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Quantitative analysis and purity evaluation of medroxyprogesterone acetate by HPLC

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Abstract: A reversed-phase high-performance liquid chromatographic method was developed for the assay of medroxyprogesterone acetate and for the detection and determination of related steroids present as impurities in the drug. The method was compared with the normal-phase technique of the USP XX and was also applied to the analysis of tablets and injectable suspensions.

Keywords: *Medroxyprogesterone acetate; reversed-phase HPLC; steroid assay; related foreign steroids; impurity identification.*

Introduction

In a previous paper a simple HPLC method to determine the purity of steroids used in pharmaceutical preparations was described [1]. Attention was focussed on the necessity of studying the selectivity of chromatographic systems in order to obtain maximum discrimination between the main compound and its foreign substances. The present study of a convenient system for analysing the related impurities of medroxyprogesterone acetate, 6α-methyl-17α-hydroxyprogesterone acetate (MPA), provides a further demonstration of the need for careful choice of a chromatographic system.

MPA is a progestational steroid prescribed for oral administration; it is frequently given intramuscularly as a long-acting contraceptive or as a palliative for endometrial carcinoma. It is generally formulated as tablets (2.5–100 mg) or as an injectable aqueous suspension (50–400 mg/ml). Procedures for the determination of MPA by normal-phase HPLC are described in the United States Pharmacopeia (USP) XX [2]; a slightly modified technique is proposed in a Supplement to the USP [3]. Das Gupta [4] described a reversed-phase chromatographic method. No mention is made of the possible presence of steroidal impurities or of the need to detect such impurities in spite of the relatively high doses that are administered. The present paper describes an HPLC procedure for both the direct determination of related substances and the assay of MPA using a suitable reference standard. The procedure has been found convenient for assaying tablets and aqueous suspensions now on the market.

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Experimental

Materials

The following samples of pharmaceutical-grade medroxyprogesterone acetate were analysed: (1) G, (2) F, (3) L, (4) L/G, (5) L/M5, (6) F/MS, (7) F/7, (8) F/8, (9) PN/9, (10) PN/10. The following compounds were used to identify impurities of medroxyprogesterone acetate: 4,5-dihydro-6α-methyl-17α-hydroxyprogesterone acetate, 6-methylene-17α-hydroxyprogesterone acetate, 6-dehydro-6-methyl-17α-hydroxyprogesterone acetate, 6β-methyl-17α-hydroxyprogesterone acetate and 6α-methyl-17α-hydroxyprogesterone.

Solvents for liquid chromatography were: HPLC-grade acetonitrile, *n*-butyl chloride, *n*-hexane, chloroform, methanol and water previously purified by passage through a Millipore Milli Q device. Solvents for thin-layer chromatography were: analytical-grade benzene, petroleum ether and ethyl acetate.

Equipment

HPLC analyses were performed with a Perkin–Elmer Series 3 liquid chromatograph, equipped with a Rheodyne 7125 injection valve (20-μl or 100-μl sample loop) and a Perkin–Elmer LC-55 B variable wavelength detector. Data were recorded and evaluated with a Perkin–Elmer Sigma 10B integrator.

Chromatographic conditions

Reversed phase chromatography. This was carried out using a 250 × 4.6 mm i.d. stainless steel column packed with 5-μm LiChrosorb RP-18. The mobile phase for isocratic elution was acetonitrile–water (60:40, v/v), prepared by mixing 600 ml of acetonitrile with about 350 ml of water in a 1-l volumetric flask at room temperature. The mixture was shaken and left to return to room temperature; water was then added to volume. All measurements were made at room temperature; the flow-rate was 1.0 ml/min^{-1}. The detector wavelength was set to 240 or 254 nm with a sensitivity of 0.2 a.u.f.s.

Normal-phase chromatography. This was carried out using a 250 × 4.6 mm i.d. stainless steel column packed with 5-μm LiChrosorb Si-100. The mobile phases for isocratic elution were: (a) the solvent described in USP XX (2) modified as described in (3); and (b) chloroform (stabilized with amylene)–methanol–water (989.4:10.0:0.6, v/v/v). All measurements were made at room temperature; the flow-rate was 1.0 ml/min. The detector wavelength was set to 254 nm with a sensitivity of 0.2 a.u.f.s.

The injection volume was 20 μl for all analyses.

