Tiocolis

Study Title:	Randomized, open-label, 2-way cross-over bioequivalence study comparing 8 mg single dose of thiocolchicoside (Thiocolchicoside Biofarm – tablets, 1 x 8 mg [Biofarm Sp. z o.o.] vs. Coltramyl – tablets, 2 x 4 mg [Sanofi]) under fasting conditions in healthy volunteers.
Name of the test product:	Thiocolchicoside Biofarm – tablets, 1 x 8 mg (Biofarm Sp. z o.o.). Each tablet contains 8 mg of thiocolchicoside.
Name of the reference product:	Coltramyl – tablets, 2 x 4 mg (Sanofi). Each tablet contains 4 mg of thiocolchicoside.
Study design:	Single centre, single dose, open-label (laboratory-blind), randomized, two-period, two-way cross-over bioequivalence study under fasting conditions in fifty (50) healthy adult Subjects with a wash-out period at least 14 days between study products administration in Period 1 and Period 2. When necessary, two-stage design (interim analysis) was to be applied for testing of thiocolchicoside bioequivalence of two products.
Sponsor:	Biofarm Sp. z o.o. Wałbrzyska St.13, 60-198 Poznań, Poland
Protocol number:	TC/BP/08/14
EUDRACT No.:	2015-002313-29
Study code in Central Register of Clinical Trials:	UR.DBL.BLE.475.0396.2015
Independent Ethics Committee Study code:	KB/1009/15
Clinical phase:	Bioequivalence study
GCP Statement:	The study was conducted in compliance with the approved Study Protocol No. TC/BP/08/14 version 1.0 of August 12th 2015 and its Amendment (No. 1) of November 16th 2015, World Medical Association Declaration of Helsinki and its amendments [1], current Good Clinical Practice guidelines (Note for Guidance on Good Clinical Practice [2], Directive 2001/20/EC [3] and Directive 2005/28/EC [4]), Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **).
Objectives and methodology	The primary objective of the study was to evaluate the pharmacokinetic properties and investigate the bioequivalence of thiocolchicoside from the test product (Thiocolchicoside Biofarm - tablets, 1 x 8 mg, Biofarm Sp. z o.o.) compared to the reference product (Coltramyl - tablets, 2 x 4 mg, Sanofi) containing the same amount (8 mg) of an active substance (thiocolchicoside), after single oral dose administration under fasting conditions. The secondary objective of the study was to evaluate the safety and tolerability of both study products. **Methodology:** The study was performed as a single center, single dose, open-label (laboratory-blind), randomized, two-period, two-way cross-over bioequivalence study under fasting conditions with a wash-out period of at least fourteen (14) days between study products administration in Period 1 and Period 2. Blood for determination of plasma level of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide (active metabolite of thiocolchicoside) was collected up to thirty (30) hours after study product administration, in eighteen (18) time points in each Period: pre-dose blood sample “0” was collected within 30 minutes before study product administration and then at the established time points 15 min, 30 min, 40 min, 50 min, 1 h, 1 h 15 min, 1 h 30 min, 1 h 45 min, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 30 hours after study product administration.

