Quality Management System

Compatibility Study Report: Gestrinone, Nimesulide and Piroxicam in Pentravan® (REE-03)

WRITTEN	REVIEWED	APPROVED
		Thalyta Raposo de Almeida

Ortofarma Laboratório de Controle de Qualidade Ltda
CRF-MG: 18266 | CNPJ: 70.796.741/0001-98
Correspondência: Caixa Postal 20.005, CEP 36016-970
Endereço comercial: BR 040, Km 800, Empresarial Park Sul, 39, CEP 36120-000
Fone: 32 3273-3560 **Fax:** 32 3273-3522

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1. AIMS

The objective of this study was to evaluate the stability of gestrinone 0.5%, nimesulide 5% and piroxicam 2% in transdermal creams compounded using Pentravan® and stored at room temperature (15-25 °C).

2. PROTOCOL

2.1. Chromatographic conditions:

The HPLC analyses were performed in a qualified and calibrated Young Lin (Korea) chromatography system composed of the following: quaternary pump (YL 9110), photodiode array detector (YL 9160), automatic injector (YL 9150), column compartment (YL 9130) and software controller (Clarity).
For gestrinone, the mobile phase was composed of a mixture of methanol, acetonitrile and water (5:2:3). The standards and the samples were diluted to a concentration of 100 mcg/mL in acetonitrile. Chromatographic separation was achieved using a Phenomenex (USA) packing L1, 4.6-mm × 25-cm, 5-µm particle column, at 25 °C; the column was connected with a pre-column with the same packing (4.0 × 3.0 mm, 5 µm) from the same manufacturer. The mobile phase was pumped using a flow rate of 1.5 mL/min. The samples and standards were injected in a volume of 20 µL and monitored using UV detection at 323 nm.
For nimesulide, the mobile phase was composed of a mixture of acetonitrile and water (1:1). The standards and the samples were diluted to a concentration of 20 mcg/mL in mobile phase. Chromatographic separation was achieved using a Phenomenex (USA) packing L1, 3.9-mm × 15-cm, 5-µm particle column; the column was connected with a pre-column with the same packing (4.0 × 3.0 mm, 5 µm) from the same manufacturer. The mobile phase was pumped using a flow rate of 1.8 mL/min. The samples and standards were injected in a volume of 20 µL and monitored using UV detection at 220 nm.

For piroxicam, the mobile phase was composed of a mixture of 0.05M sodium phosphate (pH adjusted to 3.5 with phosphoric acid), acetonitrile and methanol (55:30:15). The standards and the samples were diluted to a concentration of 50 mcg/mL in acetonitrile. Chromatographic separation was achieved using a Phenomenex (USA) packing L1, 4.6-mm × 15-cm, 5-µm particle column, at 40 °C; the column was connected with a pre-column with the same packing (4.0 × 3.0 mm, 5 µm) from the same manufacturer. The mobile phase was pumped using a flow rate of 1.5 mL/min. The samples and standards were injected in a volume of 20 µL and monitored using UV detection at 248 nm.

2.2. Forced-degradation Studies: Stability-indicating Characteristics:

Active Pharmaceutical Ingredient (API) samples were subjected to the following stressing conditions to determine the capacity of the HPLC method to determine any possible degradation product produced during storage of the transdermal cream: (i) dilution in acid (0.1M HCl); (ii) dilution in base (0.1 NaOH); (iii) exposure to ultraviolet light at 365 nm; and (iv) heating at 70 °C. These solutions were assayed by HPLC and any extraneous peaks found in the chromatograms were labeled and the resolution was determined between the degradant and the API. A resolution of 1.5 between the peaks was considered full separation.

2.3. Validation of the HPLC method:

Specificity of the method was determined using the above mentioned solutions and also running HPLC analyses of a standard solution, a Pentravan® blank solution, and a mobile phase/diluents blank solution. The acceptance criterion was defined as a percentage of discrepancy between the peak areas lower than 2%. In addition, the specificity of the method was obtained through comparison of standard chromatograms with and without the matrix. All analyses were run in triplicate.

For precision, the test was designed to assess the degree of dispersion among the series of measurements obtained by the same analyst (repeatability) and between two analysts and two days (within-lab variations, intermediate precision) for solutions of the API at work concentration. Repeatability was determined by consecutively analyzing six replicates by a single analyst in a single day. Intermediate precision was also performed in six replicates, but in two days, by different analysts. An injection precision of <5 % relative to the coefficient of variation was considered appropriate.

