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Report for the validation of the dissolution test and determination of calcium in Ketosteril® tablets

Coustomer: Labesfal – Fresenius Kabi

Start: 2010/05/07

End: 2010/05/21

Code: RGRVM_10011.1

Report validation

Analyst	Technical Director
(Rui Manadas)	(Maria Eugénia Pina)

Date of validation: 2010/10/01

1. Objective

The aim of this study is carry out the validation of dissolution test of Ketosteril® tablets by calcium release determination with atomic absorption spectrometry.

2. Table of Contents

3. Definitions and abbreviations

- AAS – Atomic absorption spectroscopy
- RSD – Relative standard deviation
- SD – Standard Deviation

4. References

- PA077 - Protocol for the validation of dissolution test of Ketosteril® (internal procedure);
- PQVAL – Procedure for the validation of analytical methods (internal procedure).

5. Formula description

5.1- Pharmaceutical form

Coated tablets.

5.2- Formulation

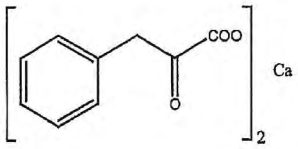
Composition	Unit (mg)
Core	
Alfa ketoisoleucine, calcium	67.0
Alfa ketoleucine, calcium	101.0
Alfa ketophenylalanine, calcium	68.0
Alfa ketovaline, calcium	86.0
Alfa hydroxymethionine, calcium	59.0
Isopropyl alcohol	101.666-108.333

Purified water	0,0016666-0,005
Povidone	26.3
Macrogol 6000	7.80
L-lisine acetate	105.0
L-threonine	53.0
L-tryptophan	23.0
L-histidine	38.0
L-tyrosine	30.0
Isopropyl alcohol	0.0375
Purified water	0.0015
Povidone	16.883
Macrogol 6000	5.0
Maize starch	44.00
Talc	19.6
Sílica colloidal anhydrous	4.40
Crospovidone	6.00
Magnesium stearate	15.00
Coating	
Isopropyl alcohol	70
Talc	9.07
Titanium dioxide	7.40
Macrogol 6000	1.20
Putified water	1.2
Quinoline yellow E 104	0.72
Putified water	6
Eudragit E 12,5% m/m	5.90
Triacetin	0.43
Macrogol 6000	0.28
Putified water	0.22
Isopropyl alcohol	2.017

5.3- Active Substances

i) α-ketophenylalanine, calcium salt

Structure:



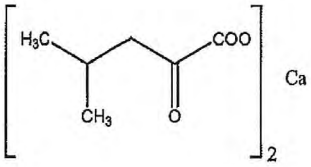
CAS number: 51828-93-4

Molecular formula: $C_{18}H_{14}O_6Ca$

Molecular mass: 366.4

ii) a-ketoleucine, calcium salt

Structure:



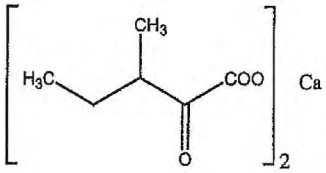
CAS number: 51828-95-6

Molecular formula: $C_{12}H_{18}O_6Ca$

Molecular mass: 298.4

iii) a-ketoisoleucine, calcium salt

Structure:



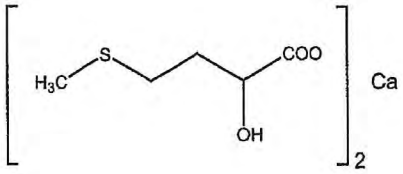
CAS number: 51828-96-7

Molecular formula: $C_{12}H_{18}O_6Ca$

Molecular mass: 298.4

iv) a-Hydroxymethionine, calcium salt

Structure:



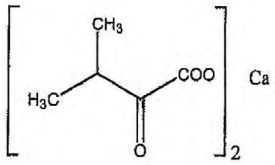
CAS number: 4857-44-7

Molecular formula: $C_{10}H_{18}O_6S_2Ca$

Molecular mass: 338.45

v) a-Ketovaline, calcium salt

Structure:



