3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

1. OBJECTIVE

Present the results obtained in the validation of the analytical method for quantitative determination of 2-Propanol in Ketosteril drug-coated tablets, by GC using FID detection.

2. DEFINITIONS AND ABBREVIATIONS

QC – Quality Control
GC – Gas Chromatography
RSD – Relative Standard Deviation
FID – Flame Ionization Detector
rt – Retention time
Rel. Resol. – Relative Resolution
USP – United States Pharmacopoeia

3. RESULTS

3.1. Formula description

3.1.1. Active Substance

Amino acids, keto acids and hydroxy acids described in the formulation (point 3.1.3)

3.1.2. Pharmaceutical form

Coated tablets

3.1.3. Formulation

- Composition of the cores

Components	Amount per unit (mg)	Function
Alfa-Ketoisoleucine, calcium**	67,0**	Active ingredient
Alfa-Ketoleucine, calcium**	101,0**	Active ingredient
Alfa-Ketophenylalanine, calcium**	68,0**	Active ingredient
Alfa-Ketovaline, calcium**	86,0**	Active ingredient
Alfa-Hydroxymethionine, calcium**	59,0**	Active ingredient
Isopropyl alcohol*	101,666 – 108,333 0,1295 – 0,138 ml	Solvent
Purified water*	0,001666 – 0,005	Solvent
Povidone	26,3	Granulating agent
Macrogol 6000	7,8	Lubricant
L-Lysine acetate	105,0	Active ingredient

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Components	Amount per unit (mg)	Function
L-Threonine	53,0	Active ingredient
L-Tryptophan	23,0	Active ingredient
L-Histidine	38,0	Active ingredient
L-Tyrosine	30,0	Active ingredient
Isopropyl alcohol*	0,0375 0,0478 ml	Solvent
Purified water*	0,0015	Solvent
Povidone	16,883	Granulating agent
Macrogol 6000	5,0	Lubricant
Maize starch***	Max. 44	Filler, balancing agent
Talc	19,6	Release agent
Silica colloidal anhydrous	4,40	Flow regulator
Crospovidone	6,00	Explosive
Magnesium stearate	15,0	Lubricant
Total		≈ 775

* eliminated during the process;
** the individual water-content is taken into account in order to achieve 100% active ingredient content in each batch;
***varies, as used as filling agent in order to achieve a constant tablet core weight with specified continuous active ingredient content.

- Composition of the film-coat

Components	Amount per unit (mg)	Function
Isopropyl alcohol*	70 89,166 ml	Solvent
Talc	9,067	Release agent
Titanium dioxide	7,40	Pigment
Macrogol 6000	1,2	Lubricant
Purified water*	1,2	Solvent
Quinoline yellow E 104	0,72	Pigment
Purified water*	6	Solvent
Eudragit E 12,5% m/m**	47,2 (5,9 solid)	Coating compound
Triacetin	0,43	Plasticizer
Macrogol 6000	0,28	Lubricant
Purified water*	0,22	Solvent

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Components	Amount per unit (mg)	Function
Isopropyl alcohol*	2,017 0,0025688 ml	Solvent
Total		≈ 25

* eliminated during the process;
** part eliminated during the process.

3.2. Method

3.2.1. Reagents and materials

2–Propanol (USP, batch: IOL393, validity: 29 Oct. 2016, activity: 100,0%)
Acetone (USP, batch: H0K208, validity: 29 Oct. 2016, activity: 100,0%)
Tolueno (Merck, batch: K44153525 306, validity: 31 Jan. 2018)
Alfa-Ketoisleucine calcium (Batch: 160400155, validity: Nov. 2018)
Alfa-Ketoleucine calcium (Batch: 150300321, validity: May. 2017)
Alfa-Ketovaline calcium (Batch: 160600190, validity: Feb. 2018)
Alfa-Ketophenylalanine calcium (Batch: 150900484, validity: June. 2018)
Alfa-Hydroxymethionine calcium (Batch: 160500145, validity: Oct. 2018)
L-Lysine Acetate (Batch: 160500195, validity: 02 Feb.2019
L-Tyrosine (Batch: 160300620, validity: Feb.2020
L-Tryptophan (Batch: 16030029; validity: 26 Feb.2018
L-Threonine (Batch: 160400321, validity: 17 Mar.2022
L-Histidine (Batch: 160400068, validity: 17 Nov. 2018
Povidone (Batch: 160300498, validity: June 2018)
Macrogol 6000 (Batch: 160100494, validity: 16 July 2019)
Maize Starch (Batch: 150500002, validity: Feb. 2017)
Talc (Batch: 160100099, validity: 18 June 2018)
Silica colloidal anhydrous (Batch: 160100499, validity: 21 Oct. 2017)
Crospovidone (Batch: 160100468, validity: 28 Oct. 2018)
Magnesium stearate (Batch: 160100057, validity: Oct. 2017)
Titanium dioxide (Batch: 150800228, validity: 07 July 2018)
Quinoline yellow E 104 (Batch: 150900495, validity: 23 July 2020)
Triacetin (Batch: 150400580, validity: Sep. 2019)
Ketosteil tablets (Batch: 18N2169, validity: June 2019)
GC headspace vials (Agilent, batch: 849-10-15/001)
GC headspace septum and caps (Agilant, batch: AGI 180740)
Paper filter 12-15µm (VWR, batch: 016025)
2µL capillary (Camag 022.7727)
Volumetric pipettes

