REPORT 4830/R

CIPAC FULL SCALE COLLABORATIVE STUDY ON THE DETERMINATION OF FOSTHIAZATE IN FOSTHIAZATE TECHNICAL MATERIAL AND FORMULATED PRODUCT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Progress Report to CIPAC on Method Development Work
Conducted by the ISK Company

By

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1 INTRODUCTION

The results of CIPAC full scale collaborative study for Fosthiazate technical material and Fosthiazate granular formulation are reported in this present study for its content of Fosthiazate.

In October 2011, CIPAC Information Sheet No. 292 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study to validate the high performance liquid chromatographic assay method for Fosthiazate in technical material and formulated product. A copy of the analytical method, protocol for the performance of the study, analysis report forms, samples and standards required for the analysis were sent to the respondents. The participants who completed the study are listed in Section 1.2.

The analytical method assessed in this study took into consideration CIPAC comments made in the frame of two previous small scale collaborative trials (reports presented in June 2010 and June 2011).

1.1 Samples

Five test samples, homogenized, and analytical standards were sent to the participants. All samples came from different batches of technical material and formulation.

Test Sample	Identification Code
Fosthiazate Technical batch 11029013	Tech-1
Fosthiazate Technical batch 11030013	Tech-2
Fosthiazate Technical batch 11031013	Tech-3
Fosthiazate 10 GR batch 11GR615	Fosthiazate 10 GR-1
Fosthiazate 10 GR batch 11GR617	Fosthiazate 10 GR-2

Fosthiazate analytical standard (batch No. Y920724, 99.3 % purity) and internal standard dimethyl phthalate (batch No. 302U1412, purity >99.5 %) were provided by ISK.

1.2 Participants

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Lab 6	Luis Manso	Laboratorio Arbitral Agroalimentario. Ministerio de Agricultura, Alimentación y Medio Ambiente Aguarón, 13. Aravaca. 28023 Madrid Spain
Lab 7	Lajos Benke	National Food Chain Safety Office, Directorate of Plant Protection Soil Conservation and Agri-environment, Pesticide Analytical Laboratory Ország út 23 H-2481 Velence Hungary
Lab 8	Dewi Lesmawaty	PT Agricon JL. Melati No.5 Desa Wanaherang Kec. Gunung Putri. Cibinong, 16965 Indonesia
Lab 9	Jim Garvey	Pesticides Control Service Laboratory Department of Agriculture, Food and the Marine, Backweston Campus, Youngs Cross, Celbridge, Co. Kildare IRELAND
Lab 10	Sunil V Waghmare	UNITED PHOSPHORUS LIMITED R & D Centre, Plot C-12, Road No 16, Wagle Industrial Estate, Thane (W) 400604 Maharashtra INDIA

Lab 11	Krste Tashev	State Phytosanitary Laboratory Ministry of Agriculture, Forestry and Water Economy bul Aleksandar the Great bb, 1000 Skopje (SPL) Jurij Gagarin br. 15 1000 Skopje (MAFWE) REPUBLIC OF MACEDONIA
Lab 12	Florentina Nedelcu	Central Phytosanitary Laboratory 11 Voluntari St, Code 077190, Voluntari Romania
Lab 13	Ana Gregorčič	AGRICULTURAL INSTITUTE OF SLOVENIA (Kmetijski inštitut Slovenije) Hacquetova ulica 17, 1000 Ljubljana, SLOVENIJA
Lab 14	Juliana Schlosserova	Central Controlling and Testing Institute in Agriculture (ÚKSÚP) Hanulova 9/A, 844 29 Bratislava 42 Slovakia
Lab 15	Andrew Plumb	The Food and Environment Research Agency Sand Hutton, York, North Yorkshire YO41 1LZ, UK

2 ANALYTICAL METHOD

2.1 Scope

This method is applicable to the determination of Fosthiazate in Fosthiazate technical (i.e. Technical Grade Active Ingredient or TGAI). This method is also applicable to the determination of Fosthiazate in the formulation Fosthiazate 10 GR.

2.2 Outline of method

Fosthiazate is determined in the test samples by reversed-phase high performance liquid chromatography using ultraviolet detection at 220 nm. Quantification is done by internal standardization (dimethyl phthalate).

2.3 Procedure

Each sample was analyzed by four independent determinations (n_i = 4). The samples were analyzed in a first run (Day 1) by duplicate injections of two weighings for each sample. The sequence was repeated on a second date (Day 2) with two other weighings of each sample. The Fosthiazate content for each injection was calculated using the mean response factor from the calibration solutions bracketing the injections of the sample solutions (single point calibration). For the calculation of the content of a sample, the mean value of the duplicate injections was used. For further details, please see attached full method description.

3 REMARKS OF PARTICIPANTS

3.1 Analytical Conditions

Lab	Liquid chromatograph; Detector; Injector	Column	Mobile phase [1]	T (°C)	Flow (ml/min)	Vol inj (µl)
1	HPLC Varian Star; Varian 9050; Varian Prostar 410, automatic	Luna C18(2) 100A, 150mm x 4.6mm x 5µm; Serial n°529946-4	Eluent A: ACN-H2O / 1-2 Eluent B: ACN	27 (amb.)	1.2	10
2	Waters Alliance 2695 Separation Module ; Waters 996 PAD; Automatic	Agilent Hypersil ODS C18, 5 µm, 125 x 4 mm i.d.; Serial No. US41B06767	Eluent A: Water Eluent B: Acetonitrile	25	1	10
3	Agilent HPLC; DAD 1200	Agilent Zorbax Eclipse XDB-C18 150×4.6 mm, 5micron; Serial No.: USKH060419	Eluent A: acetonitrile – water (1 – 2 v/v) Eluent B: acetonitrile	25	1	10
4	Agilent 1260; DAD; Automatic	Hypersil ODS 5µm, 150x4,6mm; No.: 903.068	Eluent A: demin. Water : Acetonitrile 2:1 (v/v) Eluent B: Acetonitrile	25	1.6	10
5	Agilent Technologies 1200 Serie; Automatic	LiChrospher 100 RP 18 (5µm) 4x250 mm	Eluent A: acetonitrile Eluent B: water	25	1	10
6	Agilent 1100; Automatic	Phenomenex Kinetex 2.6 µm C18 100A (100x4.6mm); Serial No. 526037-51	Acetonitrile/water (30:70)	25	1	10
7	Dionex; Dionex UVD 170S; Automatic, Dionex ASI 100	Agilent Zorbax SB-C18, 4,6x250mm, 5µm; USCL 020208	Eluent A: water Eluent B: acetonitrile	25	1	5
8	SHIMADZU 20 AT; UV, SPD 20A; Manual, CTO-10 AS VP	250 x 4.6 mm (i.d), packed with C18, 5 µm, Thermo science ODS HYPERSIL; P/No = 30105-254630, S/No = 0903388M3	Eluent A: acetonitrile – water (1 – 2 v/v) Eluent B: acetonitrile	25	1	10