CAS number: 51828-94-5

Molecular formula: $C_{10}H_{14}O_6Ca$

Molecular mass: 270.3

6. Method

6.1- Reagents and materials

- Calcium standard for AAS (equivalent to 1000mg of Ca/L). Used:
  - Standard for calibration curve: Merck, cat. N° 1.19778.0500, batch n° HC961410
  - Standard for quality control: SPC Science, cat. n° 140-001-205, batch n° SC8150168

- HCl 0,1M, prepared from HCl 37%, Merck, cat. nº 1.00317.2500, batch nº K40740917
- Lanthanum chloride solution 2%, prepared from lanthanum (III) chloride heptahydrate, Merck, cat. nº 1.12219.0250, batch nº S4561319
- MillQ water – obtained from a Elix 5 Millipore System coupled with a Milli Q advantage system from Millipore
- Filters glass fibre – Whatman, GF/D, cat. nº 1823-025, batch nº K11596196
- Volumetric glassware and calibrated equipment were used.

6.2- *Equipment*

- Atomic Absorption Spectrometer (AAS), from Analytic Yenna, model Zeenit 700, equipped with flame atomization system and autosampler AS-52S capable of dilution of standards and samples
- Dissolution equipment, from Sotax, model AT7 Smart, equipped with piston pump (CY7-50) and fraction collector (C613), validated according to USP and BP guidelines for paddle apparatus.

6.3- *Testing conditions*

i) Dissolution test

- Apparatus: Paddle
- Dissolution medium: 900ml of 0,1M HCl
- Rotation speed: 100 rpm
- Temperature: 37± 0,5ºC
- Number of samples: 6 units
- Sampling time: 40 minutes
- Sampling volume: 2 ml

ii) AAS conditions

AAS conditions were optimized to obtain the best performance.

- Lamp type: HCL
- Line: 422.7 nm
- Slit: 1.2nm
- Lamp current: 4.0mA
- Integr. Time: 3.0 s
- PMT: 249.0 V
- D2-HCL curr.: 19.0
- Analytical mode: single beam
- Flame: C2H2 / N2O
- Fuel flow: 185L/h
- Burner height: 8mm
- Nebulizer rate: 5.0 mL/min

6.4- Standard Stock Solutions

Standard Stock Solution 1 (**SS1**) was prepared using the calcium standard 1000 mg/L to achieve a concentration of 50mg/L: 10mL of lanthanum chloride solution 2% plus 5 mL of Calcium Standard Solution 1000ppm diluted with HCl 0.1M to 100mL.

Standard Stock Solution 2 (**SS2**) was prepared from **SS1** to achieve a concentration of 5mg/L of calcium: 10mL of lanthanum chloride solution 2% plus 10 mL of Calcium **SS1** diluted with HCl 0.1M to 100mL.

AAS was programmed to dilute **SS2** and trace a calibration curve with the following concentrations: 0.2mg/L, 0.5mg/L, 1mg/L, 1.5mg/L, 2mg/L and 2.5mg/L of calcium, corresponding, respectively, to about 10%, 25%, 50%, 75%, 100% and 125% of the expected concentration in the dissolution vessel.

6.5- *Sample preparation*

Each tablet was weighed and all 6 tablets immersed in dissolution vessels, after dissolution media achieved adequate temperature. After 40 minutes a sample of 2mL of dissolution media was taken by the fraction collector. Samples were automatically filtered by the system.

Samples to be analysed by the AAS were prepared in AAS vials as follows: place 1.0 mL of lanthanum chloride solution (2%), 0.4 mL of sample taken from the dissolution vessel and 10mL of HCl 0.1M.

6.6- *Blank solution*

Blank solution for the AAS was prepared by diluting 10mL of lanthanum chloride 2% solution to 100mL with HCl 0.1M.

6.7- *Placebo samples*

Placebo solution for Ketosteril® coated tablets was prepared by weighing each ingredient, except the five active substances, mixing them, weighing the correspondent amount of placebo for each tablet and placing the amount in a dissolution vessel. Placebo sample was supplied by Labesfal, Fresenius Kabi.

Placebo samples were prepared like described in "Sample preparation" (6.5-).

