

3.2.P.5.3 ENCLOSURE 5

*Method verification report of the BP method for determination of Impurity F
in Triclofem Injection*

VERIFICATION OF THE COMPENDIA BRITISH PHARMACOPOEIA (BP) TLC METHOD FOR DETERMINATION OF IMPURITY F OF MEDROXYPROGESTERONE ACETATE (MPA) IN TRICLOFEM INJECTION 150MG/ML	
PROTOCOL NO.	VMAP-1006-11
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1. PURPOSE

To verify that Thin Layer Chromatography (TLC) qualitative of BP method used for Impurity F of Triclofem Injection 150mg/ml consistently provides, at the same conditions, reproducible, and reliable results.

2. SCOPE

This protocol shall be applicable to the qualitative analytical method being done for Impurity F of Medroxyprogesterone Acetate (MPA) method for Triclofem Injection 150mg/mL performed at Quality Control Laboratory, PT Tungal Idaman Abdi.

Kind of Validation: ☐ New Method

☐ Revalidation

☒ Verification

☐ Change in the Analytical Procedure

☐ Change in Impurity Profile of Drug Substance

☐ Change in the Synthesis of the Drug Substance

3. RESPONSIBILITY

3.1 Analytical Development Analyst

- Execute analytical method validation according to the validation protocol.
- Record the results on working sheet provided.
- Report the validation execution and deviation founded during execution.

3.2 Analytical Method Pharmacist

- Prepare protocol and report of analytical method validation.
- Supervise the validation execution according to the protocol.
- Evaluate the validation report.

3.3 QA Manager

- Review and approve validation protocol and report.
- Evaluate the validation report.
- Ensure the execution of validation according to the protocol approved.

4. METHOD DESCRIPTION

This method is modification from British Pharmacopoeia (BP) 2013 since Medroxyprogesterone acetate for performance test EPCRS (containing 0.5% of impurity F) as reference standard is not available anymore in the market or withdrawn by EDQM, and replaced by combination of Related Compound A USP Reference Standard as Impurity F and Medroxyprogesterone acetate EPCRS.

4.1 Material

- Reference Standard of Medroxyprogesterone acetate EPCRS (Cat No.EPM0250000)
- Reference Standard of Related Compound A USP (Cat No.1378012)
- Finished Good Triclofem Injection 150 mg/ml
- Dichloromethane
- Tetrahydrofuran
- 1,1-Dimethylethyl methyl ether
- Hexane
- Toluenesulfonic acid
- Ethanol 96%

4.2 Product formulation

Component	Content in mg or ml
Medroxyprogesterone Acetate	150.0
Methyl Paraben	1.351
Propyl Paraben	0.147
Polysorbate 80	2.378
Sodium Chloride	8.582
Sodium Hydroxide 5%	qs
Hydrochloric Acid 5%	qs
Water for Injection ad.	1.0

4.3 Equipment

- Glass apparatus
- Calibrated Analytical Balance
- TLC silica gel plate (20x20 cm)
- Glass chamber
- Oven
- Camag UV cabinet at 365nm
- Syringe needle

4.4 Chromatographic conditions

Method : Thin Layer Chromatography (TLC)
Detector : UV cabinet at 365 nm
Plate : TLC silica gel plate (20x20 cm)
Mobile phase : Tetrahydrofuran (THF) : 1,1-Dimethylethyl methyl ether : Hexane = 10:45:45
Solvent : Dichloromethane
Application : 10 µl
Development 1 : Over a path of 10 cm
Drying 1 : in the air
Development 2 : Over a path of 10 cm in the same direction in mobile phase
Drying 2 : Dry in oven at 100 – 105°C about 10 minutes. Allow to cool.
Detection : Spray with a 200 g/L solution of toluenesulfonic acid in ethanol (96%). Heat at 120°C for 10 minutes. Allow to cool. Examine the chromatograms under UV light at 365 nm

4.5 Preparation of mobile phase

- Mix 100 volumes of THF, 450 volume of 1,1-Dimethylethyl methyl ether, and 400 volume of Hexane and allowed to equilibrate.
- Adjust volumes to 1000 volumes with Hexane.

