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A stability-indicating HPLC method for medroxyprogesterone acetate in bulk drug and injection formulation

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Abstract

A stability-indicating HPLC assay method has been developed and validated for medroxyprogesterone acetate (MPA) in bulk drug and injectable suspension. An isocratic RP-HPLC was achieved on a Hichrom C_{18} column (150 mm × 4.6 mm i.d., 5 μm) utilizing a mobile phase of methanol 0.020 M acetate buffer pH 5 (65:35, v/v) and a photodiode array detector at 245 nm. The stress testing of MPA was carried out under acidic and alkaline hydrolysis, and oxidation conditions. MPA was well resolved from its degradation products, a main related substance (megestrol acetate) and two preservatives (methyl paraben and propyl paraben) with the resolution ≥2. The proposed method was validated for selectivity, linearity, accuracy, precision and solution stability. The method was found to be suitable for the quality control of MPA in bulk drug and injections as well as the stability-indicating studies.

Keywords: Medroxyprogesterone acetate; Bulk drug; Injection; Stability-indicating assay; Reversed-phase HPLC

1. Introduction

Medroxyprogesterone acetate (MPA), 6-α-methyl-3, 20-dioxopregn-4-en-17-yl acetate, is a synthetic progestational agent used for contraception and treatment of hormone-dependent cancers, especially breast cancer [1,2]. According to USP 28, the current assay for MPA bulk drug and its related impurities employs an RP-HPLC, whereas the assay for MPA in an injectable suspension is a NP-HPLC assay which is a time-consuming method since the sample extraction is required prior to HPLC injection [3].

Up to now, several methods including spectrophotometry [4,5], HPLC [6–9], LC–MS [10–16], GC–MS [16–20] and immunoassay [21] have been published for determining MPA in pharmaceutical preparations and biological fluids. Two stability-indicating HPLC methods for MPA tablet formulations have been reported. However, MPA is not well resolved from its related impurities and degradation products [8,9]. Moreover, a stability-indicating method for MPA injection has not been developed.

Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of MPA in bulk drug and injection formulations. The established method was validated with respect to specificity, linearity, precision, accuracy and ruggedness. In addition, forced degradation studies were performed in order to prove the suitability of the method for the stability-indicating assay of MPA.

2. Experimental

2.1. Chemicals

MPA reference standard was purchased from Bangkok Chemart (Bangkok, Thailand). Progesterone (PG) and megestrol acetate (MGA) standard were kindly supported by Bureau of Drug and Narcotic, Department of Medical Sciences, Ministry of Public Health, Thailand. MPA raw material was kindly supplied by V & S Chemical Group Co. Ltd., Thailand. A

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3-ml MPA injection used in this study was labeled to contain 150 mg of MPA and the excipients, i.e., polyethylene glycol 4000, methyl paraben (MP), propyl paraben (PP) and sodium chloride. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). MP, PP and other chemicals used were analytical grade.

2.2. Instrumentation and chromatographic conditions

An HPLC system consisted of an Agilent 1100 series pump, a solvent degasser, an autosampler, a photodiode-array detector (DAD) and Chemstation software version A.08.01 (Agilent, USA). The column used was a 150 mm × 4.6 mm, 5 μm, Hichrom C18 column. The separation was carried out under isocratic elution with 65:35 (v/v) methanol/acetate buffer (0.02 M sodium acetate, adjusted to pH 5.0 with glacial acetic acid). The flow rate was 1.0 ml min^{-1}, the column temperature was 35 °C, the wavelength was monitored at 245 nm, and the injection volume was 20 μl. The assay procedure was performed using internal standard method with PG as internal standard (IS).

2.3. Preparation of standard and sample solutions

2.3.1. Standard preparation

Standard stock solutions of 0.5 mg ml^{-1} of MPA and PG in mobile phase were prepared in separate volumetric flasks. Working solutions were prepared by diluting the stock solutions with the mobile phase to contain 100–300 μg ml^{-1} of MPA and 150 μg ml^{-1} of PG as IS. The standard mixture solution of MPA, IS and all the excipients as presented in the suspension was prepared for a specificity test. MGA, a known potential related impurity, was also added in the standard solution for a purity test of bulk drug. The resolution should not be less than 1.5 according to the USP 28 [3].