7. Validation Parameters

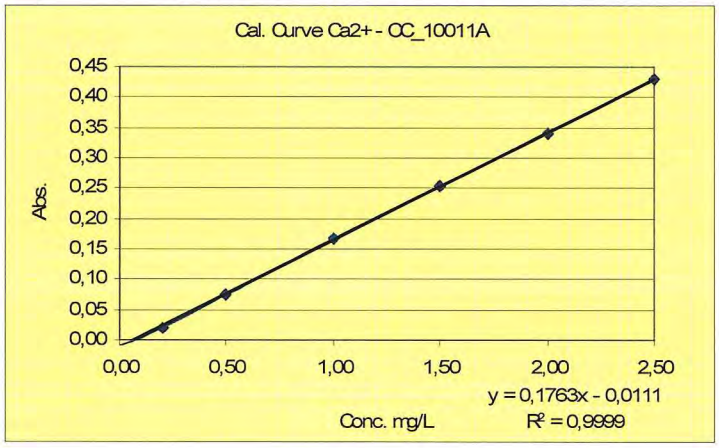
7.1- Linearity and range

Linearity of the quantification method was studied based on data from the manufacturer of the AAS equipment and on the expected concentrations of calcium after 40 minutes of dissolution. Each standard solution was measured 3 times and the average absorbance calculated.

Following are presented all the calibration curves traced:

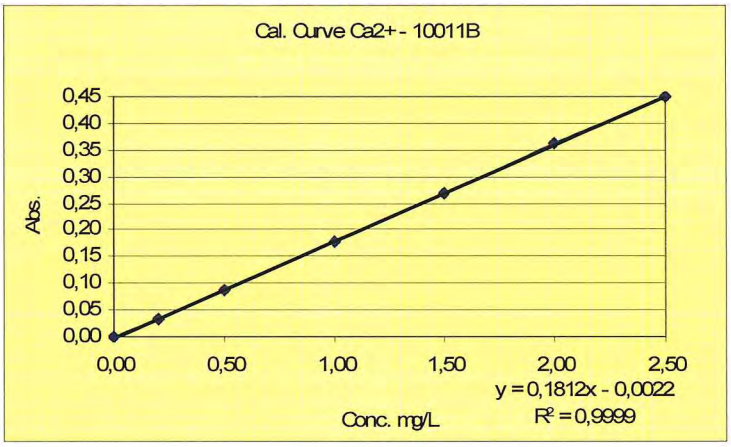
i) Calibration curve 10011A

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00891	0,00086	-9,7
P1	0.2	0,02135	0,00044	2,1
P2	0.5	0,07620	0,00142	1,9
P3	1.0	0,16633	0,00240	1,4
P4	1.5	0,25440	0,00121	0,5
P5	2.0	0,34140	0,00399	1,2
P6	2.5	0,42910	0,00675	1,6



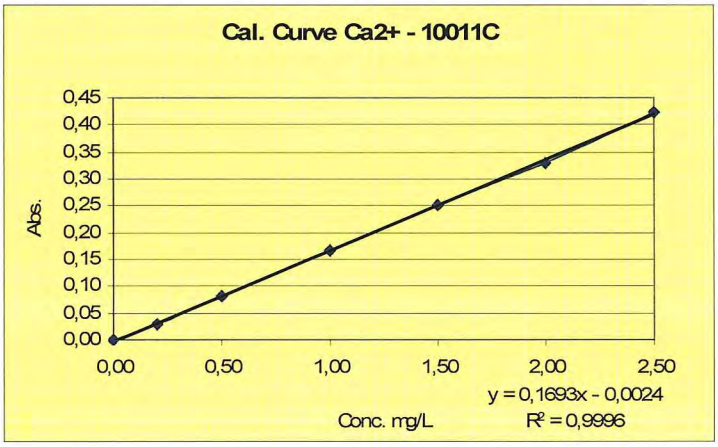
ii) **Calibration curve 10011B**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00090	0,00014	15,3
P1	0.2	0,03256	0,00007	0,2
P2	0.5	0,08778	0,00150	1,7
P3	1.0	0,17660	0,00216	1,2
P4	1.5	0,26967	0,00072	0,3
P5	2.0	0,36123	0,00046	0,1
P6	2.5	0,45107	0,00603	1,3