3.2.2. Equipment

5140-II-0201: GC chromatograph equipped with Flame Ionization Detector, auto-injector with headspace and software of acquisition and treatment of data.
5140-II-9010 and 5140-II-9004: Balances
5140-II-0359, 5140-II-0360, 4951NI1015: Micropipettes

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

3.2.3. Standard stock solution (200%): High range linearity- reference standard

1543,1 mg (2 mL) of 2-Propanol were diluted to 4 mL with toluene.
Conc. 2-Propanol = 0,5 mL/mL (385,775 mg/mL)

3.2.4. Standard working solution: High range linearity

Standard working solutions were prepared by dilution of the standard stock solution, according with table 1.

Conc. (%)	Volume of standard stock solution (µL)	Volume of acetone (µL)	Volume of toluene (µL)	2-propanol concentration (ppm) in 2 µL
9,64	50	250	700	192,89
28,93	150	250	600	578,66
48,22	250	250	500	964,44
77,16	400	250	350	1543,10
86,80	450	250	300	1735,99
96,44	500	250	250	1928,88
106,09	550	250	200	2121,76
115,73	600	250	150	2314,65
144,67	750	250	0	2893,31

Table 1: Standard working solutions – high range linearity

For each concentration level, a filter paper of ±1 cm^2 was placed on a headspace vial. 2,0 µL of the previous standard solutions were placed on the filter paper and the vial was immediately closed with a butyl/PTFE septum. Each concentration level was prepared in triplicate.

3.2.5. Standard stock solution (200%): High range linearity – matrix effect

746,4 mg (1 mL) of 2-Propanol were diluted to 2 mL with toluene.
Conc. 2-Propanol = 0,5 mL/mL (373,2 mg/mL)

3.2.6. Standard working solution: High range linearity – matrix effect

Standard working solutions were prepared by dilution of the standard stock solution, according with table 2.

Level (%)	Volume of standard stock solution (µL)	Volume of acetone (µL)	Volume of toluene (µL)	2-propanol concentration (ppm) in 2 µL
9,33	25	125	350	186,60
27,99	75	125	300	559,80
46,65	125	125	250	933,00

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Level (%)	Volume of standard stock solution (µL)	Volume of acetone (µL)	Volume of toluene (µL)	2-propanol concentration (ppm) in 2 µL
74,64	200	125	175	1492,80
83,97	225	125	150	1679,40
93,30	250	125	125	1866,00
102,63	275	125	100	2052,60
111,96	300	125	75	2239,20
139,95	375	125	0	2799,00

Table 2: Standard working solutions – high range linearity (matrix effect)

For each concentration level, a filter paper of ±1 cm^2 was placed on a headspace vial and it was added placebo amounts corresponding to the 100% composition. 2,0 µL of the previous standard solutions were placed on the filter paper and the vial was immediately closed with a butyl/PTFE septum. Each concentration level was prepared in triplicate.

3.2.7. Standard solution

400 µL of 2-Propanol were accurately weighed to a vial. 400 µL of acetone and 800 µL of toluene were added to the vial, mixing well.
A filter paper of ±1 cm^2 was placed on a headspace vial. 2,0 µL of the previous standard solution were placed on the filter paper and the vial was immediately closed with a butyl/PTFE septum.

3.2.8. Samples

About 10 tablets of ketosteril were finely grounded and 200 mg of the powder were accurately weighted into a headspace vial containing a filter paper (± 1cm^2). The vial was closed immediately with a butyl/PTFE septum.

3.2.9. Blank

A filter paper (±1 cm^2) was added into a headspace vial and the vial was closed immediately with a butyl/PTFE septum.

3.2.10. Chromatographic conditions

Head space conditions:
Vial temperature: 90ºC
Loop temperature: 95ºC
Transfer line temperature: 105ºC
Vial equilibration time: 9 minutes
GC cycle time: 34 minutes

GC conditions:
Column: Teknokroma TRB-624, 30mx0,32mm, 1,8 µm , Part number: TR-601833, Serial number: NF-15839, Internal number: GC10/15

Column: Teknokroma TRB-624, 30mx0,32mm, 1,8 µm , Part number: TR-601833, Serial number: NF-38228, Internal number: GC15/16
Carrier gas: Helium
Initial pressure/flow: 3,50
Mode: Constant flow
Split: 10:1
Detector: Flame ionization
Injection temperature: 140ºC
Oven temperature:
35ºC (5 min)
10ºC/min increase to 80ºC
80ºC (6 min)
15ºC/min increase to 180ºC
180ºC (3 min)
Detection temperature: 200ºC
Injection volume: 1000 µL
Run time: 25,17 minutes

3.3. Validation Parameters

3.3.1. System Suitability

Acceptance criteria:

Standard Solution:
- The resolution between the peaks due to acetone and 2-propanol should be not less than 2;
- The RSD between 5 injections of reference solution should be not more than 10%;

Sample solution:
- the RSD between sample preparations should be not more than 10%.

