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Purity evaluation of 6α-methylprednisolone acetate by HPLC

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Abstract: A high-performance liquid chromatographic method is described for the analysis and identification of related impurities in pharmaceutical-grade 6α-methylprednisolone acetate (MPA). Eight MPA impurities derived from synthesis and/or degradation processes were identified. The method is compared to other analytical procedures recently proposed for the European Pharmacopoeia.

Keywords: *6α-Methylprednisolone acetate; reversed-phase chromatography; purity evaluation; foreign substances.*

Introduction

6α-Methylprednisolone acetate (MPA) is a synthetic glucocorticoid widely used in the treatment of various acute and chronic joint diseases, by intra-articular injection of an aqueous suspension [1, 2]. It may also be administered by intramuscular injection for a prolonged systemic effect and by topical application in the treatment of various skin disorders [3].

Different HPLC methods have been reported for the determination of MPA in biological fluids, in illegal preparations used in cattle-breeding and in pharmaceutical preparations [1, 4, 5]. However, the determination of its related impurities in pharmaceutical preparations has not been described.

Proposals of procedures for the determination of MPA related substances by normal- and reversed-phase HPLC are being considered by the European Pharmacopoeia Commission [6].

This paper describes a simple and sensitive reversed-phase HPLC method for the direct determination of impurities derived from the synthesis or degradation of MPA; the method is designed to obtain maximum discrimination between MPA and its foreign substances. A comparison is also made between this method and the European Pharmacopoeia proposed procedures.

Experimental

Materials

Samples of pharmaceutical-grade methylprednisolone acetate were examined. In particular, the following samples were analysed: four samples (A, B, C and D) taken from different batches of the same manufacturer; and one sample (E) kindly obtained from the European Pharmacopoeia Commission.

To identify related impurities of MPA, the following certificated compounds (Upjohn, Milan, Italy) were used: 6α-methylprednisolone (1); 20(α + β)-dihydro-6α-methylprednisolone acetate (2); prednisolone acetate (3); 6α-methylhydrocortisone acetate (4); 6α-methylprednisone acetate (5); 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one (6); 21-dehydro-6α-methylprednisolone (7); 21-dehydro-17-deoxy-6α-methylprednisolone (8).

Solvents for liquid chromatography were: HPLC-grade acetonitrile and tetrahydrofuran (Merck, Darmstadt, Germany), methanol (Baker, Deventer, Holland), chloroform (stabilized with amylene, C. Erba, Milan, Italy) and water (previously purified by passage through a Millipore Milli-Q device).

Equipment

HPLC analyses were performed with a solvent programmer (Waters automated

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gradient controller equipped with two Waters pumps, models M-6000A and M-45) and a Hewlett–Packard 1040A spectrophotometer (DAD) equipped with an HP-9000-300 computer.

Chromatographic conditions

Reversed-phase chromatography (RP_1–RP_2 methods). A 5-μm Hypersil ODS column (200 × 4.6 mm i.d.) was used. The mobile phases for the isocratic elution were: for RP_1, acetonitrile–water (35:65, v/v) (proposed by the authors); and for RP_2, tetrahydrofuran–water (26:74, v/v) (proposed for the European Pharmacopoeia) [6].

Normal-phase chromatography (NP_1–NP_2 methods). Proposed for the European Pharmacopoeia as a general method for the analysis of related substances in corticosteroids [7–10]. A Zorbax Sil column (250 × 4.6 mm i.d.) was used. The mobile phases for the isocratic elution were: for NP_1, chloroform–methanol–water (978.6:20:1.4, v/v/v); and for NP_2, chloroform–methanol–water (954:43:3, v/v/v).

All measurements were made at room temperature or 30°C as specified in the corresponding figure. The flow rate was 1.0 ml min^{-1}; for highly retained compounds, the modified values are reported in the corresponding figure.