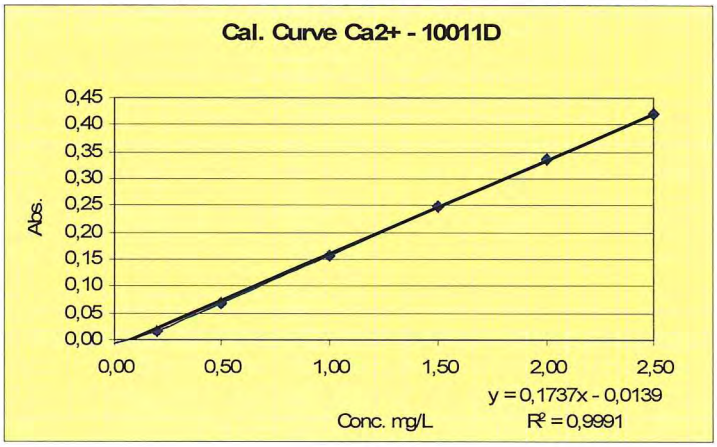
iii) **Calibration curve 10011C**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00114	0,00021	-18,0
P1	0.2	0,03063	0,00066	2,2
P2	0.5	0,08226	0,00102	1,2
P3	1.0	0,16753	0,00244	1,5
P4	1.5	0,25177	0,00211	0,8
P5	2.0	0,33030	0,00125	0,4
P6	2.5	0,42503	0,00456	1,1



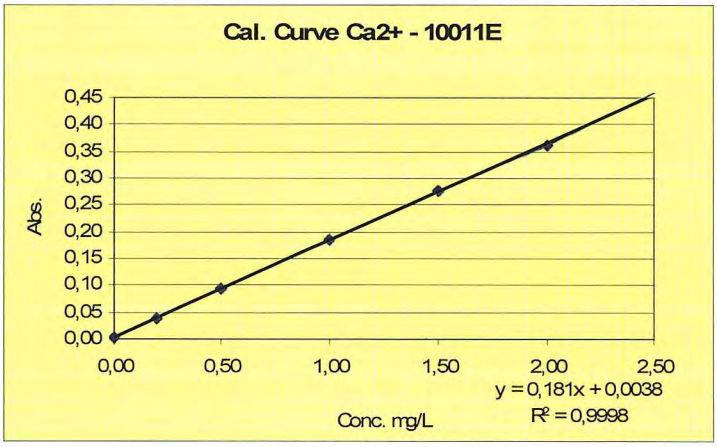
iv) **Calibration curve 10011D**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00497	0,00044	-9,0
P1	0.2	0,01681	0,00015	0,9
P2	0.5	0,06750	0,00015	0,2
P3	1.0	0,15627	0,00473	3,0
P4	1.5	0,24867	0,00401	1,6
P5	2.0	0,33473	0,00685	2,0
P6	2.5	0,42080	0,00308	0,7



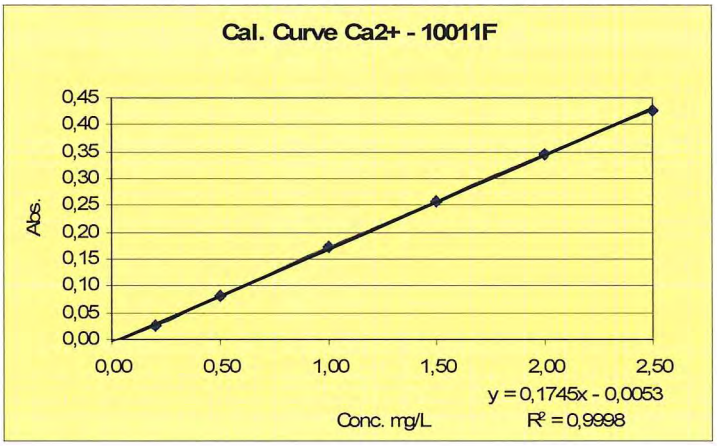
v) **Calibration curve 10011E**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00327	0,00128	39,3
P1	0.2	0,03922	0,00045	1,2
P2	0.5	0,09521	0,00075	0,8
P3	1.0	0,18680	0,00062	0,3
P4	1.5	0,27557	0,00158	0,6
P5	2.0	0,36147	0,00040	0,1
P6	2.5	0,45847	0,00233	0,5