9	AGILENT 1100 SERIES; DAD G1315B; G1313A, automatic	HYPERCLONE – PHENOMENEX – 125 X 4.00mm 5µm; 522276-3	Eluent A: Acetonitrile:H2O (1:2 v/v) Eluent B: Acetonitrile	25	1	10
10	WATERS, Alliance, Quaternaire pump. 2695; WATERS PDA 2996; Automatic	Thermo Electron Corporation SAS Hypersil 250 x 4.6 mm x 5 micron	Eluent A: Acetonitrile – Water (1:2 v/v) Eluent B: Acetonitrile	25	1.4	10
11	VARIAN Pro Star, pump 230, column thermostat 550; PDA Detector VARIAN 330; Auto-sampler 430	Brownlee Analytical C 18, 5 µm, 150 mm; 4.6 mm; Serial No: 06120457P	Eluent A: acetonitrile – water 1-2v/v Eluent B: acetonitrile	25	1	5
12	Dionex Ultimate 3000 ; DAD 100 Hz; Automatic, model WPS 300 SL	Hypersil Gold, 250 mm length, 4.6 mm I.d., 5 µm particles; SN 0764636N	Eluent A: Water Eluent B: Methanol	30	1	5
13	Agilent Techn 1200 series; Diode Array SL, G1315D; Automatic, G1367B	Spherisorb ODS 2, 5 µm, 125 x 4 mm; Serial No.: 7992402-564	Eluent A: acetonitrile / water = 1 / 2 (v/v) Eluent B: acetonitrile	25	1	10
14	HPLC Agilent 1100 Series; VWD; Automatic	Hypersil 120-3 ODS , 4.0x125 mm, 3µm; S.No.: 1014179	Acetonitrile / Acetonitrile:Water (1:2) - gradient	25	1	10
15	HP 1050; 1090 DAD; Automatic	125 x 4.0 mm Hyperclone ODS 5 µm (Phenomenex); s/n 416002-2	Eluent A: acetonitrile – water (1-2 v/v) Eluent B: acetonitrile	25	1	10

T = Temperature Vol inj = Volume injected

[(1)] Gradient elution was the same as proposed except for Labs:

Gradient	Time (min)	Eluent A (%)	Eluent B (%)
As per method: Labs: 4, 9, 10, 11, 13, 14 and 15	0	100	0
	8	100	0
	8.5	0	100
	14	0	100
	(Post time) 3	100	0
Lab 1	0	100	0
	13.5	100	0
	14	0	100
	21	0	100

	21.5	100	0
	24	100	0
Lab 2	0	66.7	33.3
	8	66.7	33.3
	8.5	0	100
	14	0	100
	14.1	66.7	33.3
	17	66.7	33.3
Lab 3	0	100	0
	12	100	0
	12.5	0	100
	18	0	100(stop time)
	(Post time)3	100	0
Lab 5	0 – 8 min	50	50
	8,5 – 14 min	100	0
	Stop Time: 14		
	Post Time: 3		
Lab 6	Isocratic		
Lab 7	0	60	40
	5	60	40
	10	10	90
	12	10	90
	13	60	40
	15	60	40
Lab 8	0	100	0
	8.5	0	100
	12.0	0	100
	12.01	100	0
	17.00	100	0
	17.01	Stop time	
Lab 12	Isocratic		

3.2 Remarks

Several labs made comments about the performance of the method and noted deviations from the method that occurred:

Lab 1 I adapted the gradient program and the flow because I used a different column dimension.
PE+5ml SI/100ml acetone =>US'15' => 2/20ml ACN.

Lab 2 No comments.

Lab 3 Only the Fosthiazate GR assay was completed.
The sonication time for sample preparation may be helpful to achieve better accuracy and precision.
An equivalent HPLC column (XDB-C18 150×4.6 mm, 5micron) was used instead of the specified column in original method. Therefore, the retention times of Fosthiazate and ISTD were delayed. In order to have the symmetrical peak shape, the time of 100% eluent A and stop time were prolonged as shown above. And the retention times of Fosthiazate and ISTD respectively were 10.2 min and 8.0 min.

Lab 4 Only Integration had to be fulfilled manually because of bad baseline.
Maybe carryover effects from sampler (see chromatogram of sample solvent).
Flow corrected from 1.0 to 1.6 ml/min for used HPLC-Column dimensions (150/4.6 mm instead of 125/4).

Lab 5 As there was no Agilent Hypersil ODS 5 µm (125 x 4 mm) column available a LiChrospher 100 RP18 (5 µm) column was used. Because of a misunderstanding also the mobile phase was changed. Retention times: Dimethylphthalat: 5.34 min, Fosthiazate: 5.87 min.

Lab 6 In my opinion, it is no necessary to use internal standard. As active ingredient is quite soluble in water, acetonitrile-water can be used as extraction solvent and avoid the use of acetone.
Less amount of standard and samples have been weihted to avoid dilution.
Acetonitrile/water (80:20) has been used as extraction solvent. Internal standard has not been used.

Lab 7 Different gradient.