Sample preparation

A weighed quantity of each MPA sample was dissolved in ethanol, evaporated to dryness and the residue dissolved in the mobile phase so that the injection volume (20 μl) contained 40 μg of MPA.

Ethanolic solutions (1 mg ml^{-1}) of each potential impurity (1–8) were also prepared. A 1 ml volume of each solution was evaporated to dryness and the residue dissolved in 20 ml of the appropriate mobile phase, according to the method adopted.

Mixtures of each sample with single impurities (0.2%) were also prepared to confirm the presence of such impurities in the main substance.

Results and Discussion

Figure 1 shows MPA and its potential impurities. Some of these impurities (1–6) could arise from the synthesis procedures (intermediate products) according to the literature [11]; two others (7–8) could be present as degradation impurities [12].

Reversed-phase chromatography (RP_1–RP_2 methods)

Quantitative evaluation of the examined samples. Samples A, B, C and D, representative of different batches of the same manufacturer, presented very similar chromatograms in respect of number and position of secondary peaks. Only slight differences in the abundance of these peaks were observed. For this reason, in the discussion of the results only one of these samples (A) is taken as representative. In contrast, the chromatographic profile of sample E, representative of a different manufacturer, presented some differences from those of the other samples.

Table 1 summarizes results of the quantitative analysis performed on two samples (A, E) representative of the two different manufacturers, with RP_1 and RP_2 as mobile phases. The chromatographic profiles of samples A and E, obtained with the RP_1 eluent, are reported in Fig. 2 (a) and (b), respectively. The chromatograms obtained with the RP_2 mobile phase are reported in Fig. 3(a) and (b), respectively.

Detection of related substances. A mixture of reference compounds, consisting of MPA-related foreign substances (1–6) was analysed using the RP_1 and RP_2 mobile phases. Relative retention times are reported in Table 2; chromatographic profiles are shown in Figs 4 and 5. The two degradation products, 21-dehydro-6α-methylprednisolone (7) and 21-dehydro-17-deoxy-6α-methylprednisolone (8) were not added to this reference mixture. The instability of their solutions causes numerous secondary peaks with consequent analytical problems when these compounds are added to a multicomponent mixture. When examined as isolated compounds, they showed, for the main components, relative retention times (rrt) 0.37 and 0.86 with the RP_1 eluent and 0.40 and 0.42 with the RP_2 eluent, respectively (Table 2).

Comparison of retention times of the reference mixture components and each sample impurity permitted identification of most of the observed impurities.

In sample A, seven compounds were observed with the RP_1 mobile phase [Fig. 2(a)]. Five of these presented lower retention times (rt) than that of the main compound. The