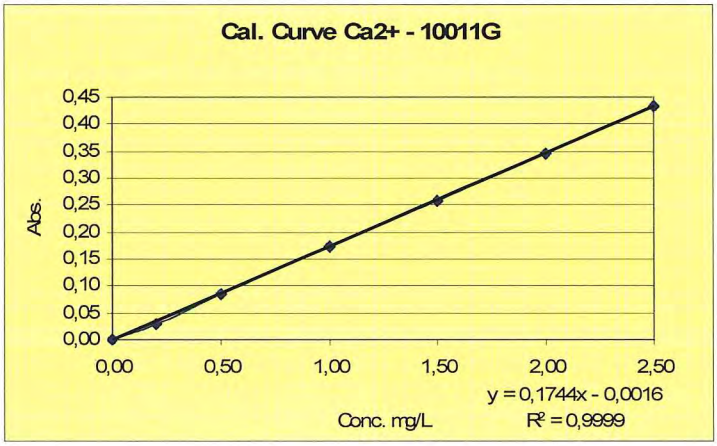
vi) **Calibration curve 10011F**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00308	0,00021	-6,7
P1	0.2	0,02574	0,00056	2,2
P2	0.5	0,08099	0,00168	2,1
P3	1.0	0,17163	0,00099	0,6
P4	1.5	0,25720	0,00218	0,8
P5	2.0	0,34540	0,00262	0,8
P6	2.5	0,42880	0,00191	0,4



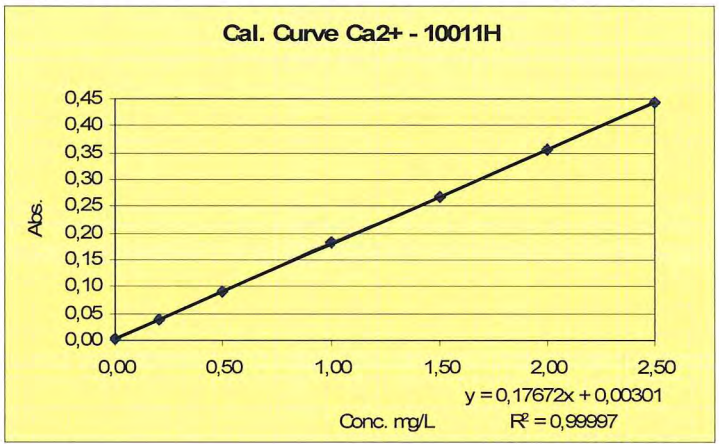
vii) **Calibration curve 10011G**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00005	0,00043	860,4
P1	0.2	0,03083	0,00053	1,7
P2	0.5	0,08627	0,00117	1,4
P3	1.0	0,17407	0,00228	1,3
P4	1.5	0,25857	0,00040	0,2
P5	2.0	0,34680	0,00255	0,7
P6	2.5	0,43507	0,00516	1,2



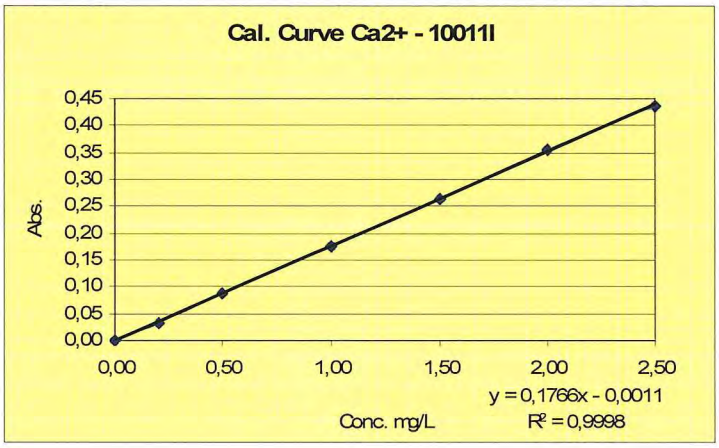
viii) **Calibration curve 10011H**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00217	0,00029	13,6
P1	0.2	0,03762	0,00036	1,0
P2	0.5	0,09209	0,00024	0,3
P3	1.0	0,18120	0,00075	0,4
P4	1.5	0,26870	0,00197	0,7
P5	2.0	0,35537	0,00424	1,2
P6	2.5	0,44460	0,00368	0,8