Main criteria for inclusion/exclusion:	All Subjects had to meet the following criteria: 1. The Subject is healthy male or female. Healthy Subjects are defined as individuals who are free from clinically significant illness or disease as determined by their medical history, physical examination, laboratory and ECG tests. Medical history check was also include verification of data concerning special precautions for study drug use, as contraindicated by the reference product characteristics as well as history of diabetes and renal impairment. 2. If males: male Subjects who are willing to use an acceptable method of contraception from the first dosing until 90 days after completion of the study (barrier methods with spermicide and sexual abstinence are allowed). 3. If females: female Subjects are eligible to enter and participate in the study if they are of: a. Non-childbearing potential (i.e. physiologically incapable of becoming pregnant) including females who: have had a hysterectomy or a bilateral oophorectomy, or a bilateral tubal ligation, or are post-menopausal (a demonstration of total cessation of menses for ≥ 12 months on the day of screening). b. Childbearing potential and have a negative result of urine pregnancy test at screening and on the evening prior to the first dose administration and agree to use one of the following contraception methods: complete abstinence from sexual intercourses or double barrier method (condom or occlusive cap used with spermicidal foam) from at least 6 days prior to administration of the study product until completion of the study or use of intrauterine non-hormonal device within at least 4 weeks prior to the first study product administration until completion of the study. No hormonal contraceptives or hormone replacement therapy are permitted in this study. 4. The Subject is ≥ 18 and ≤ 55 years of age on the day of screening. 5. BMI ≥ 18.5 to ≤ 30.0 kg/m^2 (on the day of screening). 6. Caucasian race. 7. Signed and dated written informed consent of the Subject to participate in the clinical study prior to screening evaluations. 8. The Subject is willing to refrain from the use of illicit drugs and alcohol and to adhere to other protocol-stated restrictions while participating in the study. 9. The Subject is able to understand and comply with the protocol requirements and instructions and is likely to complete the study as planned. 10. Non-smoker and non-tobacco user for at least 3 months before the day of screening. A Subject who met at least one of the following criteria couldn't enter into the study: 1. A significant abnormality in the past and/or at screening that influences present general health condition and requires pharmacological treatment during the study. 2. Any confirmed allergic reaction to any drug including allergy to study products or multiple allergies, as clinically significant in the judgement of the Investigator. 3. Any current or past disease or condition (e.g. of the alimentary tract and/or liver and/or kidneys) that may influence the absorption and/or distribution and/or metabolism and/or elimination of the drugs as assessed by the Investigator, or would constitute a risk factor when taking the study drug. 4. Clinically significant in the judgement of Investigator events of haemorrhage and syncope in medical history. 5. Episode of epilepsy or seizures anytime in medical history. 6. History of drug addiction and/or alcohol abuse (alcohol consumption of more than 500 mL of beer/day or 200 mL of wine/day or 50 mL of liquor/day) during last year preceding the day of screening. 7. Hypersensitivity to thiocolchicoside and/or its derivatives and/or to any excipient of the study products. 8. Blood loss or donation exceeding 200 mL in the last 30 days before the day of screening. 9. Positive results of HBsAg and/or anti-HCV and/or anti-HIV-1+2 tests during screening procedures. 10. Blood pressure: systolic > 140 mmHg or < 90 mmHg, diastolic < 60 mmHg or > 90 mmHg during screening procedures and on the evening prior to the first dose administration. 11. Pregnant or breast-feeding females. 12. Positive urine pregnancy test on the day of screening or on the evening before the first study product administration. 13. Heart rate < 50 or > 100 bpm at screening and on the evening prior to the first dose administration. 14. Body temperature < 36.0°C or > 37.4°C at screening and on the evening prior to the first dose administration. 15. Clinically significant abnormal laboratory values at screening. 16. Clinically significant abnormalities in 12-lead ECG recording performed at screening.