Thin-layer chromatography. This was carried out on silica gel plates with a fluorescence indicator (Merck 60 F_{254}) in saturated tanks. The solvent system was benzene–ethyl acetate–petroleum ether (70:40:10, v/v/v). Spots were detected under UV light at 254 nm and after spraying with 20% (v/v) sulphuric acid in ethanol and then heating at 120°C for about 10 min.

Preparation of sample solutions

Related substances analysis. A 62.5 mg portion of the product to be examined was weighed, dissolved in ethanol in a 25-ml volumetric flask and then diluted to volume with

the same solvent. This solution contained 2.5 mg/ml and is here referred to as Solution I. For HPLC analyses, 5 ml of Solution I was evaporated to dryness and the residue was dissolved in 5 ml of the mobile phase. For reversed-phase chromatography, the ethanol solution was used unmodified.

A calibration curve was prepared for each related substance subjected to chromatography. Medroxyprogesterone acetate was used as a reference standard; it was assumed that the sample and the related substances had similar absorptivities. Dilutions were prepared to contain 0.25, 0.5 or 1.0 mg in 10-ml aliquots, which were then injected separately as the standard 20-μl volume (equivalent to 0.5, 1.0 or 2.0 μg). The regression equation for y (integrator count) against x (amount, μg) was: $y = 2.7828x + 0.0618$; $n = 4$; correlation coefficient $r = 0.9996$. For further details of the procedure, see Ref. [1].

Single steroid assay. Solution I was prepared in the same way as described under related substances analysis. A 1 ml portion of this solution was transferred into a 50-ml volumetric flask, evaporated to dryness and the residue dissolved in the mobile phase. This solution was then diluted to volume (0.05 mg ml^{-1} = 1.0 μg 20 μl^{-1}). The same procedure was used for the reference compound; Solution I was prepared with 25.0 mg in 10.0 ml.

After testing the suitability of the equipment, both sample and reference solutions were injected according to conditions (a), (b) and (c) (see Discussion). Calculations were performed using the equation:

$$\text{Sample strength (\%)} = R_S/R_{St} \cdot C_{St} \cdot T_{St}/C_S \cdot 100$$

where R_S and R_{St} are the instrumental responses of the sample and standard, respectively, C_{St} is the standard concentration in mg ml^{-1}, T_{St} is the standard titre (theoretically equal to 100%) and C_S is the sample concentration in mg ml^{-1}.

Analysis of pharmaceutical dosage forms. Samples of the following MPA suspensions were analysed:

(1) DP, medroxyprogesterone acetate 50 mg ml^{-1}. The vehicle of the suspension contained: polyethylene glycol 4000 28.8 mg; polysorbate 80 1.92 mg; sodium chloride 8.6 mg; methyl *p*-hydroxybenzoate 1.3 mg; propyl *p*-hydroxybenzoate 0.15 mg; and water for injections to 1 ml.

(2) F, medroxyprogesterone acetate, 20% injectable suspension, in 5-ml multidose vials containing 1 g of active product. The vehicle of the suspension contained: polyethylene glycol 4000 150 mg; polysorbate 80 15 mg; methyl *p*-hydroxybenzoate 10 mg; propyl *p*-hydroxybenzoate 1 mg; sodium chloride 50 mg; and water for injections to 5 ml. The tablet formulation was: DP, medroxyprogesterone acetate 100 mg with starch, calcium stearate, sodium lauryl sulphate, talc and lactose. The following extraction procedures were adopted:

Suspensions. Samples (0.8 ml for Product (1) or 0.2 ml for Product (2)) were placed in a 50-ml glass-stoppered centrifuge tube and extracted with 25 ml of chloroform by shaking for 20 min. The layers were separated by centrifugation and 1.5 ml (2.4 mg of steroid) of the clear chloroformic layer were transferred to a 50.0-ml volumetric flask and evaporated to dryness under a stream of nitrogen. The residue was dissolved and diluted to volume with the mobile phase (0.96 μg 20 μl^{-1}).

Tablets. The mean tablet weight was calculated from 10 tablets. The tablets were finely

ground and a quantity of powder, corresponding to about 25 mg of medroxyprogesterone acetate accurately weighed, was extracted with 25 ml of chloroform in a 50-ml glass-stoppered centrifuge tube by shaking for 20 min. After centrifugation, 2.5 ml (2.5 mg of steroid) of the clear chloroformic solution was placed in a 50.0-ml volumetric flask, evaporated to dryness and diluted to volume with the mobile phase (1.0 μg 20 μl^{-1}).