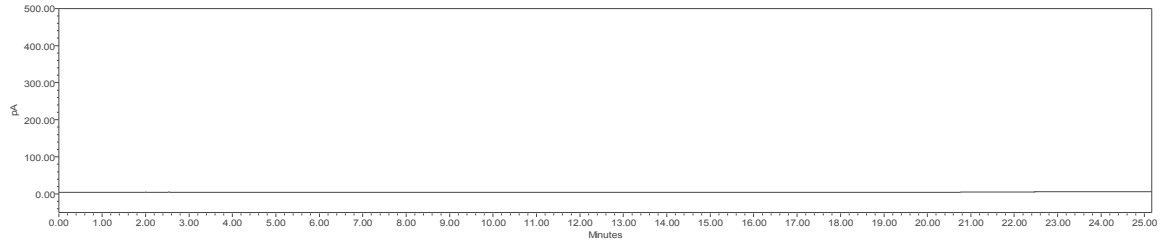
	RSD	Resolution
Standard	1,5	2,96
Sample	7,0	-

3.3.2. Selectivity

The placebo solution doesn't interfere in quantitative determination of 2-Propanol.

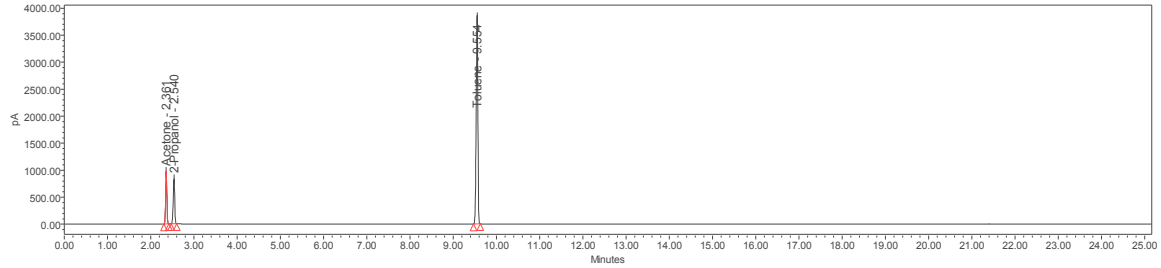
3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