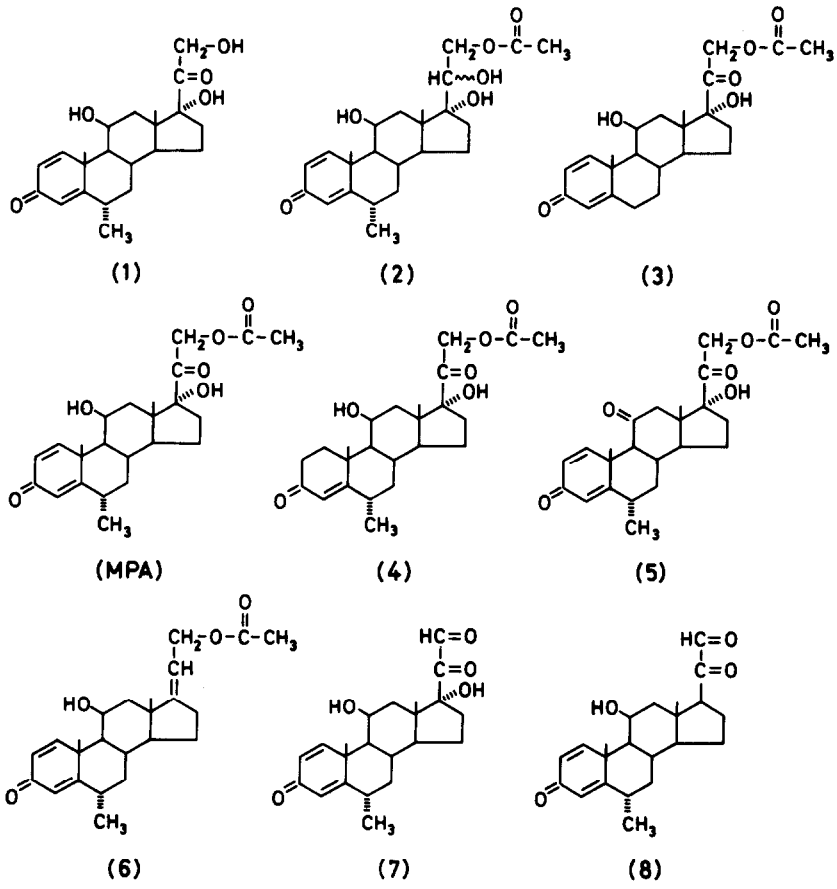

Figure 1
Structures of MPA and potential impurities. (MPA) 6α-methylprednisolone acetate; (1) 6α-methylprednisolone; (2) 20(α + β)-dihydro-6α-methylprednisolone acetate; (3) prednisolone acetate; (4) 6α-methylhydrocortisone acetate; (5) 6α-methylprednisone acetate; (6) 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one; (7) 21-dehydro-6α-methylprednisolone; (8) 21-dehydro-17-deoxy-6α-methylprednisolone.

Table 1
Relative retention time (rrt) and per cent content of MPA-related impurities in samples A and E by reversed-phase chromatography with RP_1 and RP_2 methods

RP_1 Method				RP_2 Method			
Sample A		Sample E		Sample A		Sample E	
rrt	Content (%)	rrt	Content (%)	rrt	Content (%)	rrt	Content (%)
0.22	0.01	0.23	0.01	0.38	0.17	0.38	0.02
0.38	0.01	0.36	0.03	0.61	0.01	0.62	0.04
0.47	0.17	—	—	0.92	0.11	0.92	0.24
0.54	0.01	—	—	1.00†	—	1.00†	—
0.64	0.03	0.62	0.05	1.10	0.20	1.10	0.20
—	—	0.68	0.03				
—	—	0.89	0.23				
1.00*	—	1.00*	—				
1.14	0.22	1.09	0.21				
1.28	0.13	1.22	0.05				
—	—	1.99	0.01				
	Σ0.55‡		Σ0.60‡		Σ0.48‡		Σ0.50‡

*MPA mean retention time in eluent RP_1: 16.35 min (n = 10).
†MPA mean retention time in eluent RP_2: 32.81 min (n = 10).
‡Percentages ≤0.01 omitted from the summation.

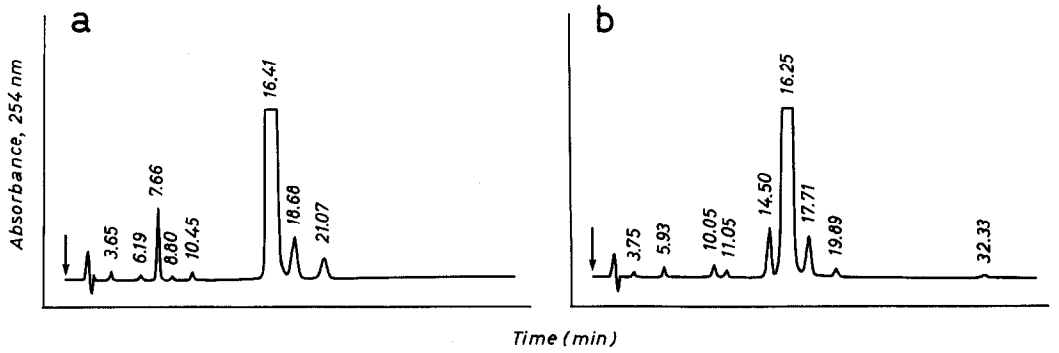

Figure 2
(a) Chromatogram of sample A; (b) chromatogram of sample E. Reversed-phase RP_1 method. Column: 5-μm Hypersil ODS, 200 × 4.6 mm i.d. Temperature 30°C. Flow rate 1 ml min^{-1}.