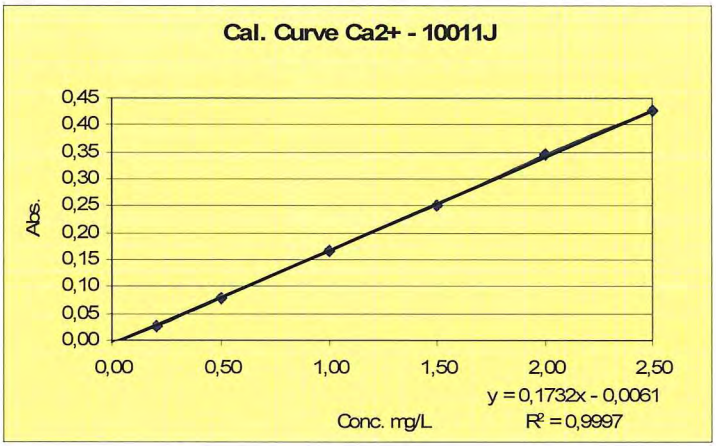
ix) **Calibration curve 10011I**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00046	0,00041	87,7
P1	0.2	0,03168	0,00023	0,7
P2	0.5	0,08642	0,00142	1,6
P3	1.0	0,17640	0,00132	0,7
P4	1.5	0,26527	0,00381	1,4
P5	2.0	0,35467	0,00575	1,6
P6	2.5	0,43780	0,00355	0,8



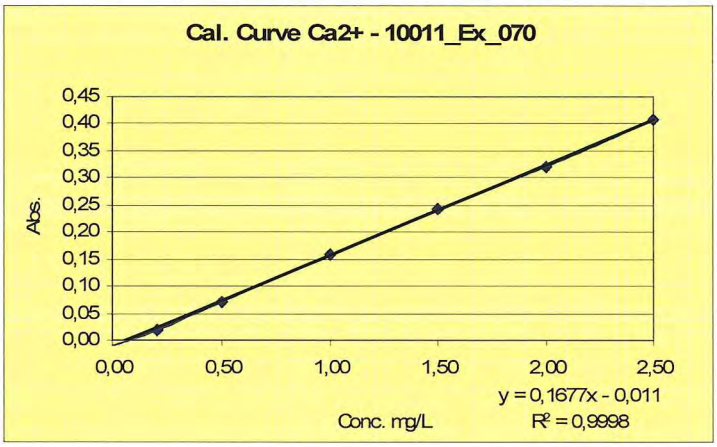
x) **Calibration curve 10011J**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00177	0,00061	-34,5
P1	0.2	0,02554	0,00014	0,5
P2	0.5	0,07915	0,00069	0,9
P3	1.0	0,16650	0,00075	0,5
P4	1.5	0,25193	0,00182	0,7
P5	2.0	0,34413	0,00476	1,4
P6	2.5	0,42593	0,00312	0,7



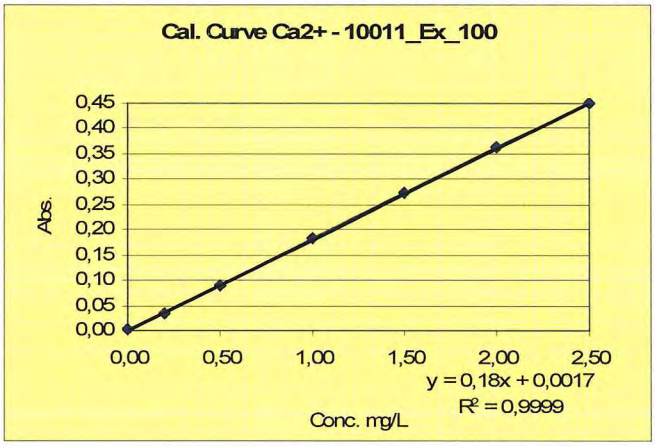
xi) **Calibration curve 10011_Ex_070**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	-0,00997	0,00102	-10,3
P1	0.2	0,01941	0,00008	0,4
P2	0.5	0,07248	0,00108	1,5
P3	1.0	0,16007	0,00172	1,1
P4	1.5	0,24250	0,00214	0,9
P5	2.0	0,32160	0,00557	1,7
P6	2.5	0,40833	0,00239	0,6