Accuracy measurements were performed by the same analyst by injecting the chromatographic samples to which the matrix was added (at the same concentrations levels performed for the linearity test (n = 3 for each concentration level)). The result was expressed as a percentage of recovery, compared with the analytical curve obtained from linearity.

For linearity, the test was conducted by the plotting of three standard curves, each constructed from the API concentrations of 70-130% of work concentration in order to assess the linear relationship between the concentration of the analyte and the obtained areas. For this purpose, the data for each concentration range of the curve after fitting by ordinary least squares method were evaluated by analysis of variance (ANOVA) and subjected to the least squares method to determine the correlation coefficient of the calibration curve.

The limit of detection (LOD) and limit of quantification (LOQ) were determined from three standard calibration curves and were calculated as shown in Eq. (1) and (2), respectively:

$$LOD = s\frac{3}{a} \quad (1)$$

$$LOQ = s\frac{10}{a} \quad (2)$$

where *a* is the slope of the calibration curve, and *S* is the standard deviation of the *y*-intercept. The LOD and LOQ were confirmed by the analysis of chromatograms generated by injecting solutions in their respective limit concentrations.

2.4. Preparation of transdermal creams: Annex I

2.5. Stability/ Compatibility Study

The API samples were HPLC-assayed in pre-determined time points to verify the stability of the API in Pentravan®. Sampling times were: initial (T=0), 15 days (T=15), 30 days (T=30), 60 days (T=60), 90 days (T=90), 120 days (T=120), 150 days (T=150) and 180 days (T=180). Aliquots were withdrawn from the creams and diluted properly in order to obtain work solutions in the concentration described in Chromatographic Conditions. All creams were assayed 6 times. The evaluation parameter was the percent recovery with respect to T = 0, using the HPLC method (results given as percentage ± standard deviation).

3. RESULTS

3.1. Forced-degradation – Gestrinone

Replicate	*Area * HCl*	*%d*	*Area* NaOH*	*%d*	*Area* UV*	*%d*	*Area* Heat*	*%d*
1	774.46	-94.73	0.00	-100.00	15013.01	2.15	14875.38	1.21
2	770.05	-94.73	0.00	-100.00	15152.40	3.62	14736.17	0.78
3	762.30	-94.80	0.00	-100.00	15050.12	2.61	14862.62	1.33
Average	768.94	**-94.76**	0.00	**-100.00**	15071.84	**2.79**	14824.72	1.10

* corrected area
%d = percentage of discrepancy.

Acid, alkaline and UV stresses led to important degradation in the analytical peak.

3.2. Forced-degradation – Nimesulide

Replicate	*Area * HCl*	*%d*	*Area* NaOH*	*%d*	*Area* UV*	*%d*	*Area* Heat*	*%d*
1	74.00	-80.55	0.00	-100.00	387.07	1.76	383.83	0.91
2	73.35	-80.62	0.00	-100.00	391.68	3.49	384.07	1.48
3	73.40	-80.72	0.00	-100.00	389.09	2.22	385.24	1.21
Average	73.58	**-80.63**	0.00	**-100.00**	389.28	**2.49**	384.38	1.20

* corrected area
%d = percentage of discrepancy.

Acid, alkaline and UV stresses led to important degradation in the analytical peak.

3.3. Forced-degradation – Piroxicam

Replicate	*Area * HCl*	*%d*	*Area* NaOH*	*%d*	*Area* UV*	*%d*	*Area* Heat*	*%d*
1	1613.28	-16.69	2070.51	6.92	2048.46	5.78	1947.33	0.56
2	1607.21	-16.54	2088.51	8.45	2055.73	6.75	1968.63	2.23
3	1644.63	-15.03	2073.53	7.12	2072.80	7.09	1979.50	2.27
Average	1621.71	**-16.09**	2077.51	**7.50**	2059.00	**6.54**	1965.15	1.69

* corrected area
%d = percentage of discrepancy.

Acid, alkaline and UV stresses led to important degradation in the analytical peak.