Lab 8 We used column from Hypersil dimension 250 x 4.6 mm (i.d), different with reference method (Agilent Hypersil, 125 x 4 mm i.d), but both of column have a same content. We had some difficulties to find same column with reference method.
The column (same gradien program) give retention time approx. 13.7 min (fosthiazate) and 12.3 (dimethyl phthalate) was different from reference method that state dimethyl phthalate (internal standard) approximately 4.7 min, fosthiazate approximately 6.4 min.).
Gradien program was different from reference method, because when we used same gradien program the response factor (f_i) of area fosthiazate and dimethyl phthalate was not stable.

Lab 9 The analysis went well. Tech 2A -1 injection for day 1 analysis showed major retention time shift and higher than expected responses compared to Tech 2A-2 injection This led to a lower result for Tech2 sample. No other major retention time shifting observed throughout day 1 run.

Lab 10

The test method is very nice and user friendly. We did not face any problem while doing the exercise. We would like to participate in any forthcoming ring tests conducted by ISK and CIPAC.
The analysis was smooth; we did not face any problem. There were no analytical variations during the analysis.
Flow rate was changed to match the retention times.

Lab 11

Preparing of the standards and samples was very easy and was not time consuming. Proposed time for sonication of the samples was not sufficient. I used for all 3 samples 3 x 10 min, and shaking between.
The chromatography system that we have it's relatively new, but we do not have UPS that will make constant current during performing the analysis.
General the test method is very nice and user friendly.

Lab 12

Very good stability of retention time.
Extractions and dilutions were performed with analytical purity methanol.
For sample preparation was used only methanol. After injections of GR formulation column was 30 min flushed with methanol at 1.0 ml/min flow rate.

Lab 13

No comments and deviations.

Lab 14

For preparation of calibration/sample solutions were used 50 ml volumetric flasks, maintaining the prescribed final concentration.

Lab 15

No comments and deviations.

4 RESULTS AND DISCUSSION

The statistical evaluation of the collaborative trial was performed according to DIN ISO 5725. Samples were sent to 15 laboratories. All laboratories sent back results and the results reported by the participants were all used. Lab 3 only determined the content of Fosthiazate in formulation samples and consequently was included only for evaluation of those data. The assay results reported by the laboratories are listed in Tables 2-6, and are presented in Figures 1-5.

Statistical evaluation of the data was done following "Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods." The data were examined for outliers and stragglers using Cochran's test on the within-lab variability, followed by Grubbs-I test on the laboratory means, and iterating where necessary. Where deemed necessary, an additional Grubbs-II test was conducted to identify two stragglers or outliers. The tests were performed at an alpha level of 0.01 for outlier, and 0.05 for straggler. Based on this procedure, the Cochran and Grubbs-I tests identified the following potential outlier and straggler lab data from the sample sets. Straggler and outlier values are reported below.

Fosthiazate determination				
Code Sample	**Cochran Straggler**	**Cochran Outlier**	**Grubbs-I Straggler**	**Grubbs-I Outlier**
Tech-1	none	Lab 8, 9, 11	none	none
Tech-2	none	Lab 1, 9, 11	none	none
Tech-3	none	Lab 11	none	none
Fosthiazate 10 GR - 1	none	none	none	none
Fosthiazate 10 GR - 2	none	Lab 12	Lab 12	none

A summary of the statistical evaluation for the labs is given in Table 1, which includes the repeatability and reproducibility values, as well as the between-lab experimental Reproducibility Relative Standard Deviation, $\%RSD_R$ and the calculated acceptable value, $\%RSD_{R(Hor)}$, based on the Horwitz curve calculation.

4.1 Fosthiazate – Method deviations not considered

Tech-1

Three outliers were detected by the Cochran test of variance homogeneity (labs 8, 9 and 11). Variance from Lab 8 and Lab 11 were significantly higher than for the other labs. Grubbs-I for all labs did not identify a straggler or outlier (nor did Grubbs-II).

The statistical analysis of the results (no outlier removed) show the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate technical 1 sample to be well below the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation. As Horwitz criteria were met with all data retained, the statistical evaluation was not repeated without identified outliers.

Tech-2

Three outliers were detected by the Cochran test of variance homogeneity. Variance from Lab 1, Lab 9 and Lab 11 were significantly higher than for the other labs. Grubbs-I for all labs did not identify a straggler or outlier (nor did Grubbs-II).

The statistical analysis of the results (no outlier removed) show the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate technical 2 sample to be well below the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation. As Horwitz criteria were met with all data retained, the statistical evaluation was not repeated without identified outliers.

Tech-3

An outlier was detected by the Cochran test of variance homogeneity for Lab 11. Variance from Lab 11 is the highest. Grubbs-I for all labs did not identify a straggler or outlier (nor did Grubbs-II).

The statistical analysis of these results (no outlier removed) show the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate technical 3 sample to be well below the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation. As Horwitz criteria were met with all data retained, the statistical evaluation was not repeated without identified outliers.

Fosthiazate 10 GR-1

No straggler or outlier was detected by the Cochran test of variance homogeneity. Grubbs-I for all labs did not identify a straggler or outlier (nor did Grubbs-II).

The statistical analysis of these results show the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate 10 GR-1 sample to be below or slightly exceeding the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation. Further refinement is proposed in 4.2.

Fosthiazate 10 GR-2

One outlier was detected by the Cochran test of variance homogeneity for Lab 12. Variance from Lab 12 is the highest and the mean value at 111.6 g/kg was significantly higher than the others, which was also detected by the Grubbs-I test as a straggler. The lab 12 value is maintained in the statistical evaluation presented in Table 1A, but was removed for evaluation in the refined Table 1B. This refinement did not have a major impact on the observed overall mean value, but did improve significantly the Reproducibility parameter.

The statistical analysis of these results show the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate 10 GR-2 sample to be below or, after refinement,

only slightly exceeding the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation. Further refinement is proposed in 4.2.

4.2 Fosthiazate – Method Deviations considered

Two laboratories deviated significantly from the suggested extraction procedure. Lab 6 used acetonitrile/water (80:20) as extraction solvent and Lab 12 used methanol. Although the results of both labs did not result in a statistical outlier (except for the GR-2 analyses by Lab 12) the impact of this modification was assessed by analyzing the data set without these two labs. Table 1C presents the results of this data evaluation and shows that no major change is observed compared to the results reported in Table 1B. The mean values slightly decreased mainly due to the removal of Lab 12 data. However, the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate GR-1 and GR-2 sample decreased slightly after refinement, being at or just above the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation.