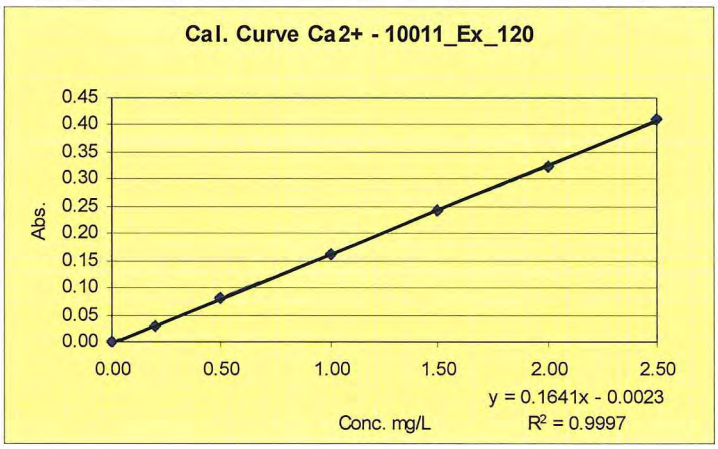
xii) **Calibration curve 10011_Ex_100**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00196	0,00006	3,2
P1	0.2	0,03583	0,00042	1,2
P2	0.5	0,09130	0,00103	1,1
P3	1.0	0,18300	0,00062	0,3
P4	1.5	0,27240	0,00304	1,1
P5	2.0	0,36410	0,00659	1,8
P6	2.5	0,44870	0,00347	0,8



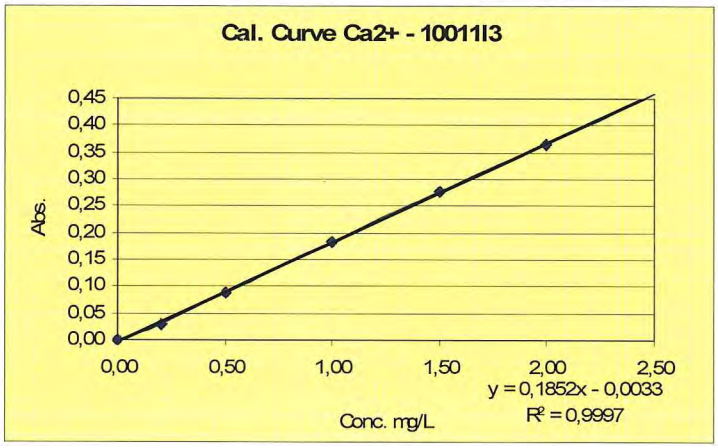
xiii) **Calibration curve 10011_Ex_120**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00110	0,00027	24,8
P1	0.2	0,02826	0,00095	3,3
P2	0.5	0,07935	0,00091	1,1
P3	1.0	0,16147	0,00107	0,7
P4	1.5	0,24363	0,00374	1,5
P5	2.0	0,32217	0,00136	0,4
P6	2.5	0,41153	0,00331	0,8



xiv) **Calibration curve 10011I3**

Standards	Conc. mg/L	Average	SD	RSD
Zero	0.0	0,00002	0,00035	1717,3
P1	0.2	0,02995	0,00085	2,8
P2	0.5	0,08661	0,00148	1,7
P3	1.0	0,18403	0,00276	1,5
P4	1.5	0,27797	0,00112	0,4
P5	2.0	0,36457	0,00140	0,4
P6	2.5	0,45947	0,00356	0,8



Calibration curves traced along the study revealed good linearity of the method - all R^2 were > 0.999.