3.3.2.1. Blank



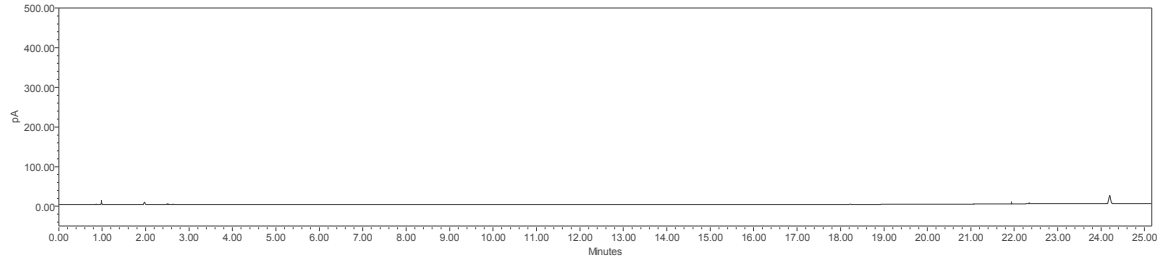
Chromatogram 1

3.3.2.2. Standard solution



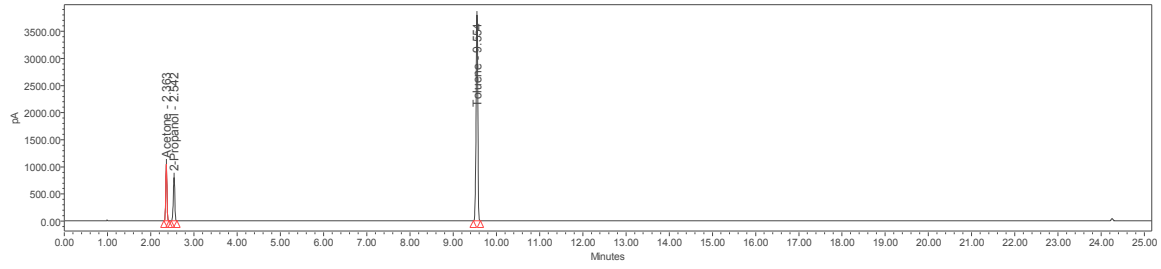
Chromatogram 2

3.3.2.3. Placebo solution



Chromatogram 3

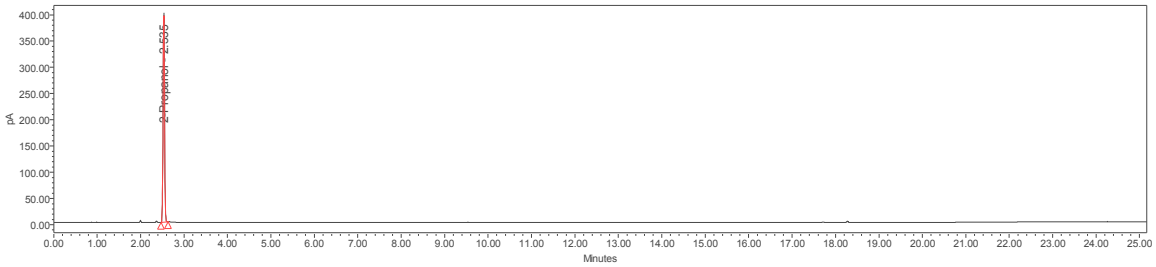
3.3.2.4. Placebo + standard solution



Chromatogram 4

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

3.3.2.5. Sample



Chromatogram 5

3.3.3. Linearity

3.3.3.1. 2-Propanol reference standard

Acceptance criteria:

- $r \geq 0{,}990$
- The residuals must have a random distribution and the 95% or 99% confidence interval for the intercept term must contain zero.
- Quantification limit calculation: $QL = 10 \times S^{[1]}/m$
- Detection limit calculation: $DL = 3{,}3 \times S/m$

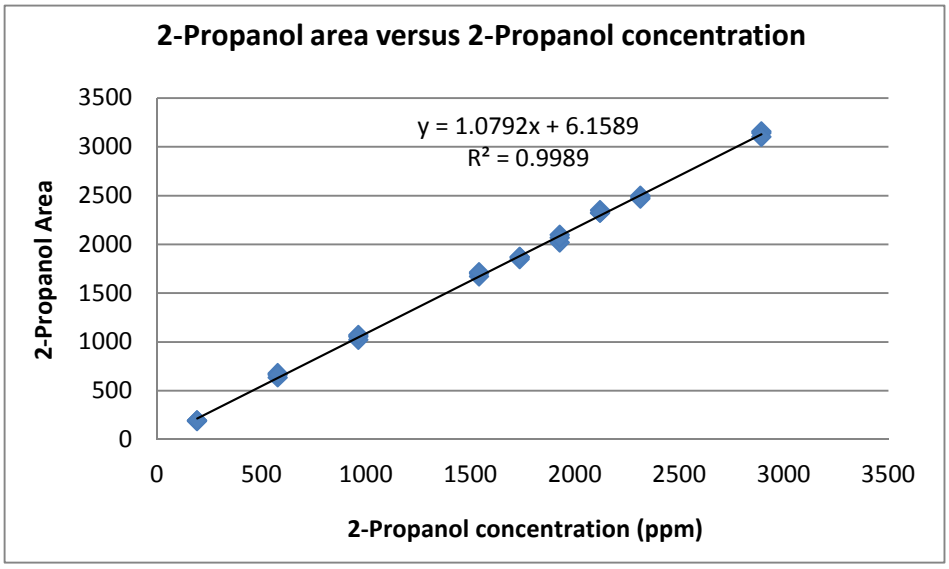
2-Propanol concentration (%)	2-Propanol concentration (ppm)	Area
9,64	192,89	194,08
		186,19
		192,53
	Average	**190,93**
	RSD (%)	**2,19**
28,93	578,66	676,70
		634,17
		664,38
	Average	**658,42**
	RSD (%)	**3,32**
48,22	964,44	1053,66
		1021,31
		1071,31

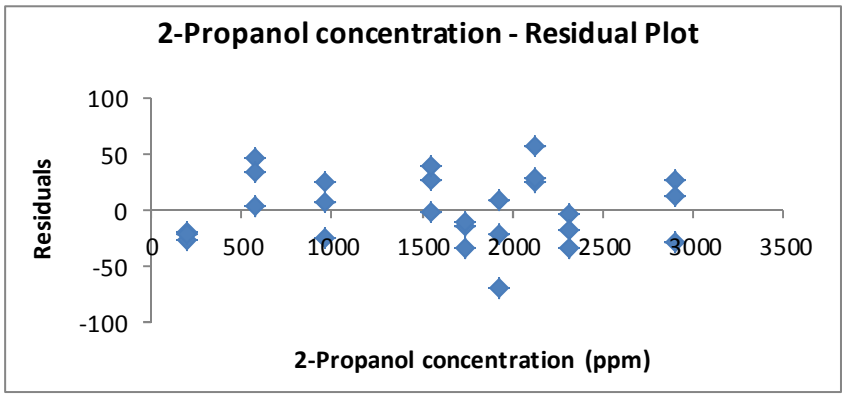
[1] S= Regression standard error
m=slope

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

2-Propanol concentration (%)	2-Propanol concentration (ppm)	Area
	Average	**1048,76**
	RSD (%)	**2,42**
77,16	1543,10	1668,54
		1698,04
		1710,78
	Average	**1692,45**
	RSD (%)	**1,28**
86,80	1735,99	1845,58
		1864,48
		1868,17
	Average	**1859,41**
	RSD (%)	**0,65**
96,44	1928,88	2096,16
		2016,96
		2066,51
	Average	**2059,88**
	RSD (%)	**1,94**
106,09	2121,76	2321,12
		2352,24
		2323,27
	Average	**2332,21**
	RSD (%)	**0,75**
115,73	2314,65	2499,48
		2485,94
		2469,48
	Average	**2484,97**
	RSD (%)	**0,60**
144,67	2893,31	3154,97
		3140,57
		3100,10
	Average Mean	**3131,88**
	RSD (%)	**0,91**