Two laboratories deviated from the proposed sample weight for the weighing of the granular formulation samples. Lab 6 and Lab 14 used less than the half (about 300-400 mg instead of 1 g) or half (about 500 mg instead of 1 g) of the recommended sample weight, respectively. For GR-1 sample, lab 14 showed the highest variance. Lab 14 also showed the highest variance for GR-2 sample after removal of the outlier value (Lab 12) for GR-2. Lab 6 showed the highest variance for GR-2 after removal of Labs 12 and 14. This can be explained by the low sample weight and therefore both Labs were excluded for the data analysis presented in Table 1D. Compared to Table 1B, the mean values slightly increased and the Repeatability Relative Standard Deviation ($\%RSD_r$) and the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for the determination of Fosthiazate in Fosthiazate GR-1 and GR-2 samples decreased after refinement below the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation.

5 CONCLUSION

After examination of all provided laboratory data and identification of stragglers and outliers, retention of all laboratory data was statistically valid for the determination of Fosthiazate in Fosthiazate technical. Labs 1, 8, 9, and 11 showed high within-lab variability (Cochran outliers), leading to somewhat higher repeatability and reproducibility, but still within acceptable range.

After examination of all provided laboratory data and identification of stragglers and outliers, deviations from the method were noticed for some labs. While the changes made to the extractions seemed to have some minor impact, the reduction of the formulation sample weight led to an inacceptable reproducibility for the determination of Fosthiazate in Fosthiazate formulation. A refinement was proposed after removal of Lab 12 for the analysis of the formulation sample GR-2 (outlier) and removal of Labs 6 and 14 for both formulation samples (insufficient sample weight). It should be noted that the minimum sample weight of 1 g is crucial for the performance of the method.

The between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) and the Repeatability Relative Standard Deviation ($\%RSD_r$) were well below the limit calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation for the determination of Fosthiazate in Fosthiazate technical, while $\%RSD_R$ exceeded the Horwitz value for Fosthiazate formulation analysis with no outlier removed. After removal of the outlier and considering deviations from the method, Horwitz criteria were met ($\%RSD_R < \%RSD_{R(Hor)}$).

The acceptance of this method as an approved CIPAC assay method for Fosthiazate in Fosthiazate technical and Fosthiazate formulation is recommended.

ACKNOWLEDGEMENTS

ISK wishes to thank all the laboratories and their staff who participated in this study.

TABLES (1-6)

TABLE 1A - Fosthiazate – Summary of the statistical evaluation of the collaborative Study Data All Test Results Retained (No outlier removed)

				Fosthiazate 10 GR	
	Tech-1	**Tech-2**	**Tech-3**	**GR-1**	**GR-2**
No. of Labs	14	14	14	15	15
No. of Stragglers	0	0	0	0	$1^{(g)}$
No. of Outliers	$3^{(C)}$	$3^{(C)}$	$1^{(C)}$	0	$1^{(C)}$
No. of Labs Retained	14	14	14	15	15
No. of Results	56	56	56	60	60
Total Mean, **X** (g/kg)	966.8	967.6	970.7	105.2	102.9
Repeatability standard deviation $\mathbf{S_r}$	6.79	6.49	3.34	2.73	2.69
"Pure" between laboratory standard variation $\mathbf{S_L}$	8.43	8.81	8.50	1.70	3.05
Reproducibility standard deviation $\mathbf{S_R}$	10.82	10.94	9.13	3.22	4.07
Repeatability **r**	19.0	18.2	9.36	7.64	7.54
Reproducibility within lab $\mathbf{R_L}$	23.6	24.7	23.8	4.77	8.54
Reproducibility between labs **R**	30.3	30.6	25.6	9.01	11.39
RSD_r (%)	0.70	0.67	0.34	2.60	2.62
RSD_R (%)	1.12	1.13	0.94	3.06	3.95
$RSD_{R(Hor)}$ (%)	2.01	2.01	2.01	2.81	2.82

Limits (g/kg)

X+R	997.1	998.2	996.3	114.2	114.3
X+r	985.8	985.7	980.1	112.8	110.4
X-r	947.8	949.4	961.4	97.5	95.3
X-R	936.5	936.9	945.2	96.2	91.5

$^{(C,c)}$ from Cochran test
$^{(G,g)}$ from Grubbs-I test

Where :

X	= average
S_r	= repeatability standard deviation
S_L	= "pure" between laboratory standard deviation
S_R	= reproducibility standard deviation = $(S_r^2 + S_L^2)^{0.5}$
r	= repeatability within-lab (2.8 S_r)
R	= reproducibility between labs (2.8 S_R)
R_L	= reproducibility within lab on different days (2.8 S_L)
% RSD_r	= repeatability relative standard deviation (100 S_r/X)
% RSD_R	=reproducibility relative standard deviation between labs (100 S_R/X)
% $RSD_{R(Hor)}$	= Horowitz value calculated from $2^{(1-0.5\log c)}$ where c is the concentration of the analyte as a decimal fraction (e.g. for 100% concentration c = 1)

TABLE 1B - Fosthiazate – Summary of the statistical evaluation of the collaborative Study Data Selected Outlier Test Results Removed

				Fosthiazate 10 GR	
	Tech-1	**Tech-2**	**Tech-3**	**GR-1**	**GR-2**
No. of Labs	14	14	14	15	15
No. of Stragglers	0	0	0	0	0
No. of Outliers	$3^{(C)}$	$3^{(C)}$	$1^{(C)}$	0	0
No. of Labs Retained	14	14	14	15	14
No. of Results	56	56	56	60	56
Total Mean, **X** (g/kg)	966.8	967.6	970.7	105.2	102.3
Repeatability standard deviation $\mathbf{S_r}$	6.79	6.49	3.34	2.73	2.10
"Pure" between laboratory standard variation $\mathbf{S_L}$	8.43	8.81	8.50	1.70	2.15
Reproducibility standard deviation $\mathbf{S_R}$	10.8	10.94	9.13	3.22	3.00
Repeatability **r**	19.0	18.2	9.36	7.64	5.88
Reproducibility within lab $\mathbf{R_L}$	23.6	24.7	23.8	4.77	6.01
Reproducibility between labs **R**	30.3	30.6	25.6	9.01	8.41
RSD_r (%)	0.70	0.67	0.34	2.60	2.05
RSD_R (%)	1.12	1.13	0.94	3.06	2.94
$RSD_{R(Hor)}$ (%)	2.01	2.01	2.01	2.81	2.82