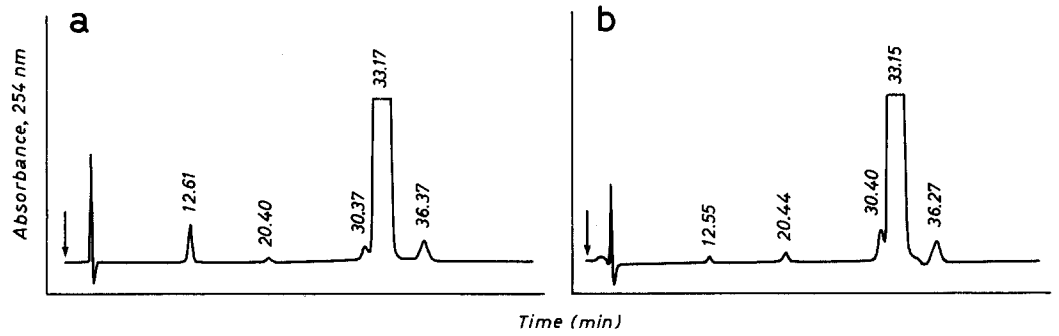

Figure 3
(a) Chromatogram of sample A; (b) chromatogram of sample E. Reversed-phase RP_2 method. Column: 5-μm Hypersil ODS, 200 × 4.6 mm i.d. Temperature 30°C. Flow rate 1 ml min^{-1}.

Table 2
Relative-retention time (rrt) of MPA-related foreign substances by reversed-phase and normal-phase chromatography with RP_1, RP_2 and NP_1, NP_2 methods

Compound		Relative retention time*			
Number (Fig. 1)	Chemical name	RP_1	RP_2	NP_1	NP_2
(1)	6α-methylprednisolone	0.37	0.41	—	3.41
(2)	20(α + β)-dihydro-6α-methylprednisolone acetate	0.46	0.42	2.36	—
(3)	prednisolone acetate	0.63	0.64	1.09	—
(4)	6α-methylhydrocortisone acetate	1.11	1.13	0.77	—
(5)	6α-methylprednisone acetate	1.25	0.95	0.55	—
(6)	21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one	7.85	2.05	0.35	—
(7)	21-dehydro-6α-methylprednisolone	0.37	0.40	2.04	1.69
(8)	21-dehydro-17-deoxy-6α-methylprednisolone	0.86	0.42	0.58	—

*Mean values (n = 10) of rrt with reference to MPA (retention time with RP_1 method: 16.04 min; with RP_2 method: 31.20 min; with NP_1 method: 16.21 min; with NP_2 method: 5.02 min).

impurity with rrt 0.47 (rt 7.66 min), found in a noticeable amount, was identified as 20(α + β)-dihydro-6α-methylprednisolone acetate (2); the impurities with rrt 0.38 (rt 6.19 min) and 0.64 (rt 10.45 min) were 6α-methylprednisolone (1) and prednisolone acetate (3), respectively. The minor compounds (about 0.01%) with rrt 0.22 (rt 3.65 min) and 0.54 (rt 8.80 min) were not identified. Finally, the impurities with rrt higher than that of MPA (rrt 1.14 and 1.28; rt 18.68 and 21.07 min) were identified as 6α-methylhydrocortisone acetate (4) and 6α-methylprednisone acetate (5).