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)





Linear regression equation (y=1,0792x + 6,1589)
m= 1,0792
b= 6,1589
r= 0,9994
Regression Standard Error= 30,54

$QL = 282{,}99$ ppm $\qquad$ $DL = 93{,}39$ ppm

3.3.3.2. Matrix effect: placebo + amino acids reference standards

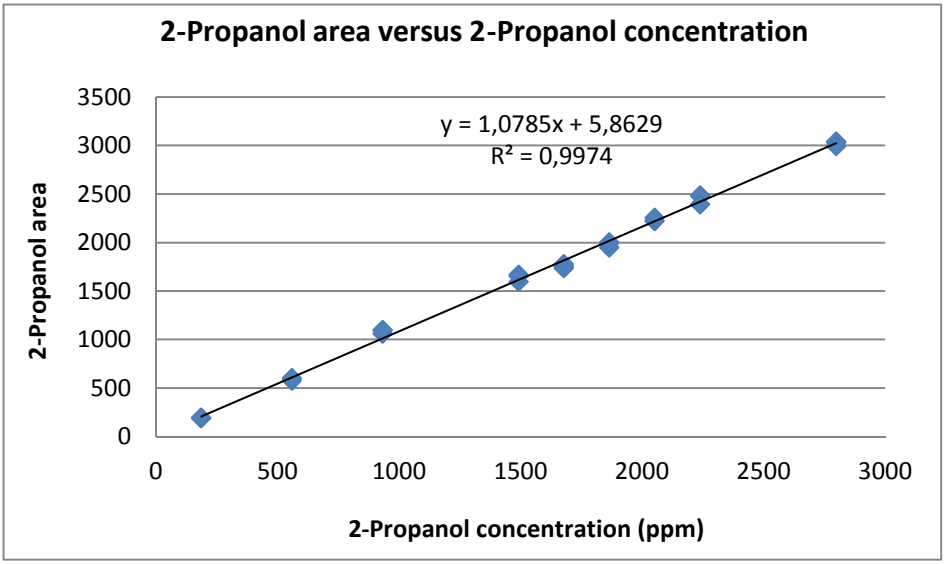
Acceptance criteria:
- $r \geq 0{,}990$
- The residuals must have a random distribution and the 95% or 99% confidence interval for the intercept term must contain zero.

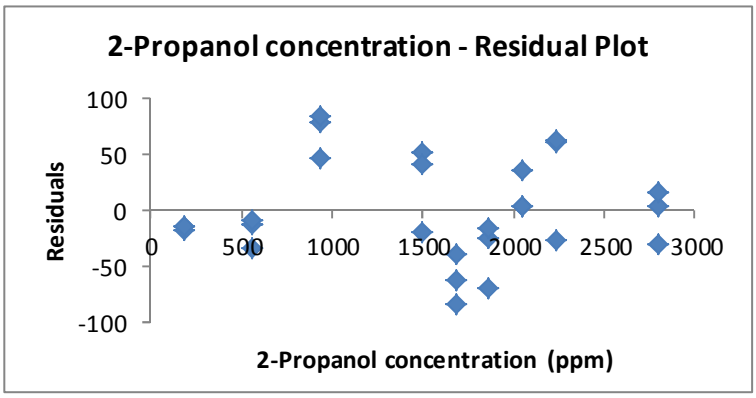
3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

2-Propanol concentration (%)	2-Propanol concentration (ppm)	Area
9,33	186,60	192,72
		188,08
		192,15
	Average	**190,98**
	RSD (%)	**1,32**
27,99	559,80	574,85
		596,79
		600,66
	Average	**590,77**
	RSD (%)	**2,36**
46,65	933,00	1090,70
		1058,03
		1095,91
	Average	**1081,55**
	RSD (%)	**1,90**
74,64	1492,80	1596,20
		1667,73
		1655,89
	Average	**1639,94**
	RSD (%)	**2,34**
83,97	1679,40	1753,32
		1777,75
		1733,19
	Average	**1754,75**
	RSD (%)	**1,27**
93,30	1866,00	1947,22
		1993,40
		2001,44
	Average	**1980,69**
	RSD (%)	**1,48**
102,63	2052,60	2223,00
		2223,36

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

2-Propanol concentration (%)	2-Propanol concentration (ppm)	Area
		2255,69
	Average	**2234,02**
	RSD (%)	**0,84**
111,96	2239,20	2480,94
		2483,34
		2393,23
	Average	**2452,50**
	RSD (%)	**2,09**
139,95	2799,00	3028,06
		3039,41
		2993,33
	Average	**3020,27**
	RSD (%)	**0,79**





Linear regression equation (y=1,0785x + 5,8629)