	17. Use of any over-the-counter medication (except for paracetamol at dose ≤ 1 g daily) including vitamins, lozenges, herbal and dietary supplements within 7 days before Day 0 in Period 1. 18. Use of any prescription drug within 14 days before Day 0 in Period 1. 19. Use of steroids or anabolic or hormonal therapy within 30 days before Day 0 in Period 1. 20. Special diet (e.g. low calories, vegetarian, etc.) during 4 weeks before the day of screening. 21. Any significant change in lifestyle: dietary or exercise habits during 4 weeks before the day of screening. 22. Consumption of products (e.g. food or drink) containing caffeine or other methylxanthines (i.e. coffee, tea, coke, chocolate, cocoa, energy drinks) within 48 hours prior to the first product administration. 23. Drinking grapefruit juice and/or other citrus juices and/or eating citrus products for 7 days prior to the first drug administration. 24. Alcohol consumption within 72 hours prior to the first product administration. 25. Positive urine test results for drugs of abuse on the day of screening (opiates, cannabinoids) or on the evening before the first study product administration (opiates, barbiturates, benzodiazepines, amphetamines, cocaine, cannabinoids). 26. Positive urine test result for cotinine on the day of screening or on the evening before the first study product administration (in 10%-20% of randomly selected volunteers). 27. Positive result of breath alcohol test on the day of screening or on the evening before the first study product administration. 28. Participation in other clinical trials where at least one dose of study product was administered within 30 days prior to the first dose administration. 29. For any reason the Subject is considered by the Investigator to be an unsuitable candidate to participate in the study.
Criteria for evaluation:	**Pharmacokinetics:** Primary parameters: C_{max} and $AUC_{(0-t)}$ for 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide. These primary pharmacokinetic (PK) parameters of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide were used for bioequivalence assessment. Secondary parameters: $AUC_{(0-\infty)}$, T_{max}, K_{el}, $AUC_{\%Extrap\_Obs}$, and $T_{1/2}$ for 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide. Secondary PK parameters were evaluated to characterize the pharmacokinetic profile of the test and reference products, they were not used for bioequivalence assessment. **Safety and tolerability evaluation:** All Subjects exposed to the study at least once were part of the safety analysis. Clinical safety of the test and the reference product after a single dose administration in healthy Subjects was assessed taking into consideration medical history, physical examination, vital signs (heart rate (HR), blood pressure (BP), body temperature), 12-lead electrocardiogram (ECG), clinical laboratory tests (haematology, biochemistry, urinalysis, serology, and pregnancy urine tests – females only) and Adverse Events monitoring. Information on AEs was obtained by medical observations, laboratory test analysis and spontaneous Subjects' reporting after product administration and recorded by Investigators using CTCAE v. 4.03 **Statistical analysis:** Descriptive statistics was performed for all Subjects pharmacokinetic parameters including the arithmetic mean, geometric mean, standard deviation, coefficient of variation, median, range, minimum (min.) and maximum (max.). Both test and reference products were assessed to be bioequivalent on the ground of analysis of variance (ANOVA) with factors for sequence, Subject within sequence, period and treatment. The decisive criterion was constituted by $AUC_{(0-t)}$ and C_{max} parameters for 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide, whereas $AUC_{(0-\infty)}$ and the other variables were considered as the secondary pharmacokinetic parameters and constituted supportive data. The conclusion on bioequivalence of the test product with the reference product was based on the results for ln-transformed C_{max} and $AUC_{(0-t)}$ of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide (primary pharmacokinetic study endpoints). If the 90% confidence intervals for the proportion of μ_T/μ_R (geometric means for the test and reference formulation) of primary pharmacokinetic parameters C_{max} and $AUC_{(0-t)}$ of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide were included entirely in the acceptance range of 80.00-125.00%, the test formulation (Thiocolchicoside Biofarm – tablets, 1 x 8 mg) was considered as bioequivalent to the reference formulation (Coltramyl - tablets, 2 x 4 mg). All calculations were performed using SAS (version 9.4) software.

Number of Subjects (planned and analysed):

Number of Subjects:	Planned		Screened	Randomized and dosed	Completed	Statistically evaluated
	to be randomized and dosed	to complete the study				
	50	44	58	50	49	49

Study initiation date:

On November 16th 2015 and November 17th 2015 the study initiation visits took place and on November 17th 2015 the first Subject was screened.

Study completion date:

On December 21st 2015 the last Subject completed the study

Pharmacokinetic results:

The mean values of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide pharmacokinetic parameters for 49 Subjects who completed the whole study and were included in the summary of descriptive statistics were as follows:

REFERENCE PRODUCT – Coltramyl - tablets, 2 x 4 mg

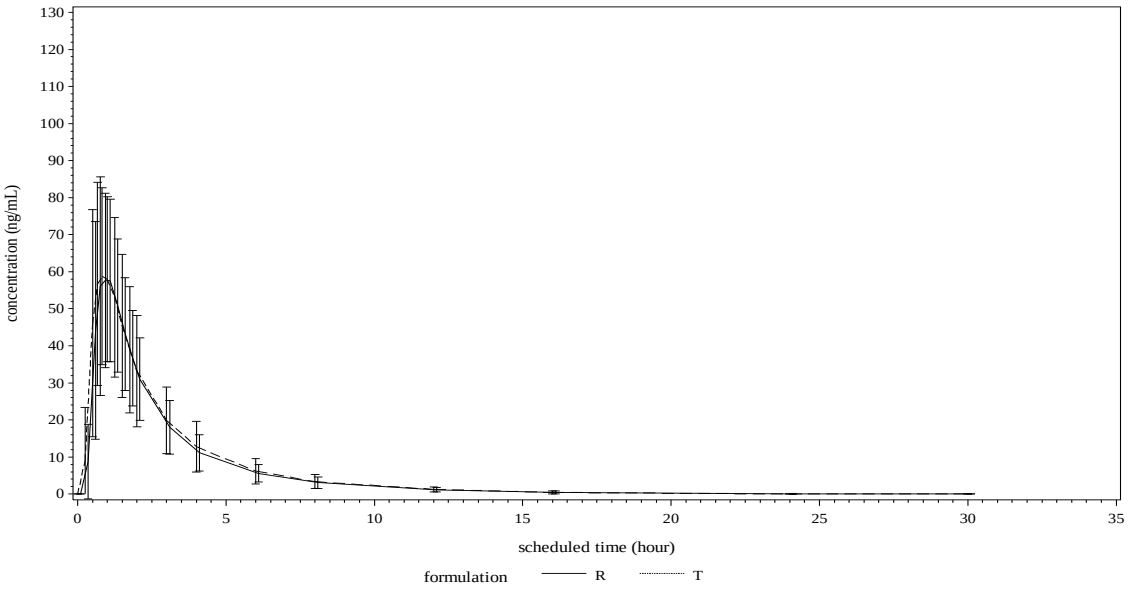
Parameter	$AUC_{0-t(last)}$ (ng*h/mL)	C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (h*ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	MRT_{0-inf} (h)	$AUC_{\%Extrap\_Obs}$ (%)
N	49	49	49	49	49	49	49
Arithmetic mean	157.783	68.311	161.770	0.888	3.419	3.265	2.565
Geometric mean	149.073	64.406	153.014	0.843	3.273	3.230	2.314
SD	52.571	23.497	53.382	0.307	1.260	0.491	1.449

TEST PRODUCT – Thiocolchicoside Biofarm – tablets, 1 x 8 mg

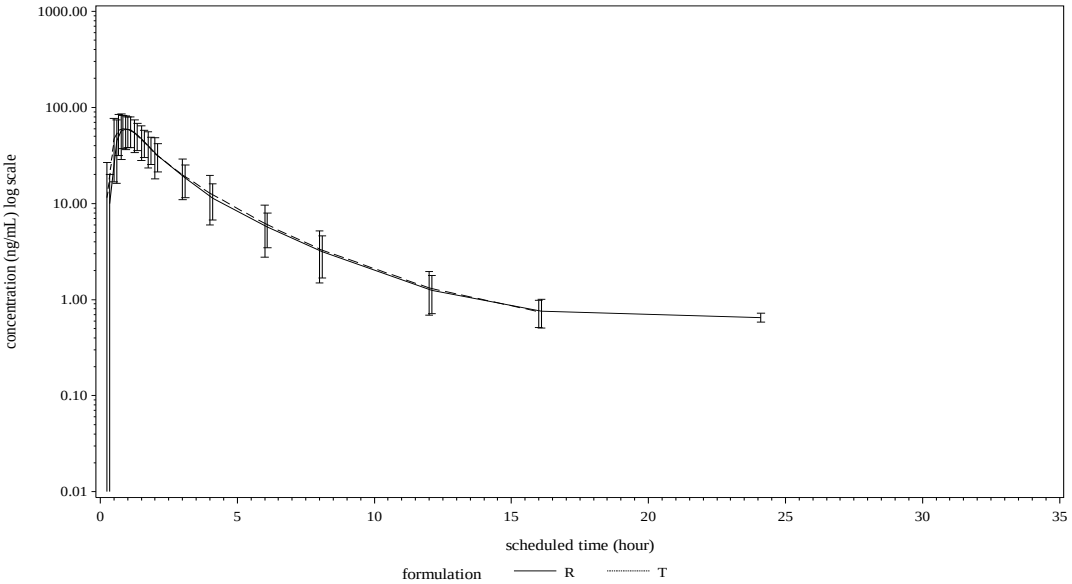
Parameter	$AUC_{0-t(last)}$ (ng*h/mL)	C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (h*ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	MRT_{0-inf} (h)	$AUC_{\%Extrap\_Obs}$ (%)
N	49	49	49	49	49	49	49
Arithmetic mean	163.384	69.682	167.149	0.913	3.159	3.248	2.368
Geometric mean	153.658	66.147	157.392	0.814	3.092	3.205	2.217
SD	59.362	21.267	60.213	0.571	0.688	0.566	0.911