Internal standard analyses

Internal standardization was performed according to the procedure described in USP XX [2], using a solution of 12.5 mg of progesterone in 100 ml of the mobile phase. Sample aliquots and dilution volumes were adjusted so that injections into the chromatograph contained 4.0 μg of medroxyprogesterone acetate and 2.5 μg of progesterone in 20 μl of the mobile phase.

Identification of impurities by mass spectrometry

Replicate injections of 100 μg of Sample (2)F were made. The impurity to be identified was isolated by collecting a suitable number of fractions corresponding to the middle part of its peak. The solvent was evaporated and the residue dissolved in chloroform at a concentration of about 1 μg μl^{-1}. Samples (1 μl) were then analysed with an LKB 2091 mass spectrometer, each sample being introduced by the direct inlet system; electron impact was 70 eV; the temperature was increased from 25° to 250°C at 60°C min^{-1}.

Results and Discussion

Detection of related substances

With the aim of studying the most efficient chromatographic system for the evaluation of related impurities, the authors followed the procedure described in a previous paper [1] by applying normal- and reversed-phase chromatographic systems to several samples of MPA obtained from different sources. This allowed the selection of the most suitable procedure, that is the procedure that would enable the greatest number of reproducible secondary peaks to be detected.

For normal-phase systems, the first approach was to study the performance of a mobile phase based on a mixture of chloroform (stabilized with amylene), methanol and water (99.0:1.0:0.06, v/v/v) with low eluent strength on silica gel columns. This particular mobile phase was derived from eluents that had given good results for the analysis of less-polar corticosteroid in previous studies [1]. The chromatographic behaviour of the main steroid was satisfactory (retention time 4.6 min), but samples from various sources and of different qualities all gave very low impurity values in terms of number and total content (from 0.26 to 0.40%). The same samples, analysed with a reversed-phase system, a 5-μm octadecyl silica column and a mobile phase consisting of acetonitrile–water (60:40, v/v) provided a satisfactory chromatogram (Fig. 1).

Nine samples of pharmaceutical grade MPA, obtained from three different manufacturers, were examined. Three samples exhibited high purity (two of the samples were working standards) and contained one detectable impurity at the most; the total content of impurity was not more than 0.3%. The other six samples exhibited virtually the same impurity profile, showing 3–5 characteristic secondary peaks in the chromatograms of the samples from different sources. All secondary peaks had the same relative retention times with reference to the main peak.

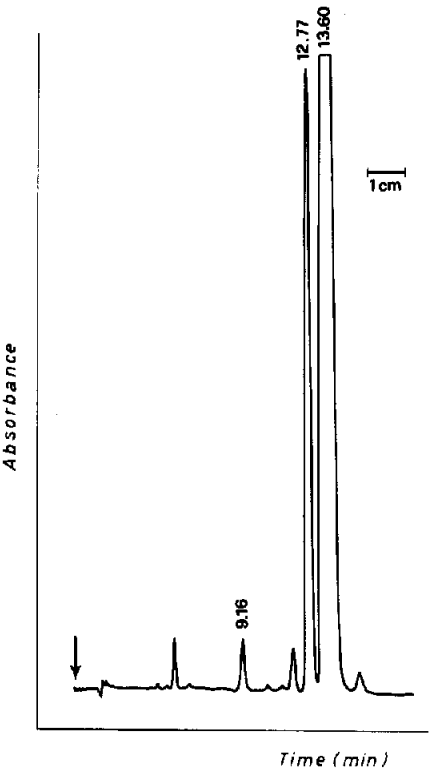

Figure 1
Separation of foreign substances related to MPA using the proposed HPLC procedure.
Sample: Medroxyprogesterone acetate, Sample (2)F, 50 μg/20 μl.
Column: 250 × 4.6 mm i.d. packed with 5-μm LiChrosorb RP-18.
Eluent: acetonitrile–water (60:40, v/v).
Flow rate: 1 ml min^{-1}.
Operating temperature: room.
Detection wavelength: 254 nm.
Chart speed: 0.5 cm min^{-1}.
Attenuation: 1 cm = 150 × 10^{-4} a.u.
Peaks (retention time, min): Medroxyprogesterone 9.16; Medroxyprogesterone acetate, β-isomer 12.77; Medroxyprogesterone acetate, α-isomer 13.60.