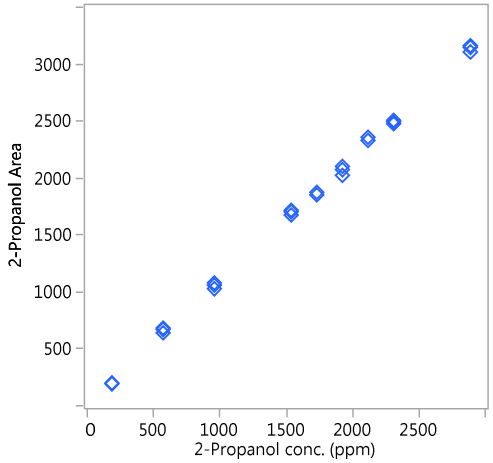
m= 1,0785
b= 5,8629
r= 0,9987

3.3.3.3. Statistical analysis

The statistical analysis was performed with assistance of the statistical software JMP Statistical Discovery from SAS, version 12 and XLSTAT 2016.

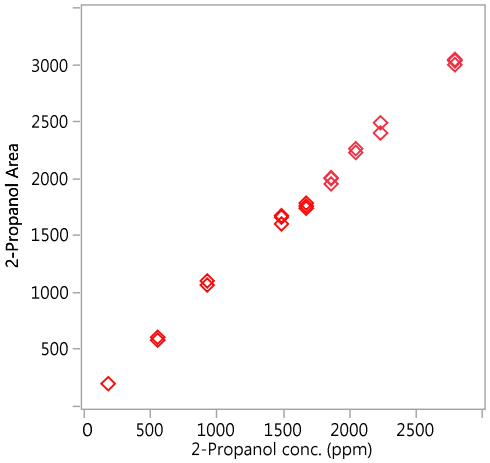
Scatterplots
In order to assess whether there is a linear relationship between the Concentration(ppm) and the Area of the analyte two scatterplots were constructed, one in which the analyte is the only component of the sample, and another in which the analyte is in a complex matrix with other constituents.
Three different areas were observed for each of the nine concentrations.

In the case where the analyte is the only component of the sample, the following scatterplot shows a nearly perfect linear relationship.



3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

It is also observed an almost perfect linear relationship between Area and Concentration (ppm) when the analyte is contained in a complex matrix.



Pearson´s correlation coefficient

Considering the correlation revealed in the two scatterplots, Pearson's correlation coefficients were estimated in order to understand if these were higher than the minimum acceptable, 0.990, which was verified. Thus, it was found that in both cases this assumption is satisfied because when the analyte is the only component of the sample r=0.9994 and when is in a complex matrix with other constituents r=0.9987.

Adjusting the linear regression lines using the Least Squares method

After having been verified a linear correlation between the two variables, through a scatterplot and its Pearson's correlation coefficient (r≥0.990), two linear regression lines were estimated, one when the analyte is the only component of the sample,

$$Area = a_1 + b_1 \times Concentration(ppm)$$

And another when the analyte is contained in a complex matrix,

$$Area = a_2 + b_2 \times Concentration(ppm)$$

Intercepts, a_1 and a_2, and slopes, $b_1$and b_2, estimates were obtained from the method of least squares.

In the first case,

	Coefficients	Std. Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	6,158	12,830	0,480	0,635	-20,266	32,582
Conc. (ppm)	1,079	0,007	150,070	0,000	1,064	1,094

In the second case,

	Coefficients	Std. Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	5,863	19,102	0,310	0,761	-33,479	45,205
Conc. (ppm)	1,079	0,011	97,450	0,000	1,056	1,101

Therefore, we have:

$$a_1 = 6{,}158,\ b_1 = 1{,}079 \qquad a_2 = 5{,}863,\ b_2 = 1{,}079$$

These estimates assume that the error in the values of *Concentration (ppm)* is constant, i.e., it is assumed that homoscedasticity exists. This means that all points $Area \times Concentration(ppm)$ have equal weight in the calculation of estimates. Below, Cochran´s test will be applied to determine whether this assumption is valid.

Cochran´s C test

Cochran´s C test was applied with the aim to evaluate the equality of variances of the dependent variable of the fitted model, Area.

In both situations, whether the analyte is the only component or contained in a matrix, the homoscedasticity hypothesis was not rejected, respectively, p=0,320 and p=0,207.

Therefore, it is concluded that the estimates found by the method of ordinary least squares are valid. The following tables show test results:

- in the first case,

C (Observed value)	0,341
C (Critical value)	0,477
p-value (unilateral)	0,320
alfa	0,05

- in the second case,

C (Observed value)	0,376
C (Critical value)	0,477
p-value (unilateral)	0,207
alfa	0,05

ANOVA and the Coefficient of Determination (R^2)

Analysis of variance, ANOVA, aims to evaluate whether the adjusted regression line explains in a satisfactory way the relationship between Area and Concentration(ppm). In ANOVA, the F test is used to test whether the slopes of each estimated lines, b_1 and b_2, differs from zero. The coefficient of determination, R^2, gives the proportion of variability in the data that is properly explained by the regression line.