The chromatographic profile of sample E

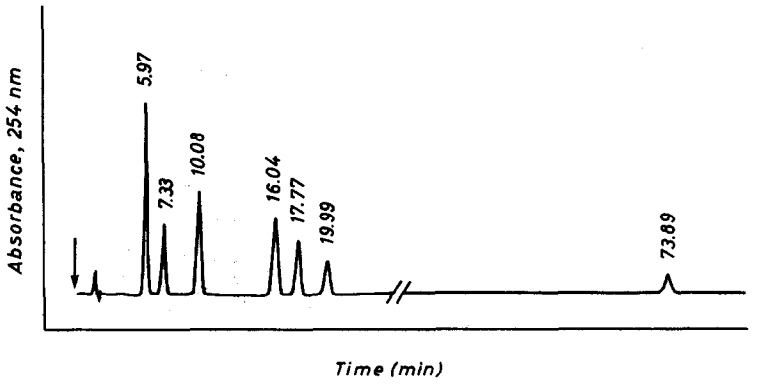

Figure 4
Chromatogram of MPA-related impurities mixture. Reversed-phase RP_1 method. Column: 5-μm Hypersil ODS, 200 × 4.6 mm i.d. Temperature 30°C. Flow rate 1 ml min^{-1} up to 22 min; 1.5 ml min^{-1} up to 25 min; 2 ml min^{-1} up to 120 min (// = Flow rate variation). Peaks: (1) 6α-methylprednisolone (rt 5.97 min; rrt 0.37); (2) 20(α + β)-dihydro-6α-methylprednisolone acetate (rt 7.33 min; rrt 0.46); (3) prednisolone acetate (rt 10.08 min; rrt 0.63); (MPA) 6α-methylprednisolone acetate (rt 16.04 min; rrt 1.00); (4) 6α-methylhydrocortisone acetate (rt 17.77 min; rrt 1.11); (5) 6α-methylprednisone acetate; (rt 19.99 min; rrt 1.25); and (6) 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one (rt 73.89 min; approximate rrt 7.85 allowing for the variation in the flow rate).

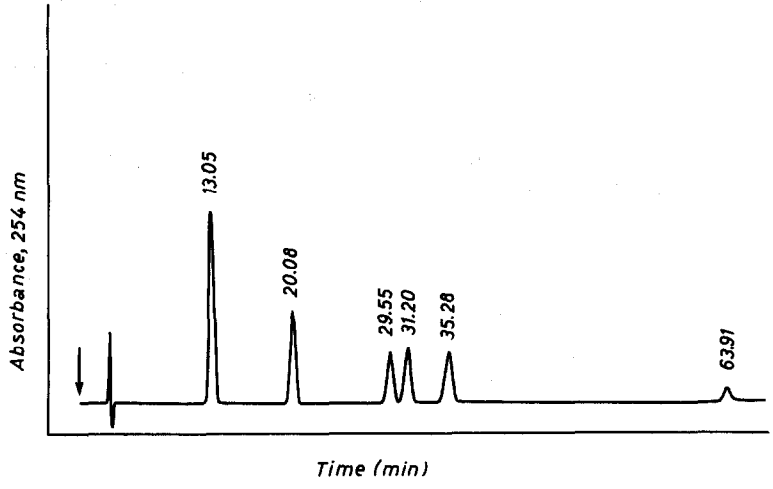

Figure 5
Chromatogram of MPA-related impurities mixture. Reversed-phase RP_2 method. Column: 5-μm Hypersil ODS, 200 × 4.6 mm i.d. Temperature 30°C. Flow rate 1 ml min^{-1}. Peaks: (1) + (2) 6α-methylprednisolone + 20(α + β)-dihydro-6α-methylprednisolone acetate (rt 13.05 min; rrt 0.42); (3) prednisolone acetate (rt 20.08 min; rrt 0.64); (5) 6α-methylprednisone acetate (rt 29.55 min; rrt 0.95); (MPA) 6α-methylprednisolone acetate (rt 31.20 min; rrt 1.00); (4) 6α-methylhydrocortisone acetate (rt 35.28 min; rrt 1.13); and (6) 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one (rt 63.91 min; rrt 2.05).