Differences were found in both the single and the combined impurity contents for the various samples, the total amount ranging from about 0.2 to 4.4%. Table 1 reports the analytical results for one sample (2)F, inclusive of peak sequence, retention times and relative retention times. The relative retention times were found to be constant and the absolute retention times were of good reproducibility after columns had been similarly equilibrated in all the chromatographic analyses reported in this paper.

Identification of impurities

The content in the analysed samples of the most abundant impurity (relative retention time 0.94), isolated by HPLC and examined by mass spectrometry, was 0.1–3.5%. The mass spectrum for this impurity showed the same fragments as did that of the main compound, similarly purified by HPLC. The patterns obtained for the samples examined show that the main impurity can only be the 6β-isomer of MPA. Indeed, from the fragments shown in Fig. 2, it can be seen that, apart from the molecular ion (*m/z* 386) with the same value of *m/z* as 6α-methyl-17α-hydroxyprogesterone acetate, the fragment sequence exhibits the same succession of ions as the 6α-isomer.

The reported fragmentation is in accordance with the general pattern described by Hammerum and Djerassi [5] for the 17-hydroxy derivatives of progesterone. For that type of substituted progesterone, those authors observed that the characteristic pattern of Δ_4-3-keto steroids fragmentation, namely the elimination of ketene from the A-ring and the B-ring cleavage with formation of the *m/z* 124 ion [6], occurred to a lesser extent. For the 6-methyl derivatives of 17α-hydroxy progesterones, the ion must have an *m/z* value of 137, as was observed in the experiment.

Table 1
Related foreign substances of medroxyprogesterone acetate Sample (2) F determined by reversed-phase chromatography. Loading 50 μg/20 μl

Peak	Retention time (min)*	Relative retention time (min)†	Related substances* (%)
1/A	5.49	0.40	0.19
2/A	9.16	0.67	0.26
3/A	11.88	0.87	0.26
4/A	12.76	0.94	3.47
MAIN	13.58	1.00	—
1/B	15.43	1.14	0.14
		Total	4.32

* Mean values of five replicates.
† Retention time relative to medroxyprogesterone acetate α-isomer (main peak).

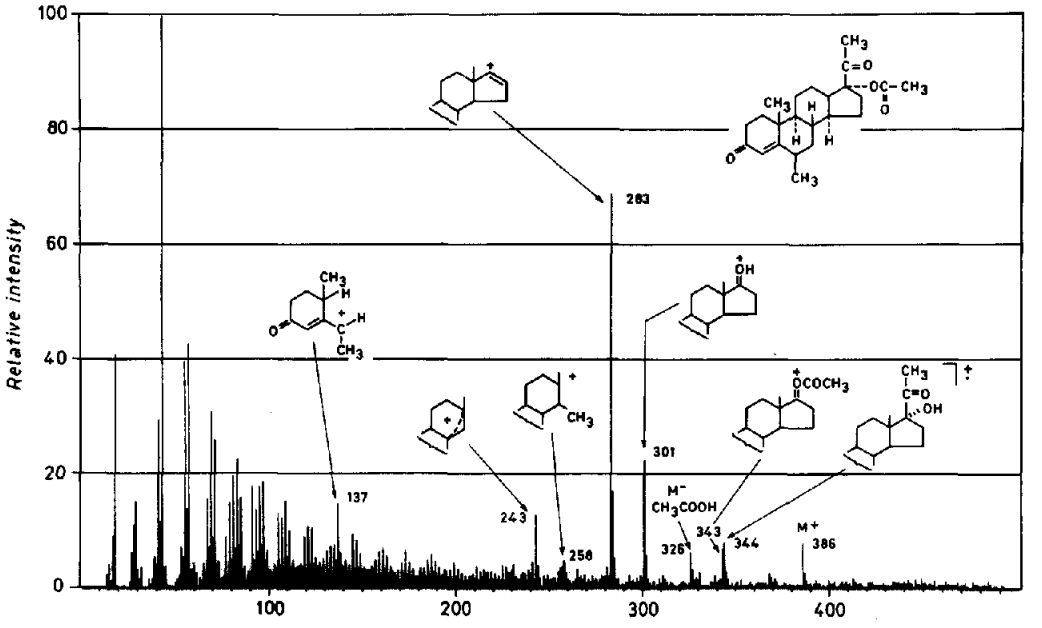

Figure 2
MS fragmentation pattern of 6β-methyl-17α-hydroxyprogesterone acetate isolated by HPLC.