2.3.2. Sample preparation

Injectable suspensions from 20 vials were pooled and shaken on a mechanical shaker for 30 min. A portion of the suspension equivalent to MPA 50 mg was weighed, and transferred into a 50-ml volumetric flask. Ten milliliters of methanol was added. The mixture was shaken for 10 min, then adjusted to volume and mixed well. A 2-ml aliquot of the solution was transferred to a 10-ml volumetric flask containing 1500 μg IS. The sample was diluted to volume with mobile phase, and filtered through a 0.45 μm nylon syringe filter.

2.3.3. Degradation of MPA in acidic, basic and oxidative condition

MPA at a concentration of 0.5 mg ml^{-1} was used in all the degradation studies. The samples were subjected to stress conditions in 1N HCl, 1N NaOH and 3% H_2O_2 at room temperature and 80 °C for 30 min. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

3. Results and discussion

3.1. Optimization of the HPLC condition

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the study of resolution factor of MPA peak from the nearest resolving peak. Peaks were identified using retention times compared with those of standards and the characteristic spectra were confirmed by photodiode array detection (range 200–400 nm).

Effects of pH (3–7) and ionic strength (5–50 mmol l^{-1}) were investigated using phosphate and acetate buffer. It was found that the retention of MPA was not significantly different at pH 5–7 and ionic strength between 20–50 mmol l^{-1}. For good column lifetime reason, a buffer with pH 5 and ionic strength at 20 mmol l^{-1} was selected for aqueous component in the mobile phase. However, the acetate buffer was preferred since the phosphate buffer has no buffering capacity at pH 5. Methanol-20 mM acetate buffer (pH 5) (65:35, v/v) was found to achieve the complete separation within 12.5 min. In the optimized conditions, MPA was well separated from MGA and PG as shown in Fig. 1A. Additionally, peaks of minor impurities in bulk drug and suspension sample were observed to separate from the peak of MPA (Fig. 1B and C). The peaks of MP and PP used as preservatives in injectable suspensions were also observed in chromatogram with no interference peak from other excipients (Fig. 1C). The retention times of MP, PP, MGA, MPA and IS in the typical chromatogram illustrated in Fig. 1A were observed to be 2.41, 3.87, 9.41, 10.25 and 11.37 min, respectively.

3.1.1. Degradation of MPA

The stability-indicating capability of the assay was examined by accelerated stress testing. Standards and samples were subjected to degradation under alkaline, acid and oxidative conditions. MPA was degraded to numerous products, especially under the basic condition. The retention times of degradation products were shorter than that of MPA, indicating that the degradation products were more polar than their parent drug.

Regarding the alkaline condition, MPA was found to decompose rapidly. The major degradant peak was eluted at 9.47 min (Fig. 2B). After heating at 80 °C for 15 min in basic solution, the most severe degradation was observed and MPA was found

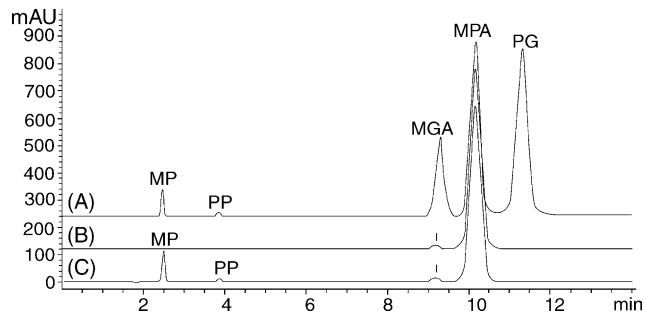

Fig. 1. Typical chromatograms of: (A) standard mixture solution with IS, (B) MPA raw material, and (C) MPA injection. For the chromatographic conditions, see Section 2.2.

to completely disappear. Accordingly, the height of the peak at 9.47 min decreased with the appearance of many new peaks having shorter retention times (Fig. 2C). MPA slowly degraded in hydrogen peroxide and the concentration of MPA was more slightly decreased after heating. Under acid condition, mild degradation was also found at room temperature and more degradation was observed after heating at 80 °C.