For the analyte to be the only component we have:

ANOVA

	SS	df	MS	F	p-value
Regression	20999899	1	20999899	22519,713	0,000
Residual	23313	25	933		
Total	21023212	26			

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Regression Statistics	
R Square	0,9989
Adjusted R Square	0,9988
Standard Error	30,54
Observations	27

This leads to the conclusion that the hypothesis of slope being zero is rejected p=0.000<0.05, and on the other hand, the line may explain 99.89% of the variability in the data.
In case the analyte is contained in a complex matrix, ANOVA was conducted considering ordinary least squares method,

ANOVA

	SS	df	MS	F	p-value
Regression	19630277	1	19630277	9496,5854	0,000
Residual	51677	25	2067		
Total	19681954	26			

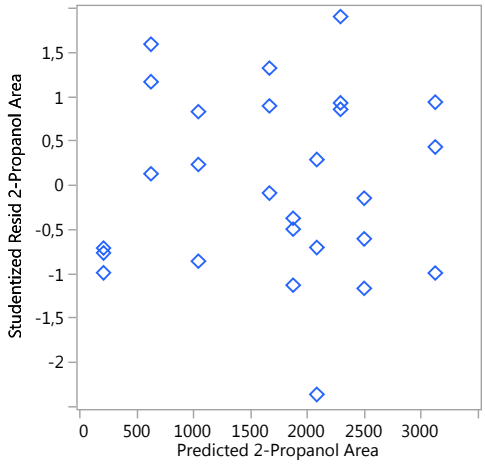
Regression Statistics	
R Square	0,9974
Adjusted R Square	0,9973
Standard Error	45,47
Observations	27

This leads to the conclusion that the hypothesis of slope being zero is rejected p=0,000<0,05, and on the other hand, the line may explain 99.74% of the variability in the data.

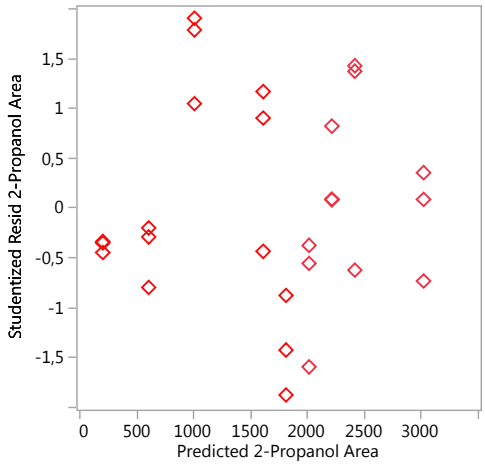
Residuals
Residuals are extremely useful to check whether a particular linear regression model is appropriate for the data. So a scatterplot must be built in which residuals and predictive values for Area are crossed. For the regression line to be appropriate is necessary that the scatterplot does not produce a marked pattern.
The scatterplots for the two estimated regression lines are:

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

- the analyte is the only component of the sample,



- the analyte is contained in a complex matrix,



The scatterplots show that there is good quality fit of regression lines.

Parallelism F test

The parallelism test aims to test the null hypothesis of equality of the slopes of the two estimated regression lines. It is intended to test the null hypothesis $b_1 = b_2$ *versus* the alternative hypothesis $b_1 \neq b_2$. If the null hypothesis is not rejected, it can be concluded that the effect of the matrix components is not significant.

The estimates obtained are:

$$b_1 = 1{,}079 \qquad b_2 = 1{,}079$$

The following table indicates the test results,

Paralell Fit SSE	*Full SSE*	*NDF*	*DDF*	*F Ratio*	*Prob > F*
74993,44	74989,99	1	50	0,002	0,962

These results show that the equality of the slopes of the regression lines is not rejected, p=0.962>0.05; therefore, no statistical evidence exists at the 5% level to assert that the matrix has a significant effect.

3.3.4. Precision

3.3.4.1. Intermediate Precision

Acceptance criteria:

- RSD between the results of determinations by the same analyst should not exceed 10%;
- RSD between the results of the two analysts should not exceed 10%.

Sample	Analyst 1: SAC Date: 06 Oct. 2016 Assay (ppm)	Analyst 2: VG Date: 03 Oct. 2016 Assay (ppm)
A1	974,84	1034,26
A2	913,39	895,57
A3	912,69	931,07
A4	888,13	904,89
A5	810,45	922,82
A6	782,30	837,10
Average (ppm)	**880,30**	**920,95**
RSD (%)	**8,13**	**7,02**

Mean between analysts: **900,6 ppm**
RSD between analysts: **7,6 %**

3.3.4.2. Repeatability

Acceptance criteria:

- The RSD between the six measurements should not exceed 10%.

Sample	Assay (ppm)
A1	1034,26
A2	895,57
A3	931,07
A4	904,89
A5	922,82
A6	837,10
Average (%)	**920,95**
RSD (%)	**7,02**

3.3.4.3. Intrumental Repeatability

Acceptance criteria:
- The RSD between the areas should not be higher than 10%.