Limits (g/kg)

X+R	997.1	998.2	996.3	114.2	110.7
X+r	985.8	985.7	980.1	112.8	108.1
X-r	947.8	949.4	961.4	97.5	96.4
X-R	936.5	936.9	945.2	96.2	93.8

$^{(C,c)}$ from Cochran test
$^{(G,g)}$ from Grubbs-I test

Where :

X	= average
S_r	= repeatability standard deviation
S_L	= "pure" between laboratory standard deviation
S_R	= reproducibility standard deviation = $(S_r^2 + S_L^2)^{0.5}$
r	= repeatability within-lab (2.8 S_r)
R	= reproducibility between labs (2.8 S_R)
R_L	= reproducibility within lab on different days (2.8 S_L)
% RSD_r	= repeatability relative standard deviation (100 S_r/X)
% RSD_R	=reproducibility relative standard deviation between labs (100 S_R/X)
% $RSD_{R(Hor)}$	= Horowitz value calculated from $2^{(1-0.5\log c)}$ where c is the concentration of the analyte as a decimal fraction (e.g. for 100% concentration c = 1)

TABLE 1C - Fosthiazate – Summary of the statistical evaluation of the collaborative Study Data Labs 6 and 12 Removed (Method Deviations – Extraction)

				Fosthiazate 10 GR	
	Tech-1	**Tech-2**	**Tech-3**	**GR-1**	**GR-2**
No. of Labs	14	14	14	15	15
No. of Stragglers	0	0	0	0	0
No. of Outliers	$3^{(C)}$	$3^{(C)}$	$1^{(C)}$	0	0
No. of Labs Retained	12	12	12	13	13
No. of Results	48	48	48	52	52
Total Mean, **X** (g/kg)	965.8	966.0	969.6	105.3	102.2
Repeatability standard deviation $\mathbf{S_r}$	6.76	6.41	2.56	2.40	1.86
"Pure" between laboratory standard variation $\mathbf{S_L}$	7.53	7.41	7.05	1.78	2.20
Reproducibility standard deviation $\mathbf{S_R}$	10.1	9.80	7.50	2.99	2.88
Repeatability **r**	18.9	17.9	7.16	6.71	5.21
Reproducibility within lab $\mathbf{R_L}$	21.1	20.7	19.7	4.98	6.17
Reproducibility between labs **R**	28.3	27.4	21.0	8.36	8.07
RSD_r (%)	0.70	0.66	0.26	2.28	1.82
RSD_R (%)	1.05	1.01	0.77	2.83	2.82
$RSD_{R(Hor)}$ (%)	2.01	2.01	2.01	2.81	2.82

Limits (g/kg)

X+R	994.1	993.5	990.5	113.7	110.3
X+r	984.7	984.0	976.7	112.1	107.4
X-r	946.9	948.1	962.4	98.6	97.0
X-R	937.4	938.6	948.6	97.0	94.1

$^{(C,c)}$ from Cochran test
$^{(G,g)}$ from Grubbs-I test

Where :

X	= average
S_r	= repeatability standard deviation
S_L	= "pure" between laboratory standard deviation
S_R	= reproducibility standard deviation = $(S_r^2 + S_L^2)^{0.5}$
r	= repeatability within-lab (2.8 S_r)
R	= reproducibility between labs (2.8 S_R)
R_L	= reproducibility within lab on different days (2.8 S_L)
% RSD_r	= repeatability relative standard deviation (100 S_r/X)
% RSD_R	=reproducibility relative standard deviation between labs (100 S_R/X)
% $RSD_{R(Hor)}$	= Horowitz value calculated from $2^{(1-0.5\log c)}$ where c is the concentration of the analyte as a decimal fraction (e.g. for 100% concentration c = 1)

TABLE 1D - Fosthiazate – Summary of the statistical evaluation of the collaborative Study Data Labs 6 and 14 Removed (Method Deviation – Formulation sample weight)

				Fosthiazate 10 GR	
	Tech-1	**Tech-2**	**Tech-3**	**GR-1**	**GR-2**
No. of Labs	14	14	14	15	15
No. of Stragglers	0	0	0	0	0
No. of Outliers	$3^{(C)}$	$3^{(C)}$	$1^{(C)}$	0	0
No. of Labs Retained	14	14	14	13	12
No. of Results	56	56	56	52	48
Total Mean, **X** (g/kg)	966.8	967.6	970.7	105.6	102.7
Repeatability standard deviation $\mathbf{S_r}$	6.79	6.49	3.34	2.31	1.55
"Pure" between laboratory standard variation $\mathbf{S_L}$	8.43	8.81	8.50	1.49	1.53
Reproducibility standard deviation $\mathbf{S_R}$	10.8	10.94	9.13	2.75	2.18
Repeatability **r**	19.0	18.2	9.36	6.48	4.34
Reproducibility within lab $\mathbf{R_L}$	23.6	24.7	23.8	4.17	4.28
Reproducibility between labs **R**	30.3	30.6	25.6	7.70	6.09
RSD_r (%)	0.70	0.67	0.34	2.19	1.51
RSD_R (%)	1.12	1.13	0.94	2.61	2.12
$RSD_{R(Hor)}$ (%)	2.01	2.01	2.01	2.81	2.82

Limits (g/kg)

X+R	997.1	998.2	996.3	113.3	108.8
X+r	985.8	985.7	980.1	112.1	107.0
X-r	947.8	949.4	961.4	99.1	98.3
X-R	936.5	936.9	945.2	97.9	96.6

$^{(C,c)}$ from Cochran test
$^{(G,g)}$ from Grubbs-I test

Where :

