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Compatibility Method Validation of Medroxyprogesterone Acetate And Estradiol Cypionate Combination Drug In Injectable Suspension Dosage Forms

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ABSTRACT

A simple RP-HPLC compatible method for the injectable suspension dosage of combined medroxyprogesterone acetate and estradiol cypionate was developed and validated according to ICH and USP guidelines. The chromatographic separation was achieved by using the Zorbax Eclipse C18 column (50 mm×4.6 mm, 2.7 µm) with gradient elution technique at a flow rate of 1.0 ml/min. The UV detection was performed at 225 nm. The linearity of medroxyprogesterone acetate over the concentration range was 49.65 to 744.69 µg/ml and 10.15 to 152.28 µg/ml for estradiol cypionate respectively. The accuracy was evaluated by means of spike recovery method and the result showed in the range of 99.7% to 101.4% for medroxyprogesterone acetate and 98.6% to 101.8% for estradiol cypionate. The specificity of the method showed that the analyte was not interfered by the presence of co-formulated substances. The robustness of the study was found agreeable; hence it proves that the method was robust. The stability of the analyte was found stable for 24 hours. The developed method was successfully employed for the determination of combined medroxyprogesterone acetate and estradiol cypionate in injectable suspension dosage forms.

Keywords: Medroxyprogesterone acetate, Estradiol cypionate, RP-HPLC, Validation

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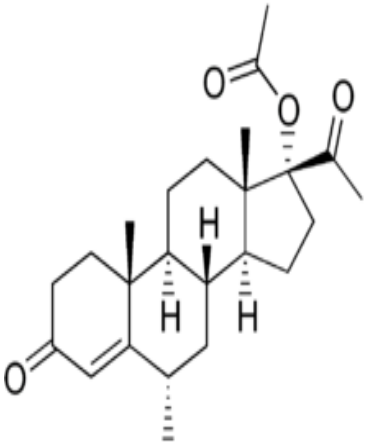
INTRODUCTION

Medroxyprogesterone acetate (MPA) also known as 17α-hydroxy-6α-methylprogesterone acetate or (Pregn-4-ene-3,20-dione,17-(acetyloxy)-6-methyl-(6(alpha)) (Figure 1), is a steroidal progestin and also a synthetic variant of the human hormone progesterone. [1, 2] It is a white to off-white, odorless crystalline powder which it freely soluble in chloroform, sparingly soluble in alcohol and practically insoluble in water[3]. MPA is a potent derivative of its medroxyprogesterone (MP). MPA is one of the listed important medication according to World Health Organization's (WHO) List of Essential Medicines[4]. Estradiol cypionate (EC) (Figure 2), is a synthetic ester, of the natural estrogen of estradiol. Chemically, it is a 3-cyclopentylpropanoyl ester or [estra-1,3,5,(10)-triene-3,17-diol,(17(beta))-,17-cyclopentanepropanoate][5, 6]. It is a white to off-white crystalline powder which is soluble in alcohol and practically insoluble in water.[7] The MPA and EC was indicated as a contraceptive medicine, and in the treatment of HRT (Hormonal replacement thepary), as well as in endometriosis[8]. MPA and EC mainly involve in the prevention of follicular maturation and ovulation. The literature survey reveals few methods are available which include the pharmacokinetics study[9, 10] and estimation by mass spectrometry[11] but there is no specific method was available for estimation of both MPA and EC in a single method. In the present study the RP-HPLC method is developed for the determination of MPA and EC in injectable suspension dosage forms and validated according to ICH and USP guidelines.



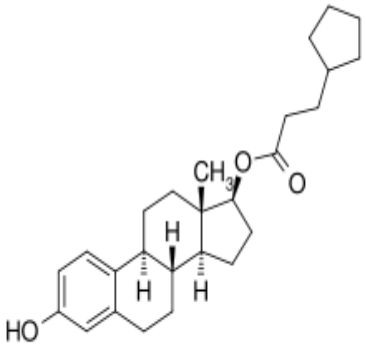


Figure 1 & 2: Structure of Medroxyprogesterone acetate & Estradiolcypionate

MATERIALS AND METHODS:

Instrumentation and Analytical Conditions:

Instrument used for the present study was of Agilent 1200 series HPLC equipped with DAD & VWD detector having single pumping method.