Injection	2-Propanol Area
1	2181,22
2	2239,77
3	2171,70
4	2261,42
5	2168,29
6	2249,21
7	2167,33
8	2191,80
9	2144,33
10	2144,95
Average	**2192,00**
RSD (%)	**1,96**

3.3.5. Accuracy

Acceptance criteria:
- The RSD between the results for three determinations of each level must be equal or less than 10%.
- The percentage recovery should be between 80% and 110%.

Level (%)	Theoretical conc. of 2-propanol (2µl) (ppm)	Experimental conc. of 2-propanol (2µl) (ppm)	Recovery (%)
10	202,00	198,03	98,04
	206,00	185,18	89,89
	198,00	170,71	86,22
	Average (%)		**91,38**
	RSD (%)		**6,62**
80	1484,00	1263,16	85,12
	1452,00	1258,25	86,66
	1478,00	1275,23	86,28
	Average (%)		**86,02**
	RSD (%)		**0,93**

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Level (%)	Theoretical conc. of 2-propanol (2µl) (ppm)	Experimental conc. of 2-propanol (2µl) (ppm)	Recovery (%)
100	1798,00	1703,49	94,74
	1998,00	1727,28	86,45
	1922,00	1687,60	87,80
	Average (%)		**89,67**
	RSD (%)		**4,96**
120	2388,00	2048,88	85,80
	2370,00	2022,55	85,34
	2288,00	1956,26	85,50
	Average (%)		**85,55**
	RSD (%)		**0,27**

3.3.6. Robustness

3.3.6.1. Variation of column batch

The column batch was changed, and it influence was evaluated on the retention time and resolution when compared with the original method conditions.

Variation of column batch	Serial number: NF-15839 Internal number: GC10/15 (method conditions)		Serial number: NF-38228 Internal number: GC15/16	
	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.
2-Propanol	2,54	2,94	3,32	3,19
Comment	-------		Not critical. Slight increase of retention time and resolution.	

3.3.6.2. Variation of the carrier gas flow rate

The carrier gas flow rate was changed in the range of 4,20 mL/min to 2,80 mL/min (3,50 mL/min ± 20%) and it influence was evaluated on the retention time and resolution when compared with the original method conditions.

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Variation of the carrier gas flow rate	2,80 mL/min		3,50 mL/min (method conditions)		4,20 mL/min	
	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.
2-Propanol	2,99	3,15	2,54	2,94	2,24	2,80
Comment	Not critical. Slight increase of retention time and resolution.		-------		Not critical. Slight decrease of retention time and resolution, fulfilling the resolution criteria.	

3.3.6.3. Variation of injector temperature

The injector temperature was changed in the range of 126ºC to 154ºC (140ºC ± 10%) and it influence was evaluated on the retention time and resolution when compared with the original method conditions.

Variation of injector temperature	126ºC		140ºC (method conditions)		154ºC	
	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.
2-Propanol	2,54	2,95	2,54	2,94	2,54	2,95
Comment	Not critical. No variation on retention time and resolution was observed.		-------		Not critical. No variation on retention time and resolution was observed.	

3.3.6.4. Variation of detector temperature

The detector temperature was changed in the range of 180ºC to 220ºC (200ºC ± 10%) and it influence was evaluated on the retention time and resolution when compared with the original method conditions.

3.2.P.5.3 Validation Report for the Determination of 2-Propanol in Ketosteril Film-Coated Tablets (GC)

Variation of detector temperature	180ºC		200ºC (method conditions)		220ºC	
	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.
2-Propanol	2,54	2,95	2,54	2,94	2,54	2,95
Comment	Not critical. No variation on retention time and resolution was observed.		-------		Not critical. No variation on retention time and resolution was observed.	

3.3.6.5. Variation of vial temperature

The vial temperature was changed in the range of 81ºC to 99ºC (90ºC ± 10%) and it influence was evaluated on the retention time and resolution when compared with the original method conditions.

Variation of vial temperature	81ºC		90ºC (method conditions)		99ºC	
	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.	rt (min)	Rel. Resol.
2-Propanol	2,54	2,95	2,54	2,94	2,54	2,95
Comment	No critical. No variation retention time and resolution was observed.		-------		Not critical. No variation on retention time and resolution was observed.	

3.4. Results analysis

3.4.1. 2-Propanol determination

The method is selective and linear in the concentration range of 187 to 2799 ppm.
The DL and QL calculated using the regression line is respectively 93,39 ppm and 282,99 ppm, however the detection and quantification limits considered corresponding to the lower concentration tested with RSD between 3 injections inferior to 10,0% (192,89 ppm).
The mean recovery percentage obtained in the accuracy test is 91,4%, 86,0%, 89,7% and 85,6% for the level 10, 80, 100 and 120% respectively.
Regarding the intermediate precision test, the mean and the RSD between the results of the two analysts are respectively 900,6 ppm and 7,6%.

3.4.2. Robustness

The variations tested in the column batch, carrier gas flow rate, injector temperature, detector temperature and vial temperature demonstrate that they are not critical when compared with the method conditions, proving the method robustness.

3.4.3. Conclusion

The method is selective, linear, precise, accurate and robust, therefore valid to perform the quantitative determination of 2-propanol in Ketosteril Tablets.