X	= average
S_r	= repeatability standard deviation
S_L	= "pure" between laboratory standard deviation
S_R	= reproducibility standard deviation = $(S_r^2 + S_L^2)^{0.5}$
r	= repeatability within-lab (2.8 S_r)
R	= reproducibility between labs (2.8 S_R)
R_L	= reproducibility within lab on different days (2.8 S_L)
% RSD_r	= repeatability relative standard deviation (100 S_r/X)
% RSD_R	=reproducibility relative standard deviation between labs (100 S_R/X)
% $RSD_{R(Hor)}$	= Horowitz value calculated from $2^{(1-0.5\log c)}$ where c is the concentration of the analyte as a decimal fraction (e.g. for 100% concentration c = 1)

TABLE 2 - Assay Results Summary by Lab for Fosthiazate in Fosthiazate Technical, Tech-1
All values in [g/kg]

	DAY 1		DAY 2				
LAB	A	B	A	B	Mean	SD	Notes
1	947.98	948.69	949.48	948.91	948.76	0.62	
2	970.54	967.74	966.76	967.82	968.21	1.62	
3							
4	961.80	962.78	965.40	964.03	963.50	1.56	
5	959.97	963.00	967.35	964.96	963.82	3.12	
6	965.33	963.42	966.43	966.99	965.54	1.57	
7	985.38	984.78	983.46	982.44	984.02	1.32	
8	968.12	1008.32	961.07	963.03	975.13	22.32	C
9	964.65	963.84	957.27	954.19	959.99	5.09	C
10	969.16	968.03	966.27	967.69	967.79	1.19	
11	952.33	957.04	970.87	969.78	962.51	9.24	C
12	980.44	982.01	982.13	978.70	980.82	1.61	
13	956.53	954.06	958.63	959.55	957.19	2.44	
14	966.61	968.16	970.26	965.70	967.68	2.00	
15	969.34	970.95	970.23	972.48	970.75	1.33	

SD = Standard deviation

c-Cochran straggler, **C**-Cochran outlier, **g**-Grubb straggler, **G**-Grubb outlier

TABLE 3 - Assay Results Summary by Lab for Fosthiazate in Fosthiazate Technical, Tech-2
All values in [g/kg]

	DAY 1		DAY 2				
LAB	A	B	A	B	Mean	SD	Notes
1	961.14	968.89	980.84	974.70	971.39	8.398	C
2	968.34	968.95	970.20	969.64	969.28	0.808	
3							
4	961.77	962.84	965.26	967.25	964.28	2.457	
5	959.69	958.88	962.48	964.17	961.31	2.455	
6	964.12	968.44	970.61	970.88	968.51	3.125	
7	984.37	984.85	984.23	983.49	984.24	0.561	
8	952.33	960.19	960.83	960.25	958.40	4.054	
9	939.80	967.62	961.41	964.12	958.24	12.550	C
10	972.14	969.88	970.39	971.04	970.86	0.973	
11	951.02	949.96	977.34	980.41	964.68	16.444	C
12	985.55	984.92	981.30	986.69	984.62	2.331	
13	948.73	943.75	951.27	955.32	949.77	4.845	
14	966.77	966.83	968.72	976.33	969.66	4.540	
15	968.78	970.29	971.67	970.82	970.39	1.212	

SD = Standard deviation

c-Cochran straggler, **C**-Cochran outlier, **g**-Grubb straggler, **G**-Grubb outlier

TABLE 4 - Assay Results Summary by Lab for Fosthiazate in Fosthiazate Technical, Tech-3 All values in [g/kg]

	DAY 1		DAY 2				
LAB	A	B	A	B	Mean	SD	Notes
1	970.38	983.57	975.23	978.00	976.79	5.51	
2	969.96	972.89	969.95	972.65	971.36	1.63	
3							
4	964.93	964.22	966.72	969.75	966.41	2.46	
5	961.68	961.42	962.52	965.21	962.71	1.73	
6	964.10	970.32	967.89	969.61	967.98	2.78	
7	985.12	984.47	983.80	983.64	984.26	0.67	
8	968.37	957.04	961.58	963.03	962.51	4.67	
9	967.46	969.26	960.11	967.17	966.00	4.03	
10	971.85	971.17	973.29	970.34	971.66	1.25	
11	976.74	978.60	990.91	973.99	980.06	7.48	C
12	988.62	987.80	988.31	986.10	987.71	1.12	
13	955.94	955.93	956.90	959.96	957.18	1.91	
14	969.57	966.45	968.09	964.67	967.20	2.11	
15	966.89	968.19	969.25	969.36	968.42	1.15	

SD = Standard deviation

c-Cochran straggler, **C**-Cochran outlier, **g**-Grubb straggler, **G**-Grubb outlier

TABLE 5 - Assay Results Summary by Lab for Fosthiazate in Fosthiazate Granules (10 GR), Fosthiazate 10 GR-1; All values in [g/kg]

	DAY 1		DAY 2				
LAB	A	B	A	B	Mean	SD	Notes
1	109.02	109.39	108.41	107.48	108.58	0.84	
2	104.53	103.72	105.04	105.09	104.59	0.64	
3	105.00	103.97	106.48	109.68	106.28	2.49	
4	101.57	101.97	103.66	104.82	103.00	1.51	
5	107.15	106.61	105.42	104.66	105.96	1.13	
6	105.71	104.62	100.80	100.49	102.90	2.65	
7	105.59	106.39	106.48	105.83	106.07	0.43	
8	105.01	106.60	110.32	111.95	108.47	3.21	
9	109.19	103.83	97.91	103.26	103.54	4.61	
10	109.21	106.40	102.43	102.66	105.17	3.25	
11	108.05	110.50	107.91	108.12	108.64	1.24	
12	109.89	100.10	107.53	103.65	105.29	4.31	
13	105.23	105.89	102.82	104.94	104.72	1.33	
14	105.84	102.23	94.57	103.97	101.65	4.94	
15	100.79	102.23	101.87	106.04	102.74	2.29	

SD = Standard deviation

c-Cochran straggler, **C**-Cochran outlier, **g**-Grubb straggler, **G**-Grubb outlier