The degradation products were well resolved from MPA and IS, confirming the stability-indicating power of the method. DAD spectral analysis was used to verify the homogeneity of the MPA peaks in all solutions. The peak purity was greater than the threshold value of 995.

3.1.2. System suitability

The system suitability parameters including capacity factor (k'), selectivity (α), resolution (Rs) and asymmetric factor (As) listed in Table 1 were established by 10 replicates. All parameters were satisfactory with good specificity for the stability assessment of MPA.

3.2. Stability of MPA and IS in solution

The stability of MPA and IS in the mobile phase was assessed by analyzing the standard mixture solution (200 μg ml^{-1} MPA and 150 μg ml^{-1} IS) at 0, 4, 8, 12, 24, 36 and 48 h. after

Table 1
System suitability parameters

Parameter	MP	PP	MGA	MPA	PG	Preferable levels
k'	0.43	1.29	4.55	5.05	5.71	
α	–	3.04	1.64	1.11	1.13	1.02–2.0
Rs	4.63	9.70	6.65	2.08	2.60	>1.5
N	5815	8465	9497	9881	10688	>2500
As	0.80	0.82	0.97	0.88	0.87	<1.5

Table 2
Summary of the method validation parameters for MPA

Linearity and range	MPA
Range (μg ml^{-1})	100–300
r^2	0.9995
Slope	0.0049
Intercept	0.0181

	Peak area[a]		Retention time (min)[a]		Peak area[b]		Retention time (min)[b]	
	MPA	PG (IS)	MPA	PG (IS)	MPA	PG (IS)	MPA	PG (IS)
Precision								
System precision (n = 10)								
Mean	9987.79	10198.86	10.25	11.37	8934.04	9720.92	10.20	11.31
%R.S.D.	0.24	0.15	0.23	0.24	0.41	0.44	0.84	0.83

	Analyst 1					Analyst 2
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Method precision (n = 6)						
%R.S.D.[c]	0.82	0.76	1.25	0.98	0.49	1.59
Intermediate precision (5 days)						
%R.S.D.	1.28					

	Mean recovery[d] (%)				
	50[e]	75[e]	100[e]	125[e]	150[e]
Accuracy					
1.	99.73	99.85	100.53	101.34	101.51
2.	98.88	99.80	101.59	101.95	102.13
3.	100.50	100.51	101.65	101.81	102.28
Mean (n = 3)	99.70	100.06	101.26	101.70	101.97
%R.S.D.	0.74	0.35	0.56	0.29	0.36

[a] Standard.
[b] Sample.
[c] Value for six replicates and three injections for each replicate.
[d] Mean value for three injections.
[e] Level (%).

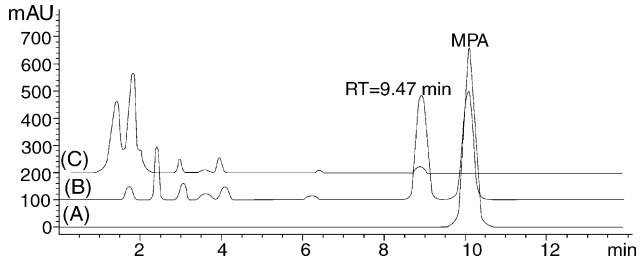

Fig. 2. Chromatograms of: (A) MPA standard, (B) MPA in 1N NaOH at room temperature, and (C) MPA in 1N NaOH at 80 °C.

preparation. The chromatogram showed no peak corresponding to the degradation products and there was no significant change in the peak area response of MP and PG. The results indicated that a standard mixture solution was stable in the mobile phase for at least 48 h.