[Fig. 2(b)] showed, in addition to some of the small peaks in sample A, two peaks corresponding to unidentified impurities with rrt 0.68, and 1.99 (rt 11.05 and 32.33 min, respectively). This sample showed also a peak, absent in the other samples, with rrt 0.89 (rt 14.50 min) which represented the highest impurity concentration (0.23%). It was not possible to identify this impurity owing to the small amount of sample E available. Finally, 20(α + β)-dihydro-6α-methylprednisolone acetate (2), present in sample A (rt 7.66 min), was not detected in this sample.

The analyses of samples A and E performed with the RP_2 mobile phase provided chromatographic profiles that were not very different from those obtained with the RP_1 eluent [Fig. 3(a) and (b)]. A remarkable exception was the behaviour of 6α-methylprednisone acetate (5) that was not completely resolved from the main compound, showing rrt 0.92 (rt 30.4 min). This incomplete resolution causes diffi-

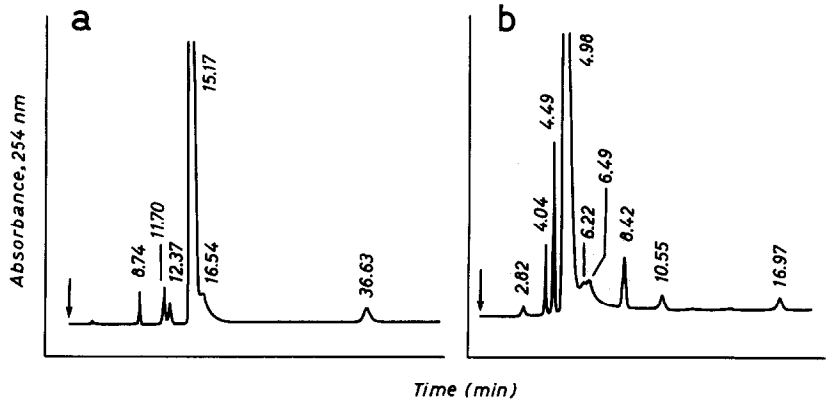

Figure 6
Chromatogram of sample A. (a) Normal-phase NP_1 method; (b) normal-phase NP_2 method. Column: Zorbax Sil, 250 × 4.6 mm i.d. Room temperature. Flow rate 1 ml min^{-1}.

culties in the quantitative evaluation of impurity (5); in addition, with RP_2 mobile phase, samples (for example, sample E) which contained an unknown impurity closely preceding the main peak, showed an overlap of this latter compound with the impurity (5). It once again caused an inaccuracy in the quantitative analysis. In addition, the RP_2 eluent did not permit the separation of 6α-methylprednisolone (1) and 20(α + β)-dihydro-6α-methylprednisolone acetate (2); a longer analytical time (*ca* ×2) was needed to obtain the elution of the main peak and of the most usual related substances (Table 2, Fig. 5). Finally, with the RP_2 eluent, the presence of a spurious peak was observed with rrt 0.86 which rapidly increased during the storage of the solution for 30 min or longer.

Consequently, the proposed RP_1 method was shown to be more suitable than the European Pharmacopoeia method [6].

Normal-phase chromatography (NP_1–NP_2 methods)

The NP_1 and NP_2 eluents showed a general comparable selectivity and order of elution [Fig. 6(a) and (b)]. In particular, the NP_2 eluent seemed to improve the separation in the zone of the chromatogram following the main compound and permitted lower elution times; this is especially important for the analysis of 6α-methylprednisolone (1) [rrt 3.41, rt 16.97 min, Fig. 6(b)].

Both these eluents (NP_1 and NP_2) showed some disadvantages. In the first place, prednisolone acetate (3) was not well separated from the main compound, especially considering the small expected percentage of this impurity (<1%) (rrt 1.09, rt 16.54 min with the NP_1 eluent).