4.6 Preparation of Standard Solution:

- Accurately weigh 100 mg of Reference Standard of Medroxyprogesterone acetate EPCRS and 25 mg of Reference Standard of Related Compound A USP to a 5 ml volumetric flask. (c-MPA = 20 mg/ml, c-Imp F = 5 mg/ml)
- Dilute to volume with solvent.

4.7 Preparation of Test Solution

- Transfer about 1.3 ml sample of Triclofem Injection 150mg/ml to a 10 ml volumetric flask. (c=19.5 mg/ml).
- Dilute to volume with solvent.

5. STRATEGY FOR VALIDATION

The following parameters shall be validated: Specificity, Repeatability, and LOD (Limit of Detection).

6. EXPERIMENTAL PLAN AND DATA EVALUATION

The analytical method validation will be executed as per the following plan:

- System suitability, the test is not valid unless the chromatogram obtained with standard solution shows two clearly separated spots.
- All parameters testing to be done as per Methodology.
- Laboratory supervisor will authorize any deviation from the plan. All deviations should be documented in the final Method Validation Report. Any deviation from the protocol will be documented and investigated. For example, if a parameter fails, it would be investigated, corrected and the tests for that parameter shall be repeated.
- All the chromatograms generated during the validation activity will be preserved.
- Printouts/picture, method file shall be attached for each activity.

7. PARAMETERS TO BE VERIFIED

7.1 Specificity

A study is to be carried out for the interference and separation of main spot of Medroxyprogesterone acetate and Impurity F in test solution of Triclofem Injection 150mg/ml. To verify that overlapping response is not occurs at the same Rf.

7.1.1 Performing the analysis

- Apply 10 μ l standard solution as described in point 4.6 on TLC silica gel plate.
- Prepare six test solutions as described in point 4.7.
- Apply 10 μ l of each test solution on TLC silica gel plate.
- Conduct the procedure under the chromatographic condition as described in point 4.4.
- Observe the appearance of MPA (main) spot by visual (naked eye).
- Observe the appearance of Impurity F spot under the chromatographic condition at UV light at 365 nm.
 - Calculate the Rf and RSD of Impurity F.
 - Observe appears of others spot.
 - Record the results on the Working Sheet IV.

7.1.2 Acceptance criteria:

Test	Description	Parameters	Acceptance Criteria
Interference	Spot observation	Rf	No overlapping between MPA (main) spot and Impurity F spot
		Rf	RSD of Rf Impurity F $\leq 2.0\%$
		Spot detection	No other spot detected under chromatographic condition

7.2 Repeatability

The method expresses the repeatability under aliquots of testing solution sample, which has been independently prepared according to the method procedure.

7.2.1 Performing the analysis

- Prepare six test solutions as described in point 4.7.
- Apply 10 μ l of each test solution on TLC silica gel plate.
- Observe the appearance of Impurity F spot under the chromatographic condition as described in point 4.4 at UV light at 365 nm.
- Calculate the Rf and RSD of Impurity F.
- Record the results on the Working Sheet V.

7.2.2 Acceptance criteria:

Test	Description	Parameters	Acceptance Criteria
Repeatability	6 replicates at 100% test concentration	Rf of Impurity F	RSD $\leq 2.0\%$

7.3 LOD (Limit of Detection)

The limit of detection is equal to or less than the reporting threshold of the test for Impurity F. The detection is described as a positive (detected) and negative (not detected).

7.3.1 Preparation of sample testing for LOD

LOD 7.5 % solution

Transfer 0.75 mL of the standard solution as described in point 4.6 to a 10 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.375 mg/ml corresponds to 75%.

LOD 5.0 % solution

Transfer 0.50 mL of the standard solution as described in point 4.6 to a 10 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.25 mg/ml corresponds to 50%.

LOD 2.5 % solution

Transfer 0.25 mL of the standard solution as described in point 4.6 to a 10 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.125 mg/ml corresponds to 25%.