3.4. Validation - gestrinone

Parameter	Results	Acceptance criteria
Specificity	$\%_{discrepancy}$ = 1.32	2%
Precision		
Repeatability	CV = 0.84	5%
Intermediate precision	CV = 1.79	5%
Accuracy	Recovery = 99.59	100% ± 2%
Linearity		
Range	71.12 – 132.08 mcg/mL	
Analytical curve	y = 41.59x + 459.85	
R^2	0.9964	> 0.99
ANOVA's significance of regression	$F_{calculated}$ = 3585.18	F >> 4.67
ANOVA's lack of fit	$F_{calculated}$ = 1.87	F < 3.71
Limit of Quantification	40.40 mcg/mL	
Limit of Detection	12.12 mcg/mL	

3.5. Validation - nimesulide

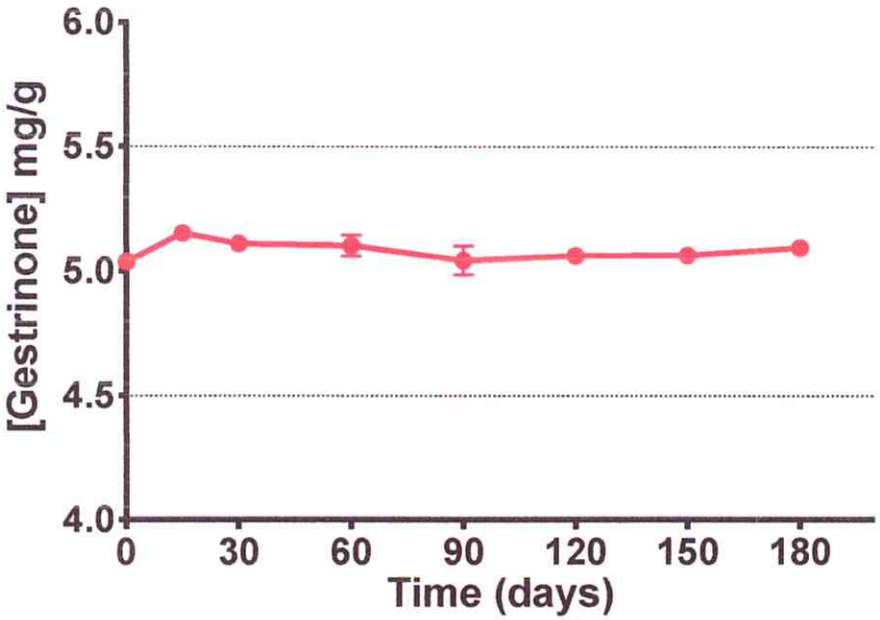
Parameter	Results	Acceptance criteria
Specificity	$\%_{discrepancy}$ = 0.99	2%
Precision		
Repeatability	CV = 0.84	5%
Intermediate precision	CV = 1.04	5%
Accuracy	Recovery = 99.63	100% ± 2%
Linearity		
Range	14.42 – 26.78 mcg/mL	
Analytical curve	y = 19.18x + 5.73	
R^2	0.9972	> 0.99
ANOVA's significance of regression	$F_{calculated}$ = 4676.69	F >> 4.67
ANOVA's lack of fit	$F_{calculated}$ = 0.75	F < 3.71
Limit of Quantification	1.50 mcg/mL	
Limit of Detection	0.45 mcg/mL	

3.6. Validation - piroxicam

Parameter	Results	Acceptance criteria
Specificity	$\%_{discrepancy}$ = 1.52	2%
Precision		
Repeatability	CV = 0.71	5%
Intermediate precision	CV = 1.61	5%
Accuracy	Recovery = 100.61	100% ± 2%
Linearity		
Range	35.56 – 66.04 mcg/mL	
Analytical curve	y = 41.69x + 25.71	
R^2	0.9922	> 0.99
ANOVA's significance of regression	$F_{calculated}$ = 1657.52	F >> 4.67
ANOVA's lack of fit	$F_{calculated}$ = 1.46	F < 3.71
Limit of Quantification	12.05 mcg/mL	
Limit of Detection	3.62 mcg/mL	