Hammerum and Djerassi [5] also observed that no differences appeared to exist in the spectra of those 6,16-dimethyl-17α-hydroxy progesterones that only differ in the 6α or 6β orientation of their methyl groups. This is also valid for the 17α and 17β isomers of corticosteroids as reported by Dekker [7]. The pattern for a second MPA impurity confirms the assumption in the present work about the nature of the main impurity. It was observed that impurity 2/A, with a relative retention time 0.67 in Table 1, had the same relative retention time as 6α-methyl-17α-hydroxyprogesterone. This related

substance, isolated by HPLC and examined by mass spectrometry (as was the main impurity) shows a molecular ion with *m/z* 344 and fragmentation with main peaks at *m/z* 301, 283, 243 and 137, all in accordance with the 6α-methyl-17α-hydroxyprogesterone suspected to be present on the basis of HPLC data only. A pharmaceutical-intermediary-grade sample of this substance was also examined by HPLC. It exhibited a head-migrating related substance (relative retention time 0.92, content 4.3% in the sample examined), which provided the same mass spectrum as that of the main compound (MPA): it is likely to be its 6β-methyl isomer. These identifications of impurities are in accordance with the synthesis pathways described by Babcock *et al.* [8] and by Camerino *et al.* [9]. In both methods, one of the key steps is represented by the preparation of 6β-methyl-17α-hydroxyprogesterone which is then converted into the 6α-methyl isomer and subsequently acetylated.

The chromatographic behaviour of other possible MPA impurities was also investigated. These impurities included: (I) megestrol acetate (6-dehydro-6-methyl-17α-hydroxyprogesterone acetate), (II) 6-methylene-17α-hydroxyprogesterone acetate and (III) 4,5-dihydro-6-methyl-17α-hydroxyprogesterone acetate. Their respective relative retention times (referred to MPA) were: (I) 0.96; (II) 0.94; (III) 1.30 (detectable only at 210 nm); and (IV) (the main impurity, the 6β-methyl isomer) 0.94. From these data it can be seen that (II) and (IV) have the same relative retention times but (II) is clearly distinguishable from the main impurity (IV) by its mass spectrum. In fact, most of the fragments are generated by the same fission mechanism as for MPA and its main impurity (IV), but the ions from (II) show *m/z* values 2 units lower, i.e. 384 (molecular ion), 342 (ketene loss), 341 (acetyl loss), 324 (acetyl and water loss), 299 (ketene and acetyl loss), 281 (ketene, acetyl and water loss), 256 (D-ring cleavage), 241 (D-ring cleavage), 43 (base peak).

Comparison with the USP XX procedure

Confirmation of the validity of the present method was effected by comparison with the procedure described in USP XX [2, 3]. That procedure for the assay of MPA involves HPLC on a silica column with *n*-butyl chloride (water saturated)–*n*-hexane (water saturated)–acetonitrile (700:300:80, v/v/v) as the solvent. Under these normal-phase conditions the primary impurity peak follows the main peak but separation is not complete (Fig. 3). The USP XX normal-phase resolution factor for the 6α-6β isomers is 1.73; this factor is lower than 2.46, resolution factor for the 6β and 6α isomers in the reversed-phase method proposed in the present work.

Elution order is also important. In the normal-phase procedure, the impurity peak is on the tail of the main peak and its evaluation is less accurate than in the separation described in the present work. In fact, recourse to the USP XX normal-phase conditions to analyse the related substances in Sample (2)F provides a β-isomer content of 4.9% instead of 3.5% as obtained by the present procedure. Another drawback of the USP XX procedure is its lower sensitivity of response to the Δ_4-3-keto steroids in the solvent specified for the method. This is due to the lower absorptivity of MPA in that solvent, coupled with the need to use a 254 nm wavelength instead of 240 nm because the solvent is less transparent; the response to a 1-μg peak of MPA using the USP XX procedure is about 30% of the value obtained when the reversed-phase procedure is used. Thus fewer secondary peaks can be detected. For example, 6α-methyl-17α-hydroxyprogesterone shows a peak with a retention time of about 29.5 min yet it is not detectable clearly owing to its flat profile at the levels at which it occurs.