Mean 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide plasma concentration vs. time curve (N = 49) following a single 8 mg oral dose of Thiocolchicoside Biofarm - tablets (Biofarm Sp. z o.o.) [test product, T] and single oral dose of Coltramyl - tablets, 2 x 4 mg (Sanofi) [reference product, R] in linear- linear and log- linear scales are presented below:

A. linear / linear scale



B. log / linear scale



Bioequivalence comparison:

Statistical analysis summary

Pharmacokinetic parameters of 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide after a single 8 mg dose of thiocolchicoside

Parameter	T (geom. mean)	R (geom. mean)	T/R [%]	90% C.I. [%]	WSV [%]
$AUC_{0\text{-}t(last)}$ [ng*h/mL]	153.658	149.073	1.0304	0.9827-1.0805	14.0605
C_{max} [ng/mL]	66.147	64.406	1.0264	0.9651-1.0917	18.3391
$AUC_{0\text{-}\infty}$ [ng*h/mL]	157.392	153.014	1.0283	0.9818-1.0769	13.6923

T – test product (Thiocolchicoside Biofarm – tablets, 1 x 8 mg)
R – reference product (Coltramyl - tablets, 2 x 4 mg)

Pharmacokinetic and statistical conclusion:

Based on the statistical results for the active metabolite of parent drug (thiocolchicoside) 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide it can be concluded that the test product Thiocolchicoside Biofarm – tablets, 1 x 8 mg (Biofarm Sp. z o.o.) **is bioequivalent** with the reference product Coltramyl - tablets, 2 x 4 mg (Sanofi). The bioequivalence criteria with respect to plasma concentrations of thiocolchicoside active metabolite 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide, as per criteria set in the Study Protocol, have been met: the 90% confidence interval for 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide $AUC_{(0\text{-}t)}$ and C_{max} T/R ratios are lying within the standard bioequivalence acceptance range of 80.00–125.00%. Such analysis was equivalent to two one-sided parametric t-tests according to Schuirmann.
The comparative statistics performed for the secondary pharmacokinetic parameters: $AUC_{(0\text{-}\infty)}$, T_{max}, $AUC\_\%_{Extrap\_Obs}$, $MRT_{0\text{-}inf}$, and $T_{1/2}$ for 3-Demethyl Thiocolchicine 3-O-β-D-Glucuronide did not show significant differences between the test and the reference products **confirming the bioequivalence** of the two formulations.

Safety results:	During the study 11 non-serious Adverse Events (AEs) were reported in nine Subjects: seven occurred after administration of test product and four after administration of the reference product. Out of the 50 Subjects who received products in Period 1, seven experienced nine AEs (five classified as not related to the study product and four as possibly related to the study product). After product administration in Period 2, two Subjects of 49 who continued the study experienced a total of two AEs (two classified as not related to the study product). Intensity of AEs were assessed as mild in eight cases and moderate in three cases. All 11 AEs were resolved. There were no deaths and serious adverse events (SAEs) during the conduct of the study.
Safety conclusion:	The clinical part of the study was completed without deaths, SAEs and suspected unexpected serious adverse reactions (SUSARs). During the study a total number of 11 non-serious AEs were reported in nine Subjects. Four AEs were considered as possibly related to the study product and seven as not related. The intensity of these AEs was classified as mild in eight cases and as moderate in three cases. The test product caused seven Adverse Events and the reference product caused four AEs. There were three AEs possibly related to the test product and one to the reference product. Based on the clinical results of the study, it was clearly demonstrated that the test formulation of thiocolchicoside was tolerated in the same way as the reference drug. The safety profiles of both formulations are similar and do not differ from information described in the Investigator's Brochure.
Date of the Clinical Study Report:	February 29th 2016