Materials:

Medroxyprogesterone acetate standard and estradiol cypionate standard were kindly provided by Caplin Point Laboratories Limited, Chennai, India. The pharmaceutical preparations of combination of cyclofem, were obtained from PT. Tunggal, Indonesia. The HPLC grade of Acetonitrile (Finar, Ahmedabad), Water (Pall Cascada AN water, New York) was used.

Preparation of Diluent solution 1:

A mixture of Ethanol and Water (90:10 v/v).

Preparation of Diluent solution 2:

A mixture of Acetonitrile and Water (80:20 v/v).

Preparation of Standard Solutions:

Standard stock solutions containing MPA and EC were prepared individually by dissolving 125 mg of MPA and 25 mg of EC, and made up to volume to 50 mL with diluent solution 1. Transferred 5mL of MPA solution and 5mL of EC solution in 25 mL volumetric flask, dilute with diluent 2 and made up the volume with diluent 2.

Preparation of Sample Solutions:

Collected 30 numbers of vials and mixed it properly. Find out the weight per mL at 25°C, Weighed 2.5 g of sample in to a 50 mL volumetric flask, diluted with diluent 1 solution and volume made with dilute 1. Transferred 5mL of this solution in 25 mL volumetric flask, diluted with diluent 2 and volume made up with diluent 2.

RESULTS AND DISCUSSION:

Method Development:

During the stage of method development initially the column of C18 (Zorbax Eclipse, 50 mm×4.6 mm, 2.7 µm) was chosen according to the basic structure of the molecules of MPA and EC. For mobile phase selection the solvents of methanol and water of varied compositions was used. The mobile phase of methanol and water showed sharp and early eluted peak for MPA and broad, higher elution timed peak for EC due to its poor solubility. Hence, decided to bring the aprotic nature of the acetonitrile solvent instead of methanol to bring down the EC peak to elute earlier with good peak shape. During this trail run the acetonitrile: water showed the good peak shape for MPA and EC, but still it take more elution time for EC due to less organic in the mobile phase composition. Finally, the ratio of acetonitrile was increased to bring the EC earlier but the MPA peak eluted too early.

Hence, decided to go with the gradient method to bring the both MPA and EC with reasonable retention time within 10 minutes. The ratio of Water: Acetonitrile (40: 60 v/v) for MPA and with Water: Acetonitrile (20: 80 v/v) for EC was finalized. The chromatogram showed the good peak shape and of lesser elution time of EC. The chromatograms were showed in Figure 3. The flow rate was settled to 1.0 mL/min. The drugs of MPA and EC showed maximum absorbance at 225nm, was selected as wavelength for further analysis. . The mobile phase was prepared and filtered by using 0.45µ membrane filter and then ultrasonicated for 15 min before use.

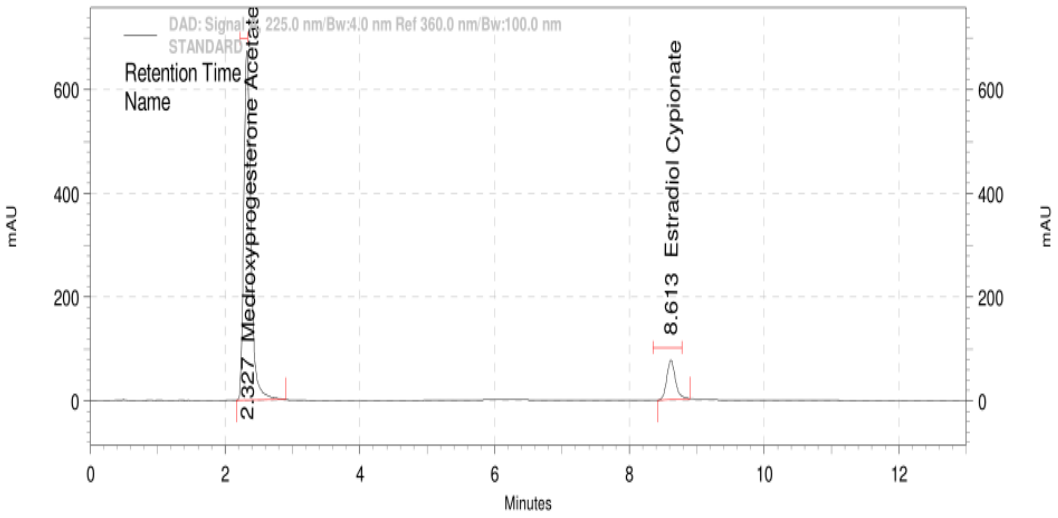


Figure 3: Chromatogram of Medroxyprogesterone acetate and Estradiol cypionate

Optimized Chromatographic Conditions:

Column : C_{18} (Zorbax Eclipse, 50 mm×4.6 mm, 2.7 µm)

Temperature : 30 °C

Inject volume : 20 µL

Wave length : 225nm

Flow rate : 1.0 mL/min

Run time : 13 min.

Mobile Phase :

Time in minutes	Water	Acetonitrile
0.00	40	60
3.30	40	60
4.50	20	80
9.00	20	80
10.00	40	60
13.00	40	60

Method Validation:

The proposed HPLC method was validated as perICH[12,13] and USP guidelines[14].

System suitability:

The system suitability of MPA and EC were assessed by comparing the Theoretical plates, Asymmetry factor, Retention time of standard MPA& EC. The results were showed in Table 1.

Table 1. System Suitability of MPA and EC

	Retention time	Theoretical plates	Tailing factor
MPA	2.327	2504	1.32
EC	8.613	22228	1.17

Linearity:

Linearity of MPA and EC was studied by preparing the standard solutions at different concentration levels. The linearity range was showed over the concentration range of 50-740 µg/ml for MPA and 10-150 µg/ml for EC respectively. The coefficient of correlation of MPA and EC (r) was found to be 0.999 and 0.999 respectively. The results obtained for the calibrations plots of MPA and EC were showed in Figure 4 and 5.

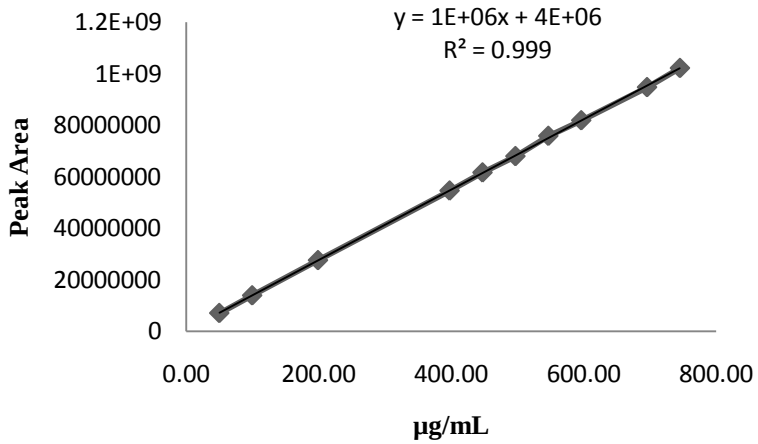


Figure 4: Calibration curve of Medroxyprogesterone acetate

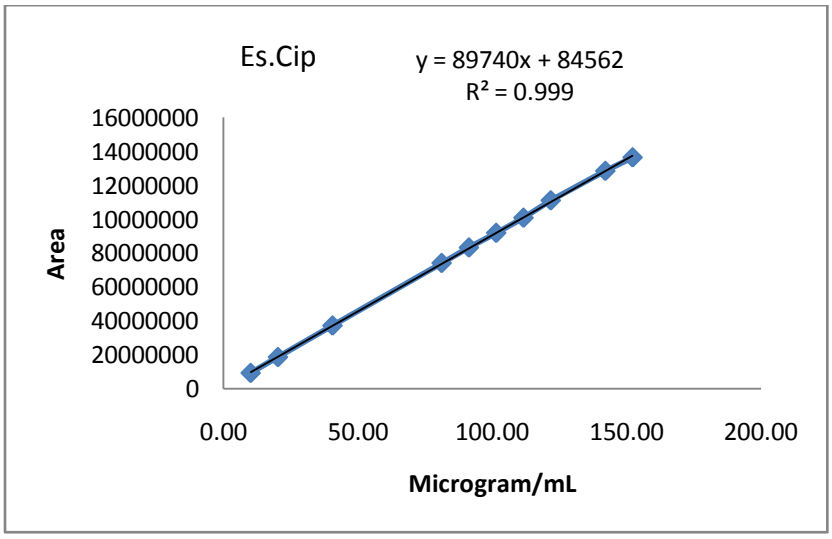


Figure 5:Calibration curve of Estradiol cypionate

Precision:

Precision was evaluated by carrying out six independent sample preparation on the same day (Intra-day) and of three consecutive days (Inter-day) of a single lot of formulation of MPA and EC. The Percentage relative standard deviation was found to be less than 2%which proves that the method was precise. The summarized results were showed in Table 2 and Table 3.