LOD 1.0 % solution

Transfer 0.10 mL of the standard solution as described in point 4.6 to a 10 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.05 mg/ml corresponds to 10%.

LOD 0.5 % solution

Transfer 0.10 mL of the standard solution as described in point 4.6 to a 20 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.025 mg/ml corresponds to 5%.

LOD 0.3 % solution

Transfer 0.15 mL of the standard solution as described in point 4.6 to a 50 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.015 mg/ml corresponds to 3%.

LOD 0.2 % solution

Transfer 0.10 mL of the standard solution as described in point 4.6 to a 50 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.010 mg/ml corresponds to 2%.

LOD 0.1 % solution

Transfer 0.10 mL of the standard solution as described in point 4.6 to a 100 mL volumetric flask, dilute to volume with solvent mixture and mix. The final concentration of this solution is 0.005 mg/ml corresponds to 1%.

7.3.2 Performing the analysis:

- Prepare one test solution per concentration
- Apply 10 μ l of each standard for LOD solution as described in point 7.3.1 on TLC silica gel plate.
- Observe the appearance of Impurity F spot under the chromatographic condition as described in point 4.4 at UV light at 365 nm.
- Calculate the R_f of Impurity F.
- Record the results on the Working Sheet VI.

7.3.3 Acceptance criteria:

Test	Description	Parameters	Acceptance Criteria
LOD	on each concentration	Spot detection	Positive (detected)

8. RESULTS & EVALUATION

The raw data of each parameter testing is provided in working sheet.

8.1 Specificity

Sample solution	Results					Others spot by UV light at 365nm
	MPA spot (main spot) by visible (naked eye)	Impurity F				
		Spot by UV light at 365nm	Spot distance (cm)	Mobile phase distance (cm)	Rf	
Std.Solution	positive	positive	5.70	10.0	0.7125	negative
Test solution (Triclofem Injection)						
1	positive	positive	5.50	10.0	0.6875	negative
2	positive	positive	5.40	10.0	0.6750	negative
3	positive	positive	5.45	10.0	0.6813	negative
4	positive	positive	5.40	10.0	0.6750	negative
5	positive	positive	5.40	10.0	0.6750	negative
6	positive	positive	5.50	10.0	0.6875	negative
Average	-	-	-	-	0.6802	negative
RSD (%)	-	-	-	-	0.904	negative

Test	Description	Parameters	Acceptance Criteria
Interference	Spot observation	Rf	No overlapping between MPA (main) spot and Impurity F spot
		Rf	RSD of Rf Impurity F $\leq$ 2.0%
		Spot detection	No other spot detected under chromatographic condition

Remark:

positive = detected

negative = not detected

The results of specificity analysis are:

- The obtained response spot of MPA (main spot) by visible (naked eyes) and clear separation (no overlapping) with Impurity F under UV light 365 nm indicating no interference between each others.
- The obtained results of Impurity F sample solution under UV light at 365 nm meet the specification with %RSD of Rf impurity F is 0.904%.
- No others spot detected under visible and UV light at 365 nm.

8.2 Repeatability

Sample preparation	Sample Solution – Impurity F		
	Spot distance (cm)	Mobile phase distance (cm)	Rf
1	4.90	10.0	0.6125
2	4.85	10.0	0.6063
3	4.90	10.0	0.6125
4	4.95	10.0	0.6188
5	4.90	10.0	0.6125
6	5.05	10.0	0.6313
Average	-	-	0.6157
RSD (%)	-	-	1.401

Test	Description	Parameters	Acceptance Criteria
Repeatability	6 replicates at 100% test concentration	Rf of Impurity F	RSD $\leq$ 2.0%

The repeatability results from 6x preparation of sample solution are within the specification with the RSD of Rf Impurity F 1.401 %.