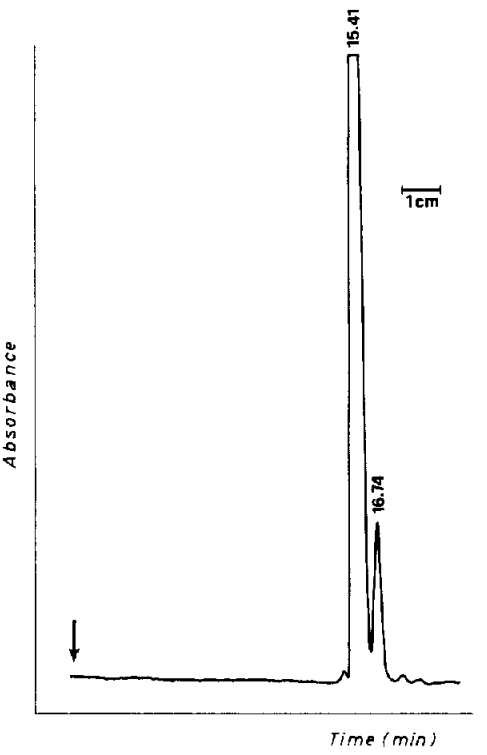

Figure 3
Separation of foreign substances related to MPA using the USP XX HPLC procedure.
Sample: Medroxyprogesterone acetate, Sample (2)F, 50 μg/20 μl.
Column: 250 × 4.6 mm i.d. packed with 5-μm LiChrosorb Si-100.
Eluent: water-saturated *n*-butyl chloride–water-saturated *n*-hexane–acetonitrile (70:30:7, v/v/v).
Flow rate: 1 ml min^{-1}.
Operating temperature: room.
Detection wavelength: 254 nm.
Chart speed: 0.5 cm min^{-1}.
Attenuation: 1 cm = 150 × 10^{-4} a.u.
Peaks: (retention time, min): Medroxyprogesterone acetate, α-isomer 15.41; Medroxyprogesterone acetate, β-isomer 16.74.

Application to the assay of MPA

The proposed procedure can also be applied to the assay of MPA by reference to a suitable standard. Table 2 reports the results obtained in the analysis of related substances and in the assay of nine different samples of MPA. Both the related substances analysis and the single steroid assay were performed on two of these samples and the USP XX normal-phase procedure was applied for purposes of comparison. Three samples appeared to be very pure by the related substances test; because of their purity those samples were used as assay references. Each result of the related substances test was expressed as a "purity index", which was the difference between 100% and the percentage content of contained related substances. These values for purity index are reproducible as may be seen in the replicated analyses reported in the table. Good reproducibility was also obtained in the replications of the assay. The standard deviation, obtained on 6 pairs of replicates was 0.65%, i.e. in agreement with analogous data reported by Walters in her report on the validation of an HPLC procedure for the assay of hydrocortisone, proposed as a USP method [10].

In the comparison experiments performed by applying the USP XX method, the analyses were replicated using progesterone as the internal standard. With a fixed loop injector, no significant differences with or without the internal standard were observed.

A different comparison between the values obtained in the related substances test, expressed as purity index, and the values obtained in the assay can be attempted. In general good agreement was observed, as was to be expected in samples with values for loss on drying and sulphated ash within normal limits. Any disagreement may be due to the presence of impurities whose structures are not closely related to that of the main

Table 2
Results obtained in the analysis of nine samples of medroxyprogesterone acetate by reversed-phase and normal-phase chromatography.

Sample	Reversed-phase chromatography		Normal-phase chromatography	
	Purity index* (%)	Single steroid assay† (%)	Purity index* (%)	Single steroid assay† (%)
(1) G	97.6 97.2	98.0[a] 96.5[b]	not performed	not performed
(2) F	95.6 95.7	95.5[a] 95.8[c]	94.5 94.5	95.2[a] 95.4[a']
(3) L (St)	99.8 —	— —	not performed	not performed
(4) L/M5	99.8 99.8	99.0[a] 97.8[b]	not performed	not performed
(5) F/7	98.9 98.9	97.2[a] 98.2	not performed	not performed
(6) F/8	98.2 98.1	95.3[a] 95.1[a]	not performed	not performed
(7) PN/9	98.8 98.8	99.1[b] 99.6[b]	98.2 98.3	99.7[a'] 98.6[b]
(8) PN/10 (St)	99.7 —	— —	not performed	not performed
(9) F/MS (St)	99.8 —	— —	not performed	not performed