Table 2. Summarized data of Method Precision

S. No.	Peak Area of MPA			Peak Area of EC		
	Std	Sample	Assay %	Std	Sample	Assay %
1	672784342	669229847.0	102.3	90083302	87956320.0	102.7
2	670655458	671111133.0	102.6	89603509	87285457.0	101.9
3	670972112	675727678.0	103.3	89771230	86996012.0	101.5
4	669614104	668053434.0	102.1	89419932	87777286.0	102.5
5	669618262	676727672.0	103.5	89425502	87358817.0	102.0
6	669975440	665612856.0	101.8	89745976	86078305.0	100.5
Mean	670603286.33	671077103.3	102.6	89674908.50	87242032.8	101.9
SD	1203220.99	4381115.4	0.66710	250371.00	667385.2	0.78930
% RSD	0.18	0.65	0.65	0.28	0.76	0.77

Table 3. Summarized data of Intra-day Precision

S. No.	Peak Area of MPA			Peak Area of EC		
	Std	Sample	Assay %	Std	Sample	Assay %
1	682784524	678499942.0	102.6	89503511	86056358.0	101.3
2	680656008	680145837.0	102.9	89833537	86306466.0	101.6
3	680857117	679527975.0	102.8	89811297	87456514.0	102.9
4	679681125	688553678.0	104.2	89559898	87195774.0	102.6
5	671118201	682957231.0	103.3	89685709	86959889.0	102.3
6	670976085	681892766.0	103.2	89213934	87147521.0	102.6
Mean	677678843.33	681929571.5	103.2	89601314.33	86853753.7	102.2
SD	5234711.78	3623477.8	0.54647	230862.24	550131.9	0.63061
% RSD	0.77	0.53	0.53	0.26	0.63	0.62

Compatibility Analysis of Commercial injection Results:

"Cyclofem" (MPA-25 mg + EC-5mg) commercial injection were analyzed (obtained from PT. Tunggal, Indonesia) and the Results are accurate and reproducible with respect to the label claim proves that the method is valid and compatible. The results are showed in Table 4.

Table 4. Summarized data of Commercial (Cyclofem) Injection

S. No.	Peak Area of MPA			Peak Area of EC		
	Std	Sample	Assay %	Std	Sample	Assay %
1	693458924	679845871.0	100.4	88759248	86345862.0	101.4
2	695468920	681548248.0	100.7	89045862	86297845.0	101.4
3	691254852	679854965.0	100.4	89235482	87045892.0	102.3
4	695489124	687895426.0	101.6	89578542	87197895.0	102.4
5	689548562	685894252.0	101.3	89987546	86987542.0	102.2

6	692563142	683548621.0	101.0	89984521	87045892.0	102.3
Mean	692963920.67	683097897.2	100.9	89431866.83	86820154.7	102.0
SD	2351293.94	3302506.0	0.48409	505114.32	392525.6	0.46904
% RSD	0.34	0.48	0.48	0.56	0.45	0.46

Recovery studies:

To check the degree of closeness of the method, recovery studies were performed at 50%, 80%, 100%, 120% and 150% levels. Known amounts of standard MPA and EC were added to pre-analyzed samples and were subjected to the proposed HPLC method. The summarized results of recovery studies were showed in Table 5.

Table 5.Accuracy data of MPA and EC

Accuracy of MPA				
Level (%)	**Peak Area**	**Amount recovered**	**Amount Added**	**Recovery (%)**
50	345360677	251.56	248.11	101.4
80	545523796	397.36	396.97	100.1
100	679217335	494.75	496.21	99.7
120	818201320	595.98	595.46	100.1
150	1021779172	744.27	744.32	100.0
Accuracy of EC				
50	46911698	51.01	50.76	100.1
80	74784439	81.01	81.22	99.7
100	92368024	100.05	101.52	98.6
120	114435376	123.96	121.82	101.8
150	141462761.7	153.23	152.28	100.6

Robustness:

The robustness was evaluated by changing the small deliberate variations in the optimized method conditions. The summarized data of robustness parameters were showed in Table 6.The results are found within the acceptance criteria.