3.3. Validation of assay method

3.3.1. Linearity and range

Linearity of system was determined by analysis of three replicates of five concentrations of standard solutions (range from 100 to 300 μg ml^{-1}) containing 150 μg ml^{-1} IS. The calibration curve showed good linearity over the concentration range. The regression line was $y = 0.0049x + 0.0181$ with a correlation coefficient (r^2) of 0.9995. The relative standard deviation (R.S.D.) of the slope of the five linear regressions prepared on 5 different days was 1.19%. Linearity of method, determined by plotting the amount of MPA found against the amount added over the range of 50–150% of label amount, showed good linearity with $r^2 = 0.9998$.

3.3.2. Precision

3.3.2.1. System precision. Ten replicates ($n = 10$) of a standard mixture solution (200 μg ml^{-1} MPA and 150 μg ml^{-1} IS) and a sample solution were analyzed to assess system precision. The R.S.D. of peak area response and retention time in Table 2 showed the satisfactory repeatability of the system (<1%).

3.3.2.2. Method precision and intermediate precision of the method. Six replicates ($n = 6$) of sample solutions were analyzed in the same day to determine method precision and in five different days to evaluate intermediate precision. The low R.S.D. (<2%) showed the suitability of the method for the determination of MPA in an injectable suspension. The method precision and intermediate precision were summarized in Table 2.

3.3.3. Accuracy

The accuracy was evaluated by the recovery studies which were carried out by spiking five known amounts of MPA in placebo suspension (range from 50–150% label amount). Three samples were prepared at each concentration. The recovery of added drug was calculated by comparing the ratio of MPA peak area to IS peak area of the test samples with that of the standard solutions. As shown in Table 2, the average recovery at each level was within 100 ± 2% and the R.S.D. at each level was ≤1%.

Table 3
Summary of the validation parameters for MP and PP

	MP	PP
Linearity and range		
r^2	0.9991	0.9996
Slope	104.84	93.489
Intercept	36.81	−0.1233
Range (μg ml^{-1})	3.6–10.8	0.4–1.2
Precision (%R.S.D.)		
System precision ($n = 10$)		
Peak area	0.36	0.86
Retention time	0.16	0.23
Method precision ($n = 6$)[a]		
Day 1	3.00	3.91
Day 2	2.86	3.08
Day 3	4.81	5.48
Day 4	0.57	1.51
Day 5	0.53	2.02
Intermediate precision (5 days)	3.07	5.06
Accuracy at 100% level ($n = 6$)		
Mean recovery (%)	93.00	94.58
R.S.D. (%)	0.83	3.07

[a] Value for six replicates and three injections for each replicate.

3.3.4. Ruggedness

The ruggedness was established by determining MPA in injections using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision (Table 2). Additionally, good separations were always achieved which suggested that the method was selective for all components under the test.

3.3.5. Limit of detection (LOD) and limit of quantitation (LOQ) for MGA

The LOD and LOQ were obtained from the calibration curve of MGA, the potential impurity in bulk drug. The LOD and LOQ were calculated based on the standard deviation (S.D.) and the slope (S) of the calibration curve using the formulae 3.3 and 10 δ/S, respectively. The LOD and LOQ concentrations were found to be 0.85 and 2.84 μg ml^{-1} for 20 μl injection volume.

3.4. Determination of MP and PP

The proposed method was also applied for monitoring MP and PP presented in MPA injectable suspensions. However, the external standard method was preformed according to the great difference between the concentration of these preservatives and IS. The validation data of MP and PP was reported in Table 3.

4. Conclusion

A simple isocratic RP-HPLC method was successfully developed to separate MPA from related substances, impurities, degradation products and two preservatives (MP and PP) simultaneously. The method was found to be specific, linear, precise and accurate. All validation parameters were within the

acceptance range. The advantages of the proposed method in comparison to the compendial method are shorter analysis time, less toxic organic solvent used and no sample extraction required. The developed method is considered to be reliable and suitable for the routine quality control and stability-indicating studies of MPA. Besides, the method can be applied for the determination of MP and PP in injection formulations.

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