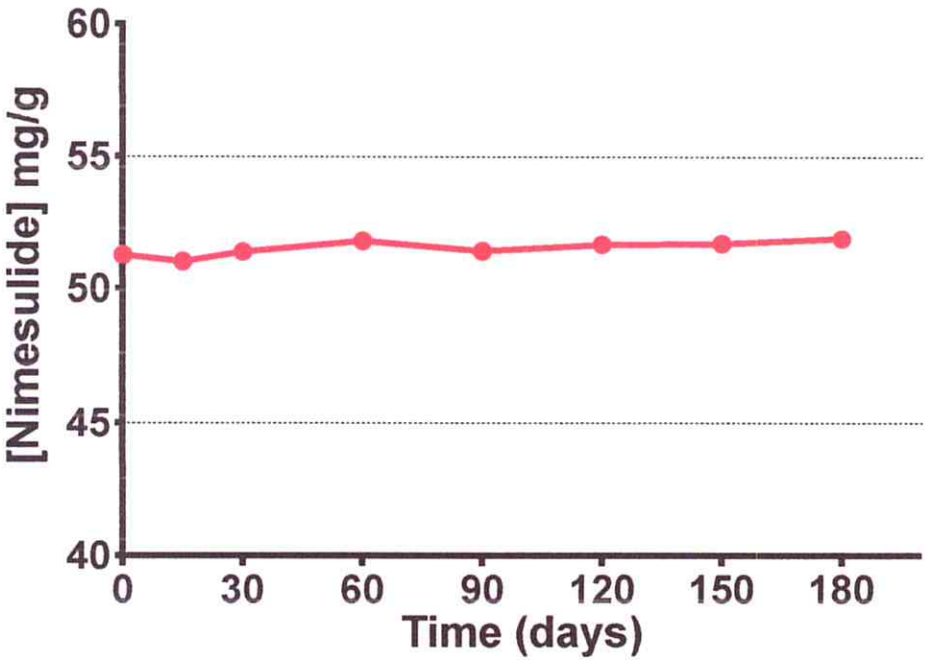
3.7. Compatibility Study – Gestrinone 0.5% in Pentravan®

Elapsed time (days)	*% Recovery* *Room Temperature (20-25 °C | 58-77 °F)*
T = 0	100 ± 0.23
T = 15	102.27 ± 0.46
T = 30	101.45 ± 0.28
T = 60	101.29 ± 0.81
T = 90	100.11 ± 1.17
T = 120	100.49 ± 0.14
T = 150	100.53 ± 0.28
T = 180	101.15 ± 0.42



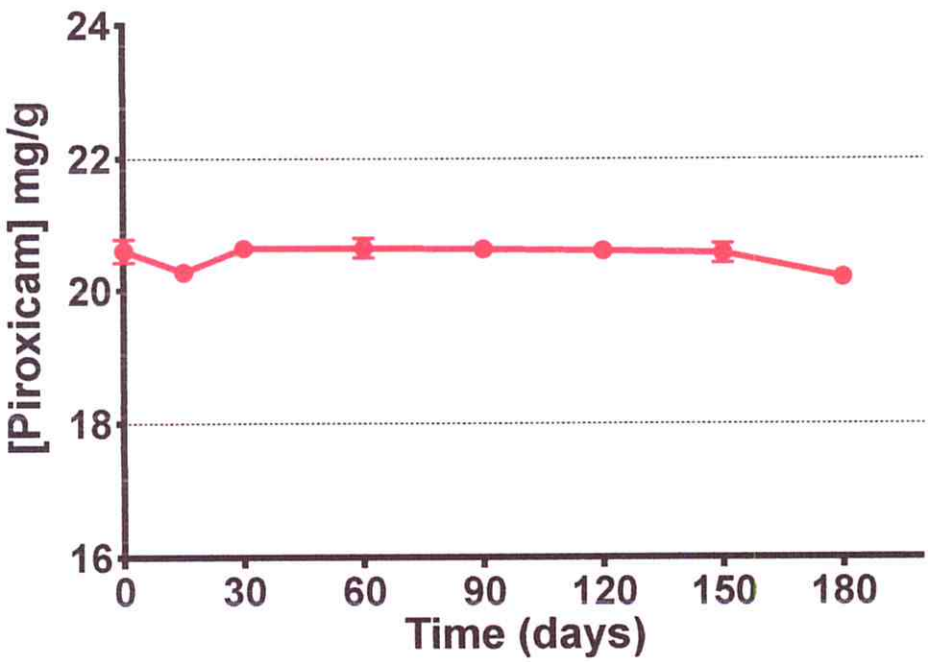
3.8. Compatibility Study – Nimesulide 5% in Pentravan®