Table 6.Robustness Study of MPA and EC

Parameter			**Area**	**RT**	**Plate**	**Tailing**
Flow rate (mL)	0.9	MPA	741013106.67	2.56	2199	1.38
		EC	99346113.00	9.15	19133	1.25
	1.1	MPA	741165496.33	2.55	2163	1.39
		EC	101213280.67	9.15	19037	1.28
Column temperature (°C)	25	MPA	657875511.67	2.42	2525.7	1.29
		EC	91195590.33	9.05	17946	1.27
	35	MPA	668125415.00	2.23	2215	1.32
		EC	91699222.33	8.18	19489	1.29
Wavelength (nm)	223	MPA	615651061.33	2.28	2241	1.37
		EC	96863266.83	8.53	21120	1.35
	227	MPA	780036772.33	2.29	2144	1.33
		EC	76705340.00	8.53	21126	1.30

Stability:

The prepared drug standard and sample solution was found to be stable, and it was performed by injecting the standard and sample solution up to 24 hours in interval of every 4 hours. The results are showed in Table 7.

Table 7. Stability Study of MPA and EC

Hours	MPA		EC	
	STD	SPL	STD	SPL
0	0.00	0.00	0.00	0.00
8	-0.11	0.16	-0.15	-0.11
16	0.10	0.03	0.10	0.05
24	0.13	0.08	0.07	0.15

Acceptance criteria is ±2.0.

CONCLUSION:

In conclusion, the proposed gradient RP-HPLC compatible method was developed and validated as per the ICH and USP guidelines. The method was of simple, stable, accurate, precise and robust technique as per the results found. Hence, it can be used for routine estimation of any Medroxyprogesterone acetate and Estradiol cypionate injectable suspension dosage forms.

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REFERENCES:

1. Medroxyprogesterone: Drug Information Provided by Lexi-Comp". Merck Manual. 2009-12-01. Retrieved 2010-07-08.
2. Lenco W, Mcknight M, Macdonald AS. Effects of cortisone acetate, methylprednisolone and medroxyprogesterone on wound contracture and epithelization in rabbits. Ann Surg1975; 181: 67–73.
3. http://www.rxlist.com/depo-subq-provera-drug.htm
4. WHO Model List of Essential Medicines. World Health Organization. October 2013. Retrieved 22 April 2014.
5. Roberts D. Dictionary of Steroids: Chemical Data, Structures, and Bibliographies. CRC Press. 1991; 415.

6. Index Nominum 2000: International Drug Directory. Taylor & Francis US. 2000; 405. Retrieved 20 May 2012.
7. http://www.rxlist.com/depo-estradiol-drug.htm
8. Product Information: Lunelle,medroxyprogesterone acetate and estradiol cypionate. Pharmacia & Upjohn, Kalamazoo, MI(PI Issued 10/2000) PI Reviewed 12/2000.
9. Zhou XF, Shao QX, Han XJ, Weng LJ, Sang GW. Pharmacokinetics of medroxyprogesterone acetate after single and multiple injection of Cyclofem in Chinese women. Contraception 1998;57:405–11.
10. Thurman A, Kimble T, Schwartz JL, Hall P, Archer DF. Medroxyprogesterone acetate and estradiol cypionate injectable suspension (Cyclofem) monthly contraceptive injection: steady-state pharmacokinetics. Contraception 2013; 87: 738-743.
11. Diaz-Cruz MS, Lopez de Alda MJ, Lopez R, Barcelo D. Determination of estrogens and progestogens by mass spectrometric techniques (GC/MS, LC/MS and LC/MS/MS). J Mass Spectrom 2003;38:917–23.
12. Anonymous, ICH Guidelines: Validation of Analytical Procedures: Text and Methodology Q2 (R1), 1996; 1-17.
13. ICH, Guidance for Industry Q2B Validation of Analytical Procedures: Methodology. 1996; 1-12.
14. United States Pharmacopeia-National formulary (USP-NF 35) section <1225> Validation of Compendial Procedures. 2010; 1-10.