8.3 Limit of Detection (LOD)

Concentration of Imp.F		Spot by UV light 365 nm	Spot distance (cm)	Mobile Phase distance (cm)	Rf
(%)	mg/ml				
Std (100)	5.000	positive	5.10	10.0	0.6375
75	0.375	positive	4.75	10.0	0.5937
50	0.250	positive	4.65	10.0	0.5812
25	0.125	positive	4.70	10.0	0.5875
10	0.050	positive	4.65	10.0	0.5812
5	0.025	positive	4.60	10.0	0.5750
3	0.015	positive	4.95	10.0	0.6187
2	0.010	positive	5.00	10.0	0.6250
1	0.005	positive	5.00	10.0	0.6250

Test	Description	Parameters	Acceptance Criteria
LOD	on each concentration	Spot detection	Positive (detected)

Remark:

positive = detected

negative = not detected

The results of LOD test shows that ability of TLC method to detect until the concentration of Impurity F is 0.005 mg/ml.

9. CONCLUSION

The modification of BP method by TLC for Impurity F of MPA in finished product (Triclofem Injection 150mg/ml) has been validated according to the specified protocol.

All the acceptance criteria are achieved satisfactorily:

a. Specificity

The method is proven for Impurity F of MPA in Triclofem Injection with no overlapping spot or clear separation between MPA and impurity F observed, and no others spot detected under specified procedure.

b. Repeatability

The RSD of Rf Impurity F (sample solution) is achieved at 1.401% within the acceptance criteria of $\leq 2.00\%$.

c. Limit of Detection

The TLC method is able to detect the concentration of Impurity F of MPA until 0.005 mg/ml.

10. RECOMMENDATION

10.1 Apply EP TLC procedure for Impurity F of MPA procedure at the condition as follows:

- Method : Thin Layer Chromatography (TLC)
- Detector : UV cabinet at 365 nm
- Plate : TLC silica gel plate (20x20 cm)
- Mobile phase : Tetrahydrofuran (THF) : 1,1-Dimethylethyl methyl ether : Hexane = 10:45:45
- Solvent : Dichloromethane
- Application : 10 μ l
- Development 1 : Over a path of 10 cm
- Drying 1 : in the air
- Development 2 : Over a path of 10 cm in the same direction in mobile phase
- Drying 2 : Dry in oven at 100 – 105°C about 10 minutes. Allow to cool.
- Detection : Spray with a 200 g/L solution of toluenesulfonic acid in ethanol (96%). Heat at 120°C for 10 minutes. Allow to cool. Examine the chromatograms under UV light at 365 nm

10.2 Apply this BP TLC method for routine analysis to detect the Impurity F of MPA in finished product (Triclofem Injection 150mg/ml).