Moreover, Fig. 7 shows that 21-dehydro-17-deoxy-6α-methylprednisolone (8) (rrt 0.58) and 6α-methylprednisone acetate (5) (rrt 0.55), were unresolved (rt 9.33 min); a long analysis time (>2.5 times as long as the main peak) is necessary for the detection of 20(α + β)-dihydro-6α-methylprednisolone acetate (2) (rrt 2.36, rt 38.30 min), with the NP_1 eluent. On the other hand, these chromatographic procedures enabled 6α-methylprednisone

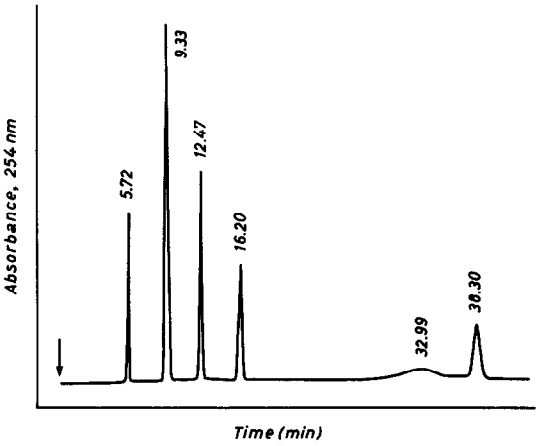

Figure 7
Chromatogram of MPA-related impurities mixture. Normal-phase NP_1 method. Column: Zorbax Sil, 250 × 4.6 mm i.d. Room temperature. Flow rate 1 ml min^{-1}. (6) 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one (rt 5.72 min; rrt 0.35); (5) + (8) 6α-methylprednisone acetate + 21-dehydro-17-deoxy-6α-methylprednisolone (rt 9.33 min; rrt 0.55 and 0.58); (4) 6α-methylhydrocortisone acetate (rt 12.47 min; rrt 0.77); (MPA) 6α-methylprednisolone acetate (rt 16.20 min; rrt 1.00); (7) 21-dehydro-6α-methylprednisolone (rt 32.99 min; rrt 2.04); and (2) 20(α + β)-dihydro-6α-methylprednisolone acetate (rt 38.30 min; rrt 2.36).

acetate (5) (rrt 0.55, rt 9.33 min), 6α-methylhydrocortisone acetate (4) (rrt 0.77, rt 12.47 min) and 21-acetoxy-11β-hydroxy-6α-methylpregna-1,4,17(20)-trien-3-one (6) (rrt 0.35, rt 5.72 min) to be well separated. This permits the detection of the last mentioned impurity (6), that could pass unnoticed with the reversed-phase method, unless high flow rates are used with the consequent increase of the operating pressure.

The quantitative analysis of sample A performed with NP_1 and NP_2 methods gave comparative results, as reported in Table 3.

In conclusion, the results demonstrate the need for complementary information resulting from different chromatographic systems, for a careful confirmation of the identity of the related substances. The proposed method (RP_1 reversed phase) was more suitable than the other methods reported. The proposed method has a greater general resolution capability in reasonable analysis time and is sensitive and easy to perform. In addition, none of the substances examined showed any decomposition in the mobile phase. Consequently, the proposed method could also be carried out for the quantitative assay of MPA.

Table 3
Relative retention time (rrt) and per cent content of MPA-related impurities in sample A by normal-phase chromatography with NP_1 and NP_2 methods

NP_1 method		NP_2 method	
rrt	Content (%)	rrt	Content (%)
0.58	0.12	0.57	0.02
0.77	0.17	0.81	0.12
0.81	0.13	0.90	0.46
1.00*	—	1.00†	—
1.09	0.30	1.25	0.06
2.41	0.16	1.30	0.10
		1.69	0.17
		2.12	0.06
		3.41	0.04
	Σ0.88		Σ1.03

*MPA mean retention time in eluent NP_1: 15.17 min (n = 10).
†MPA mean retention time in eluent NP_2: 4.98 min (n = 10).

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