*The purity index is the difference between 100% and the percentage content of related substances.
†The terms a, b, c, a' relate to procedures (a), (b), (c) and (a') (see text). Data obtained with procedure (c) are mean values of four replicates of assay.

compound and hence do not absorb or only very weakly radiation at 240 or 254 nm; therefore, values from the single steroid assay can be expected to be lower than purity index values. In this instance a further investigation can be performed by either TLC with strong visualization systems or HPLC at low wavelengths (210 nm). This is shown by sample (6)F/MS, where values for the single steroid assay differ by about 3.0% from purity index values. In addition to the normal thin-layer chromatogram detectable by examination at 254 nm, an extra spot was also detected by spraying with 20% sulphuric acid in ethanol and heating. The R_f corresponded to that of an authentic sample of 4,5-dihydro-6α-methyl-17α-hydroxyprogesterone acetate (Compound III)) (main spot $R_f = 0.30$). The same sample, analysed by the proposed HPLC method but monitoring at 210 nm, showed a detectable peak with a retention time of 18.1 min, coincident with that of Compound III.

Suitability tests for the method

Some important considerations that have recently emerged from papers on the use of HPLC procedures in the assay of pharmaceutical product [11] were taken into account when the present method was devised.

Suitability tests for the chromatographic system. For system efficiency, a convenient range of N values (calculated on the MPA peak) must be fixed. In the present

investigation, good results were obtained when N was 58 012–60 112 plates m^{-1}. Hence, these values were prescribed. A second suitability test was the determination of the resolution factor between the 6β-methyl and 6α-methyl isomers. The value prescribed was not less than 2.0; the usual values obtained were 2.5–2.7.

For system reproducibility, only values of relative standard deviation lower than 2.0%, obtained on six replicated injections of the sample, are acceptable.

Suitability tests for the comparison of sample and reference solutions. The following three different approaches are possible: (a) comparison between mean values of the response for n replicated injections of the sample solution and for n replicated injections of the reference solution. In this instance an assay suitability test is included by establishing a relative standard deviation of 2.0% for each mean value ($n = 6$ at least). (b) comparison between alternate injections of sample and reference solutions, replicated n times ($n = 6$ at least). The mean value (± relative standard deviation) of the response ratios gives the result. In this instance an assay suitability test is included by limiting the acceptable data with a relative standard deviation of ± 2%. (c) similar to (b) but restricted by imposition of a different assay suitability test as described by Roman [12], in which there is total response agreement between duplicate samples which are bracketed by standards and likewise between standard replicates. It is preferable to establish some form of limitation, as introduced by Walters and Dunbar [13]; that is the response values for three consecutive standard injections must agree within 1.0% before the sample is injected. For each approach (a), (b) and (c), a parallel series (a′), (b′) and (c′) can be used in which the internal standard (progesterone) is included in the measurements. Of the three approaches tested, (c) was found to be the most convenient because three or four complete assay replicates can be obtained with about the same number of injections as for a single (a) or (b) experiment. This obviously increases confidence levels of the analytical procedure. Furthermore, the introduction of replicated procedures in the assays described in official compendia has recently been advised by Tuckerman [14].

Assay of formulated products

Finally, the procedure described in the present work was applied as a purity test and MPA assay of three oral and parenteral route formulations. Results are summarized in

Table 3
Results obtained in the analysis of medroxyprogesterone acetate in dosage forms

Sample	Reversed-phase chromatography		Normal-phase chromatography	
	Purity index* (%)	Single steroid assay (%)	Purity index* (%)	Single steroid assay (%)
Tablets DP (100 mg)	98.8	100.1	—	97.2
	—	102.5	—	99.9
Suspension DP (50 mg/ml)	98.7	104.7	—	104.2
	—	103.9	—	103.8
Suspension F (200 mg/ml)	98.7	102.3	—	103.4
	—	102.6	—	101.7

*The purity index is the difference between 100% and the percentage content of related substances.
†Percentage of the amount of steroid claimed on the label.