Elapsed time (days)	*% Recovery* *Room Temperature (20-25 °C | 58-77 °F)*
T = 0	100 ± 0.38
T = 15	99.52 ± 0.52
T = 30	100.23 ± 0.53
T = 60	101.03 ± 0.54
T = 90	100.29 ± 0.42
T = 120	100.79 ± 0.10
T = 150	100.86 ± 0.41
T = 180	101.23 ± 0.12



3.9. Compatibility Study – Piroxicam 2% in Pentravan®

Elapsed time (days)	*% Recovery* *Room Temperature (20-25 °C | 58-77 °F)*
T = 0	100 ± 0.86
T = 15	98.40 ± 0.47
T = 30	100.16 ± 0.24
T = 60	100.19 ± 0.72
T = 90	100.11 ± 0.27
T = 120	100.01 ± 0.14
T = 150	99.83 ± 0.73
T = 180	98.06 ± 0.47



4. CONCLUSIONS

Transdermal emulsions of gestrinone 0.5%, nimesulide 5% and piroxicam 2% compounded using Pentravan® and stored at room temperature (15-25 °C) are stable in terms of drug content for at least 180 days. No phenomena such as colour and/or odour changing, disproportionation, sedimentation, flocculation, creaming, coalescence or phase separation were observed throughout the study.

Responsible for the study: _____

Hudson Polonini, PhD
R & D Manager
Pharmacist
CRF-MG 34340

Anexo I

Ortofarma Laboratório de Controle de Qualidade Ltda
CRF-MG: 18266 | CNPJ: 70.796.741/0001-98
Correspondência: Caixa Postal 20.005, CEP 36016-970
Endereço comercial: BR 040, Km 800, Empresarial Park Sul, 39, CEP 36120-000
Fone: 32 3273-3560 **Fax:** 32 3273-3522

1. FORMULA NAME: Gestrinone 0.5 % in Pentravan®

DOSAGE FORM: Transdermal cream

FORMULA (℞):

Ingredients	For: 100 g	Lot#	Supplier
Gestrinone	0.5 g	75901509001	Fagron
Ethoxydiglycol	4.5 g	14L17-U09-023117	Fagron
Pentravan®	95 g	16D3-B047-004728	Fagron

USE/TYPE: Human Use **CATEGORY:** Non-Sterile Preparation

METHOD OF PREPARATION:

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Accurately weigh and/or measure each ingredient.
3. Place the API in a mortar and add ethoxydiglycol to powder and mix to form a smooth mixture.
4. Add sufficient Pentravan® to final weight in portions and mix well.
5. Package and label.
6. Store at room temperature.

2. FORMULA NAME: Nimesulide 5% in Pentravan®

DOSAGE FORM: Transdermal cream

FORMULA (℞):

Ingredients	For: 30 g	Lot#	Supplier
Nimesulide	1.5 g	DCPL/15NIM/011	Fagron
Ethoxydiglycol	2.4 g	14L17-U09-023117	Fagron
Pentravan®	26.1 g	16D3-B047-004728	Fagron

USE/TYPE: Human Use **CATEGORY:** Non-Sterile Preparation

METHOD OF PREPARATION:

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Accurately weigh and/or measure each ingredient.
3. Place the API in a mortar and add ethoxydiglycol to powder and mix to form a smooth mixture.
4. Add sufficient Pentravan® to final weight in portions and mix well.
5. Package and label.
6. Store at room temperature.

3. FORMULA NAME: Piroxicam 2% in Pentravan®

DOSAGE FORM: Transdermal cream

FORMULA (℞):

Ingredients	For: 100 g	Lot#	Supplier
Piroxicam	0.6 g	20141202	Fagron
Ethoxydiglycol	1.5 g	14L17-U09-023117	Fagron
Pentravan®	27.9 g	16D3-B047-004728	Fagron

USE/TYPE: Human Use **CATEGORY:** Non-Sterile Preparation

METHOD OF PREPARATION:

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Accurately weigh and/or measure each ingredient.
3. Place the API in a mortar and add ethoxydiglycol to powder and mix to form a smooth mixture.
4. Add sufficient Pentravan® to final weight in portions and mix well.
5. Package and label.
6. Store at room temperature.