TABLE 6 - Assay Results Summary by Lab for Fosthiazate in Fosthiazate Granules (10 GR), Fosthiazate 10 GR-2; All values in [g/kg]

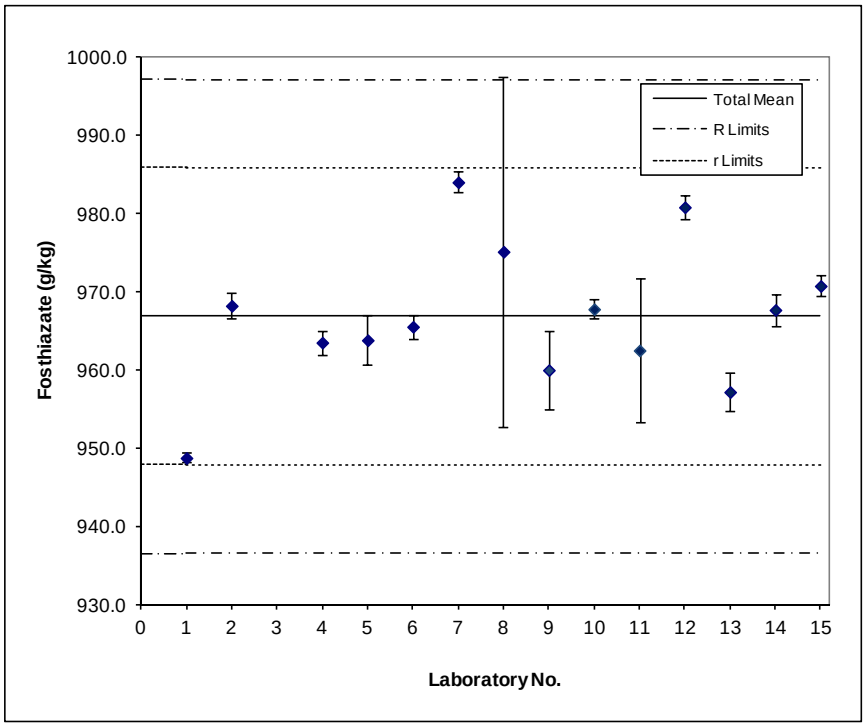
	DAY 1		DAY 2				
LAB	A	B	A	B	Mean	SD	Notes
1	105.97	104.91	104.57	105.33	105.19	0.60	
2	105.13	103.11	101.74	103.19	103.29	1.39	
3	102.03	101.12	107.26	107.58	104.50	3.40	
4	102.67	102.69	102.06	102.05	102.37	0.36	
5	101.85	103.50	102.00	101.98	102.33	0.78	
6	102.02	108.11	103.25	99.40	103.20	3.65	
7	105.09	104.28	105.71	105.01	105.02	0.59	
8	103.53	106.00	98.42	102.75	102.67	3.16	
9	98.70	99.65	101.39	99.03	99.69	1.20	
10	104.60	102.28	102.56	102.93	103.09	1.04	
11	102.09	102.55	104.08	104.41	103.28	1.13	
12	106.05	105.33	117.92	117.16	111.62	6.85	C, g
13	100.01	99.15	102.55	100.80	100.63	1.45	
14	99.35	99.20	91.21	95.59	96.34	3.84	
15	99.07	97.78	101.19	101.27	99.83	1.70	

SD = Standard deviation

c-Cochran straggler, **C**-Cochran outlier, **g**-Grubb straggler, **G**-Grubb outlier

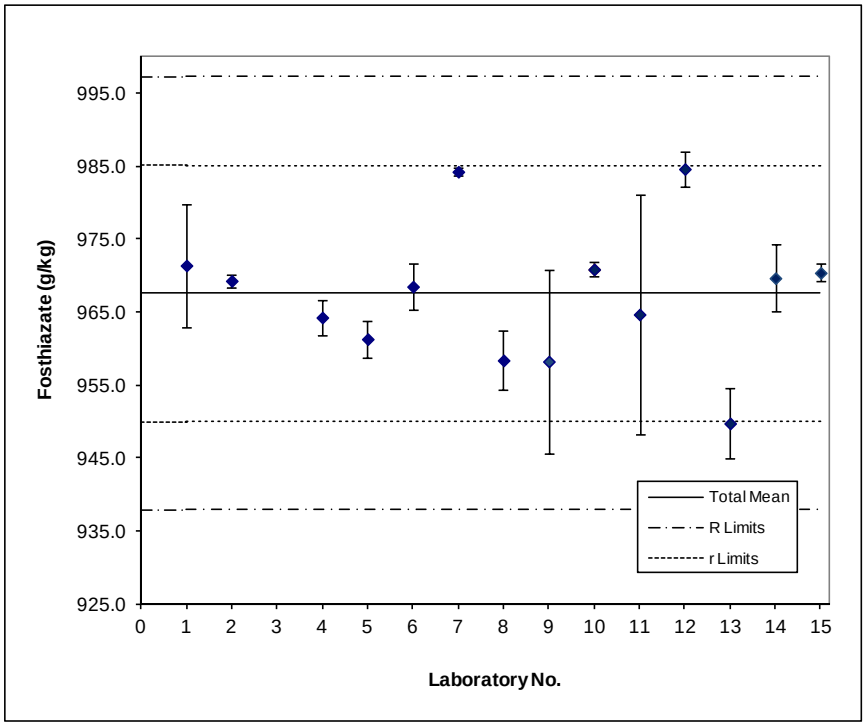
FIGURES (1-5)