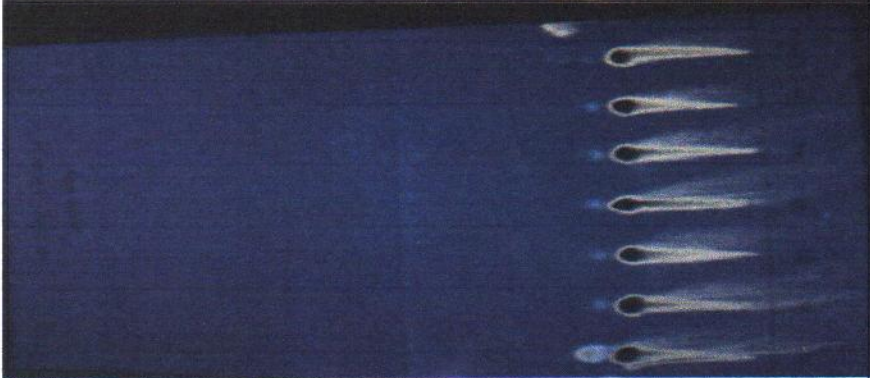
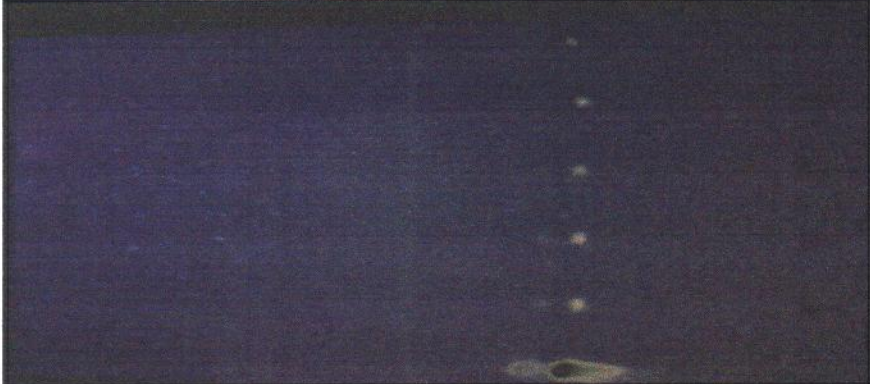
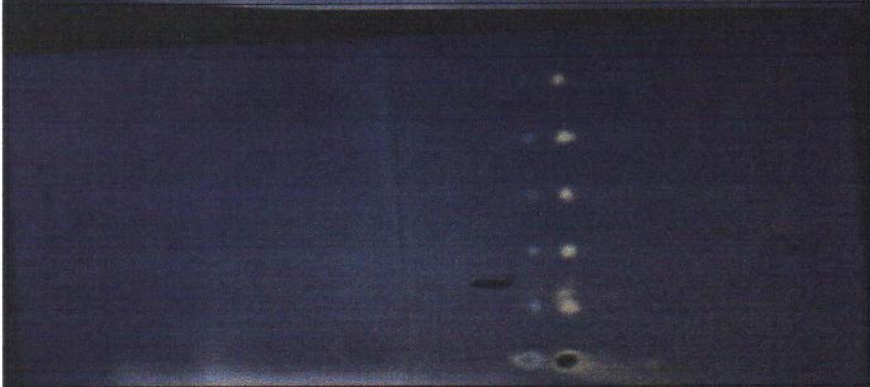
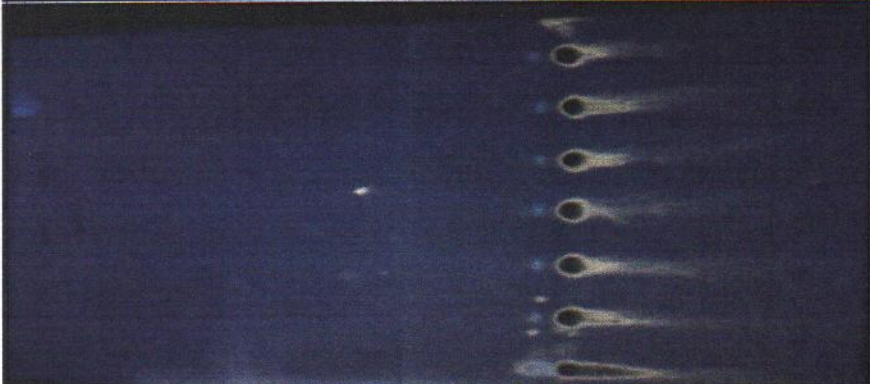
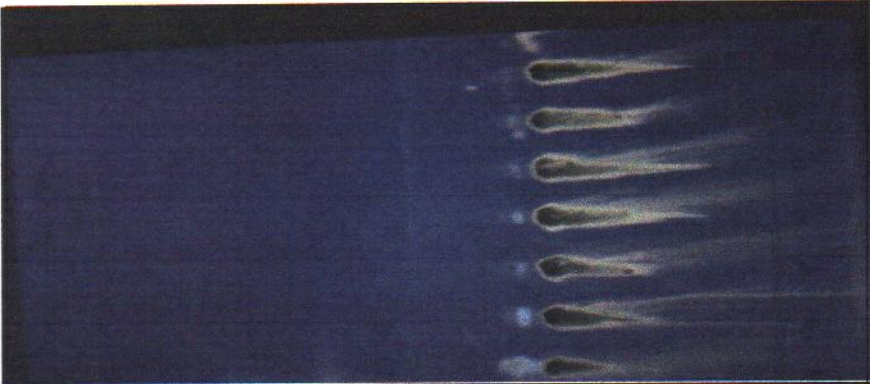
11. REFERENCES