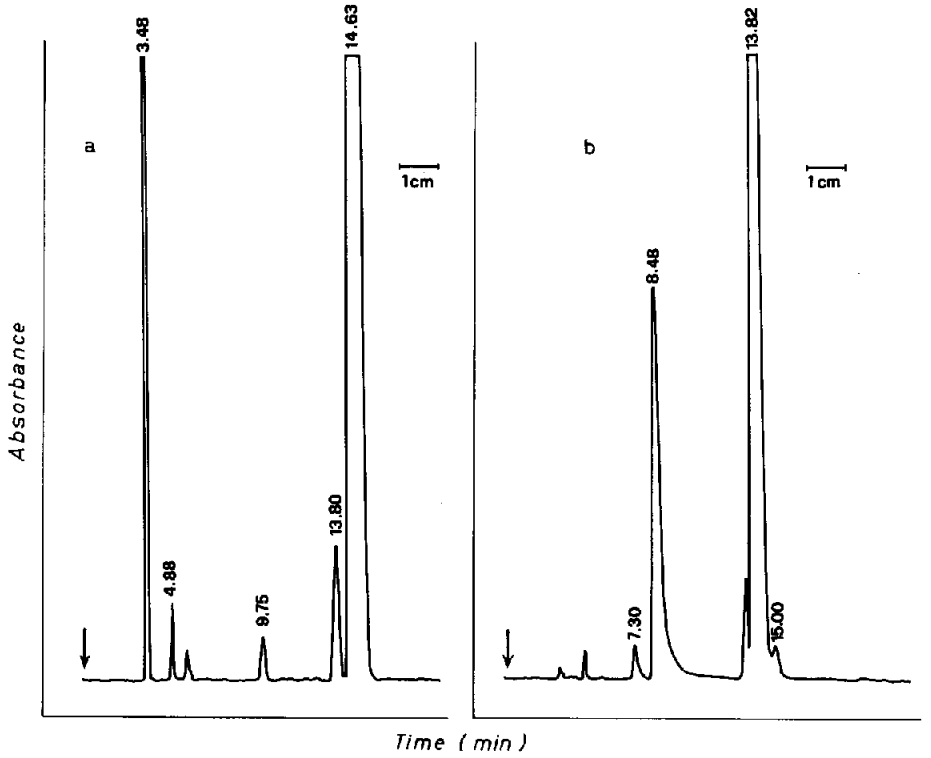

Figure 4
Chromatograms of an MPA suspension
(a) *Sample*: MPA suspension F, 50 μg/20 μl.
Column: 250 × 4.6 mm i.d. packed with 5-μm LiChrosorb RP-18.
Eluent: acetonitrile–water (60:40, v/v).
Flow rate: 1 ml min^{-1}.
Operating temperature: room.
Detection wavelength: 254 nm.
Chart speed: 0.5 cm min^{-1}.
Attenuation: 1 cm = 150 × 10^{-4} a.u.
Peaks (retention time, min): Methyl *p*-hydroxybenzoate 3.48; Propyl *p*-hydroxybenzoate 4.88; Medroxyprogesterone 9.75; Medroxyprogesterone acetate, β-isomer 13.80; Medroxyprogesterone acetate, α-isomer 14.63.
(b) *Sample*: MPA suspension F, 50 μg/20 μl.
Column: 250 × 4.6 mm i.d. packed with 5-μm LiChrosorb Si-100.
Eluent: water-saturated *n*-butyl chloride–water-saturated *n*-hexane–acetonitrile (70:30:7, v/v/v).
Flow rate: 1 ml min^{-1}.
Operating temperature: room.
Detection wavelength: 254 nm.
Chart speed: 0.5 cm min^{-1}.
Attenuation: 1 cm = 150 × 10^{-4} a.u.
Peaks (retention time, min): Propyl *p*-hydroxybenzoate 7.30; Methyl *p*-hydroxybenzoate 8.48; Medroxyprogesterone acetate, α-isomer 13.82; Medroxyprogesterone acetate, β-isomer 15.00.

Table 3 and a comparison is made with those obtained with the USP XX procedure. Figures 4a and 4b give the profiles obtained with both procedures. Use of the proposed procedure results in clearer separation of related substances and preservative peaks (Fig. 4a). Hence the present procedure enables related substances to be detected easily and the purity of the bulk substances used in the manufacturing of pharmaceutical forms to be characterized.

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