FIGURE 1 - Fosthiazate in Fosthiazate Technical, Tech-1



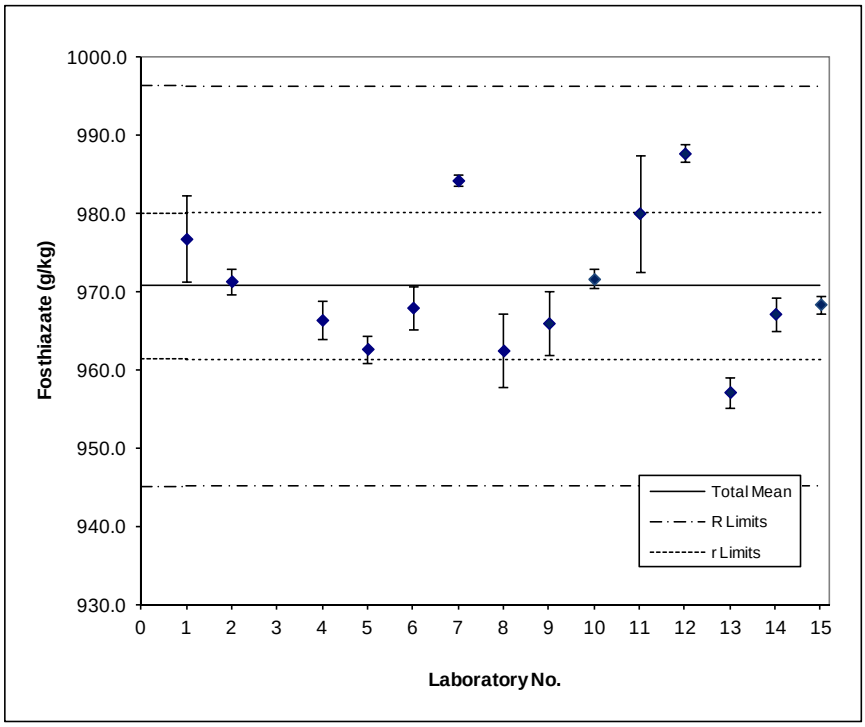
ni = 4 for all Labs

FIGURE 2 - Fosthiazate in Fosthiazate Technical, Tech-2



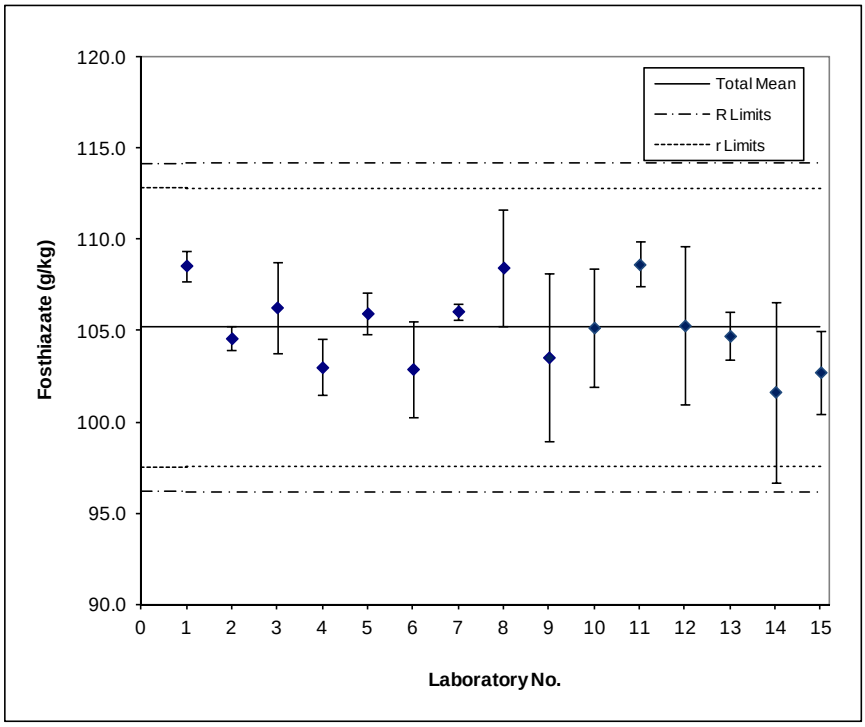
ni = 4 for all Labs

FIGURE 3 - Fosthiazate in Fosthiazate Technical, Tech-3



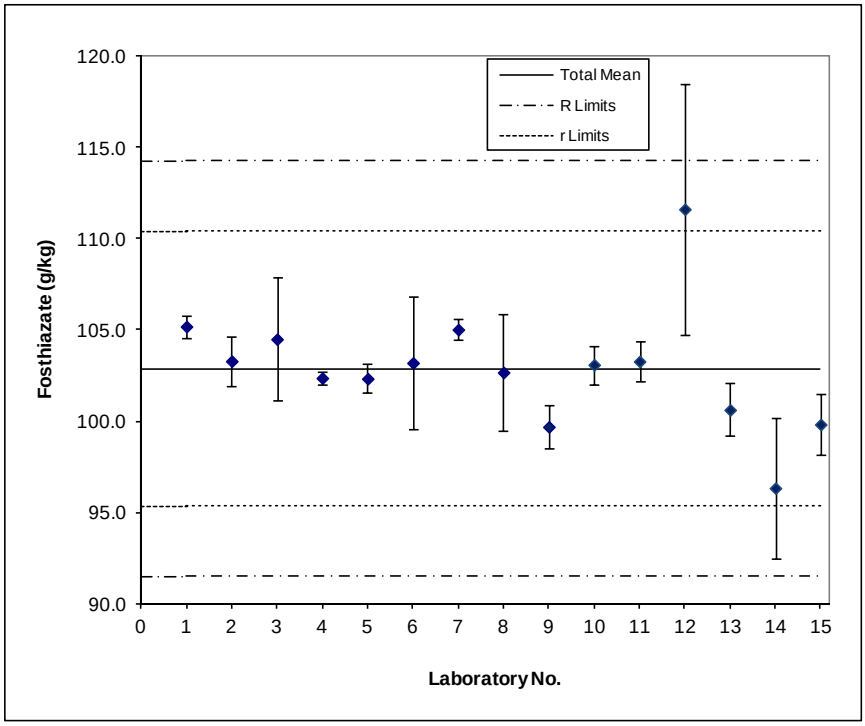
ni = 4 for all Labs

FIGURE 4 – Fosthiazate in Fosthiazate Granules (10 GR), Fosthiazate 10 GR-1



ni = 4 for all Labs

FIGURE 5 - Fosthiazate in Fosthiazate Granules (10 GR), Fosthiazate 10 GR-2



ni = 4 for all Labs