- ICH Q2A "Guidance on Validation of Analytical Methods: Definitions and Terminology".
- ICH Q2B "Guidance for Industry – Validation of Analytical Procedures: Methodology".
- British Pharmacopoeia 2013
- CPOB (Local GMP) 2012.
- Testing method of Triclofem Injection #TM-FG1006
- Analytical Method Validation & Instrument Performance Verification, 2004
- SOP Validasi Metoda Analisa (Analytical Method Validation) #QAV-003

13. HISTORY LIST

Report version No.:	Reason for Compilation	Date
01	First version for approval	8 / 4 / 2014

TRICLOFEN INJ

				
Intermediate Precision	Limit of Detection (I)	Limit of Detection (II)	Repeatability	Specificity

20

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Verification Column : Fill with Available or Not available

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Verification Column : Fill with Available or Not available

VMAR-1006-11

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Verification Column : Fill with Available or Not available

IV. Specificity

Sample solution	Results					Others spot by UV light at 365nm
	MPA spot (main spot) by visible (naked eye)	Impurity F			Rf	
		Spot by UV light at 365nm	Spot distance (cm)	Mobile phase distance (cm)		
Std.Solution	positive	positive	5.70	10.0	0.7125	negative
Test solution (Triclofem Injection)						
1	positive	positive	5.50	10.0	0.6875	negative
2	positive	positive	5.40	10.0	0.6750	negative
3	positive	positive	5.45	10.0	0.6813	negative
4	positive	positive	5.40	10.0	0.6750	negative
5	positive	positive	5.40	10.0	0.6750	negative
6	positive	positive	5.50	10.0	0.6875	negative
Average	-	-	-	-	0.6802	negative
RSD (%)	-	-	-	-	0.904	negative

Test	Description	Parameters	Acceptance Criteria
Interference	Spot observation	Rf	No overlapping between MPA (main) spot and Impurity F spot
		Rf	RSD of Rf Impurity F $\leq 2.0\%$
		Spot detection	No other spot detected under chromatographic condition

Remark:

positive = detected

negative = not detected

Analyst : <i>Ms</i>	Reviewed : <i>for</i>
Date : <i>07/04/14</i>	Date : <i>8/4/14</i>

V. Repeatability

Sample preparation	Sample Solution – Impurity F		
	Spot distance (cm)	Mobile phase distance (cm)	Rf
1	4.90	10.0	0.6125
2	4.85	10.0	0.6063
3	4.90	10.0	0.6125
4	4.95	10.0	0.6188
5	4.90	10.0	0.6125
6	5.05	10.0	0.6313
Average	-	-	0.6157
RSD (%)	-	-	1.401

Test	Description	Parameters	Acceptance Criteria
Repeatability	6 replicates at 100% test concentration	Rf of Impurity F	RSD $\leq$ 2.0%

Analyst : <i>ML</i> Date : <i>07/04/14</i>	Reviewed : <i>[Signature]</i> Date : <i>8/4/14</i>
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VI. Limit of Detection (LOD)

Concentration of Imp.F		Spot by UV light 365 nm	Spot distance (cm)	Mobile Phase distance (cm)	Rf
(%)	mg/ml				
Std (100)	5.000	positive	5.10	10.0	0.6375
75	0.375	positive	4.75	10.0	0.5937
50	0.250	positive	4.65	10.0	0.5812
25	0.125	positive	4.70	10.0	0.5875
10	0.050	positive	4.65	10.0	0.5812
5	0.025	positive	4.60	10.0	0.5750
3	0.015	positive	4.95	10.0	0.6187
2	0.010	positive	5.00	10.0	0.6250
1	0.005	positive	5.00	10.0	0.6250

Test	Description	Parameters	Acceptance Criteria
LOD	on each concentration	Spot detection	Positive (detected)

Remark:

positive = detected

negative = not detected

Analyst : <i>mg</i>	Reviewed : <i>Jan</i>
Date : <i>07/04/14</i>	Date : <i>8/4/14</i>