are 362, 321, 303, 292, 283, 265, 225, 161 and 123, and the molecular ion 422 for 9α-fluorohydrocortisone acetate; similarly the resultant masses *m/z* are 400, 360, 319, 301, 299, 281, 223, 147 and 121, and the molecular ion 420 for 9α-fluoroprednisolone acetate. All these data are in agreement with the spectrum described by Florey for 9α-fluorohydrocortisone acetate [11] and with the general behaviour of MS of corticosteroids, as described by Genard *et al.* [12] and by Compernolle *et al.* [13].

The spectra registered for 9α-bromoprednisolone acetate using the DIP technique did not allow verification of the molecular ion. The ion of higher mass is at *m/z* 400, which might indicate the loss of HBr from the molecule. Such a loss could be either of thermal nature or due to the fragmentation upon electronic impact. The more reliable hypothesis is that of thermal decomposition: in fact, the ionic current due to the ions at *m/z* 79–82 tends to decrease when the total ionic current increases. This is probably the consequence of the fact that the product begins to pass into the vapour state only after having lost HBr. The fragmentation is in agreement with the hypothesized structure with further losses from *m/z* 400 of: (−60), acetic acid from the lateral chain, (−101), the entire lateral chain, H_2O, CH_3OH with resultant masses *m/z* 382, 368, 340 and 299, and the masses 223, 197, 147 and

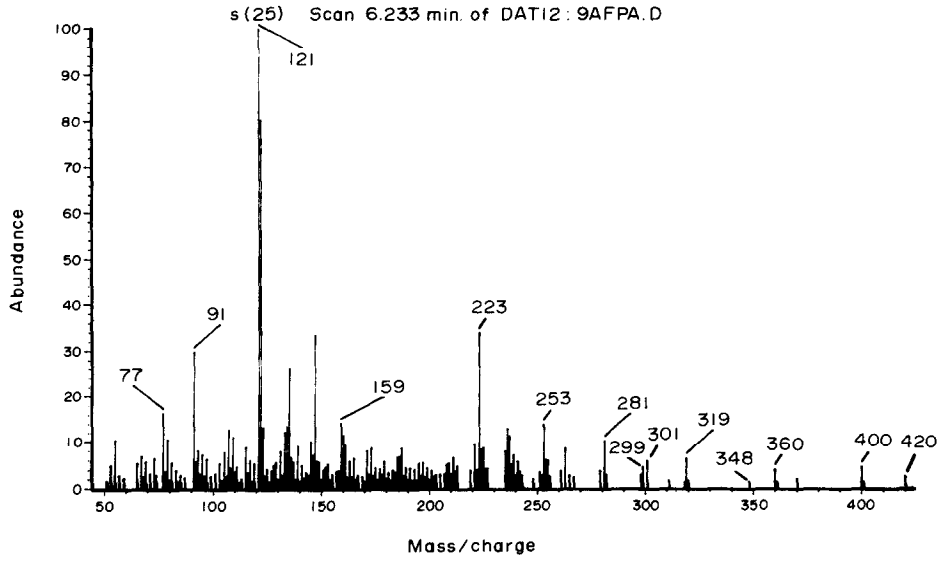

Figure 5
Mass spectrum of the reference compound 9α-fluoroprednisolone acetate. Technique DIP, 70 eV.

121 characteristic of the steroid nucleus. Attempts to derivatize 9α-bromoprednisolone acetate in order to render it more volatile and perhaps separable by GLC–MS, using reactions with TFA, methoxyamine HCl and successive reactions with MT BSTFA (with 20% TMBrS) and Tri-Sil-TBT* produced no positive results in any case.

Working with the DEI technique (direct electron impact: direct introduction of sample into the electronic source beam), which in an analogous case of an unstable 9α-bromine steroid derivative, improved the mass-spectrum [10], it was possible to show the molecular ion of the product at *m/z* 480 with the analogous peak at *m/z* 482† and all the further fragmentation in accordance with the structure of 9α-bromoprednisolone acetate as illustrated in Fig. 6.

Since the DEI technique has been demonstrated to be much more selective and reliable for the identification of the molecule 9α-bromoprednisolone acetate, the fraction containing the impurities Nos 5 and 6 [Table 1, eluent (b)], isolated using normal-phase preparative HPLC, was examined by mass spectrometry with this technique (Fig. 7). From this spectrum it is possible to confirm the presence of the two hypothesized compounds based on preceding chromatographic and spectrophotometric data. In fact, there are peaks at *m/z* 480 (with an isotopic peak at 482), 420, 400, 322, 289, 281, 263, 237, 223 and 121, characteristic of 9α-bromoprednisolone acetate and peaks at *m/z* 422, 362, 321, 292, 283, 225, 161 and 123, characteristic of 9α-fluorohydrocortisone acetate. All these peaks have already been described by the spectra of the single substances. The hypotheses previouisly formed about the nature of the two principal impurities of the examined compound, based on the chromatographic and spectrophotometric behaviour, are thus confirmed. These impurities are 9α-fluorohydrocortisone acetate and 9α-bromoprednisolone acetate. This study confirms our previous finding [10]: the use of high resolution chromatography with a diode array detector and an examination of the second derivatives of the spectra is a convenient procedure for the differentiation of the peaks corresponding to the impurities present in samples of synthesized 9-halogen substituted corticosteroids and could be of considerable utility in their identification.