7.2- *Precision*

7.2-1. Repeatability

Repeatability was studied by preparing 6 placebo samples and adding to each one the five actives substances equivalent to 100% content in calcium. Each placebo sample was submitted to the same procedure as the sample (Ketosteril® tablets).

The calcium content was determined and results obtained are as follows:

Placebo samples fortified (% calcium determined)

Time (min.)	PI-1	PI-2	PI-3	PI-4	PI-5	PI-6	Average	SD	RSD
40	98,3	98,7	100,1	99,5	101,5	102,3	100,1	1,6	1,6

Results evidenced good repeatability (RSD = 1.6%), smaller than the specified (RSD<2.0%).

7.2-2. Intermediate precision

Intermediate precision was studied by analysing the same sample in two different days, 6 tablets each day, by the same analyst.
The calcium content was determined and results obtained are as follows:

Sample code 10011I (% calcium determined)									
Day	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Average	SD	RSD
1	98,8	99,1	94,7	97,2	91,8	95,7	96,2	2,7	2,9
2	96,9	92,4	95,0	96,7	93,7	99,6	95,7	2,6	2,7
Average	**97,9**	**95,7**	**94,8**	**96,9**	**92,8**	**97,7**	**96,0**	**2,6**	**2,7**

Results evidenced good intermediate precision (RSD = 2.7%), smaller than the specified (3%).

7.3- Selectivity

Selectivity was studied by evaluating the interference of all the ingredients, except Active Substances, in the determination of calcium by the adopted methodology.
Placebo samples were prepared according to described in 6.7- and 6.3-i), and analysed according to described in 6.3-ii).

Placebo Sample - % calcium dissolved

Time (min.)	PI1	PI2	PI3	PI4	PI5	PI6	Average	SD	RSD
40	0,8	0,6	0,8	1,0	0,9	0,5	0,8	0,2	23,6

Results evidenced little interference of the placebo in the determination of calcium (<2% as specified).

7.4- Accuracy

Accuracy was studied by performing 18 determinations of calcium content in a placebo matrix fortified with 3 levels of concentration (70%, 100% and 120%), 6 independent samples for each concentration, and submitted to the dissolution conditions like samples. One supplementary sample was taken and analysed at 60 minutes.

Percentage recovered was determined and, for each fortification, the average value and the RSD were also evaluated.

% Recovery - fortification 70%									
Time (min.)	**Vessel 1**	**Vessel 2**	**Vessel 3**	**Vessel 4**	**Vessel 5**	**Vessel 6**	**Average**	**SD**	**RSD**
40	98,1	96,8	97,5	99,3	98,7	98,1	98,1	0,9	0,9
60	98,4	98,9	98,1	99,8	100,5	98,1	99,0	1,0	1,0

% Recovery - fortification 100%									
Time (min.)	**Vessel 1**	**Vessel 2**	**Vessel 3**	**Vessel 4**	**Vessel 5**	**Vessel 6**	**Average**	**SD**	**RSD**
40	98,3	98,7	100,1	99,5	101,5	102,3	100,1	1,6	1,6
60	99,5	101,5	99,7	100,5	101,7	104,4	101,2	1,8	1,8

% Recovery - fortification 120%

Time (min.)	**Vessel 1**	**Vessel 2**	**Vessel 3**	**Vessel 4**	**Vessel 5**	**Vessel 6**	**Average**	**SD**	**RSD**
40	99,4	100,7	101,8	99,9	100,5	101,3	100,6	0,9	0,9
60	99,4	101,9	103,0	101,9	101,5	100,7	101,4	1,2	1,2

After 40 minutes and 60 minutes of dissolution time, all samples complied the specifications for recovery (95-105%).

8. Conclusions

We can conclude that the proposed method of dissolution and determination of calcium from Ketosteril® coated tablets is suited for its purpose because it complies with specifications for validation parameters.

9. History

First edition approved in 2010-05-21.

2010-10-01 – Correction of specification from 5% to 3% according to PA077 – Chapter 7.2.2, page 18.