* Registered names of Pierce derivatization reagents.

† It is characteristic of the brominated ions to show one isotopic ion at an intensity of M + 2 practically equal to that of ion M.

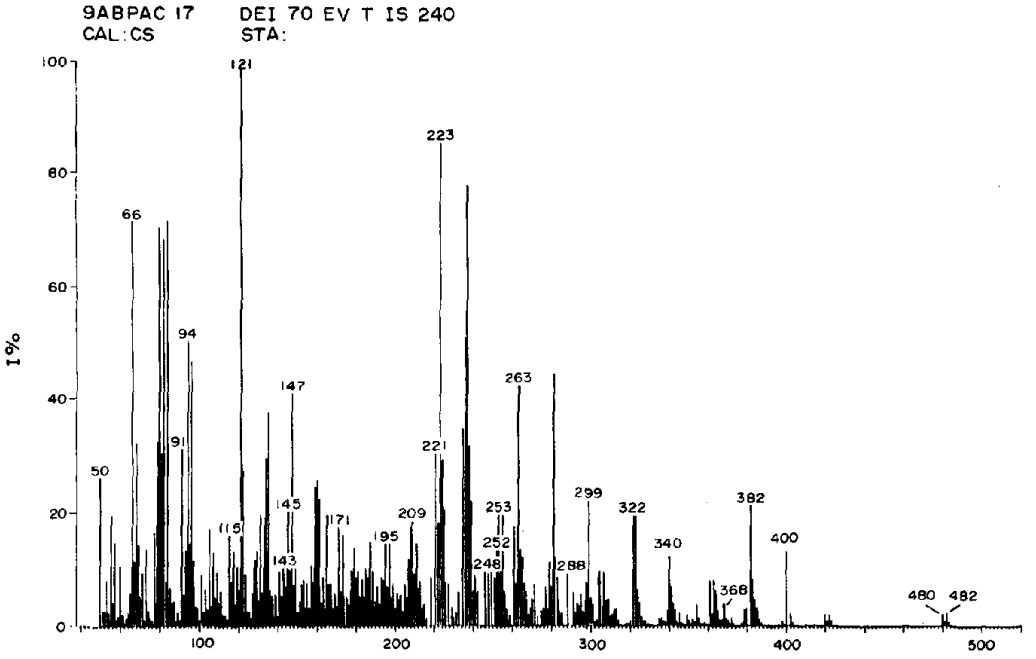

Figure 6
Mass spectrum of the reference compound 9α-bromoprednisolone acetate. Technique DEI, 70 eV.

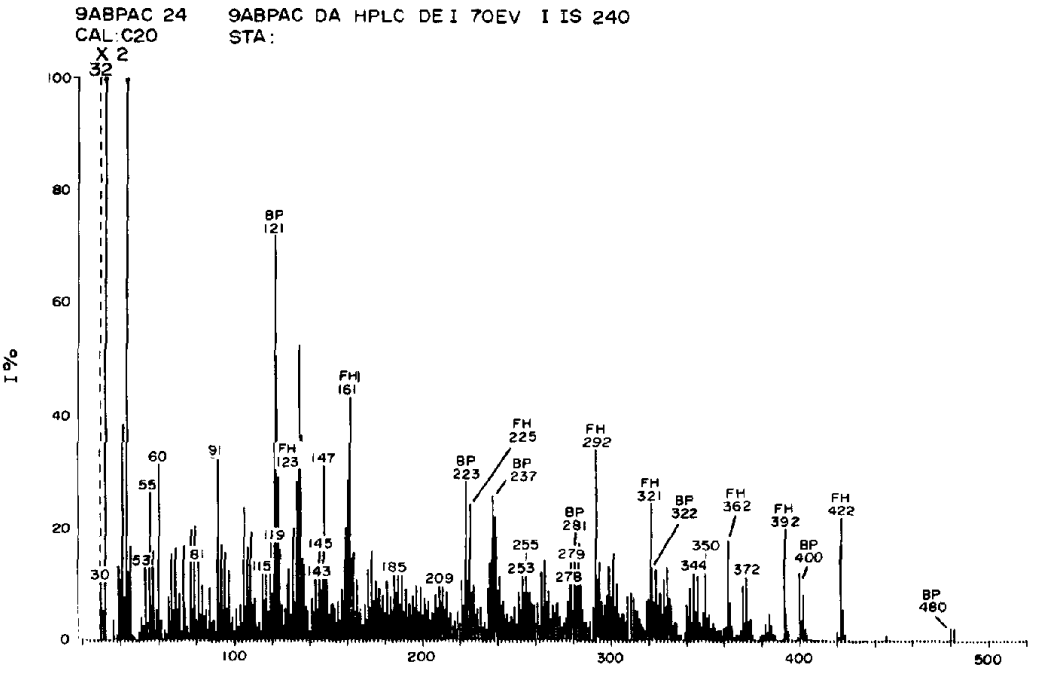

Figure 7
Mass spectrum of the fraction at r.r.t. 0.72 containing the two major related substances isolated by HPLC (normal-phase chromatography) from sample 01212M. Technique DEI, 70 eV; BP, bromoprednisolone acetate peak; FH, fluorohydrocortisone peak.

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