Part 7. List of Supporting Data and Information in GRAS Notice

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Appendices

Appendix A. Certificates of Analysis

Appendix A. Batch Data and Certificate of Analysis

In this appendix the analytical data for three independent batches of Arcofolin®, certificates of analysis, and Swissmedic certificate of GMP compliance are provided.

Summary of Analytical Batch Release Data for Arcofolin®

Batch	**ESY0008-XX**	**ESY0009-XX**	**ESY0010-XX**	**Specification**
Inspection Lot	**890000066482**	**890000066484**	**890000066483**	
Appearance				
Appearance color	Beige	beige	beige	white to yellow or beige
Appearance texture	Powder	powder	powder	Powder
Identity (IR-Spectrum)				
Identity / IR	Ok	ok	ok	ok**
Water content (KF, Coulometric)				
Water content, %w/w	0.2	0.2	0.2	<= 1.0 %*
IC (Cations)				
Identity retention time sodium	conforms to reference	conforms to reference	conforms to reference	conforms to reference*
Assay Sodium (IC), %w/w	4.5	4.7	4.5	4.0 to 5.0 %*
Residual Solvent (GC)				
Assay Ethanol, %w/w	0.06	0.07	0.06	<= 0.5 %
Assay Isopropanol, %w/w	0.00	0.00	0.00	<= 0.5 %
Specified Elemental Impurities				
Boron (ICP-OES), ppm	< LOQ (5)	< LOQ (5)	< LOQ (5)	<= 10 ppm
Platinum (ICP-MS), ppm	< LOQ (5)	< LOQ (5)	< LOQ (5)	<= 10 ppm
Arsenic (ICP-MS), ppm	< LOQ (1.5)	< LOQ (1.5)	< LOQ (1.5)	<= 1.5 ppm
Cadmium (ICP-MS), ppm	< LOQ (0.5)	< LOQ (0.5)	< LOQ (0.5)	<= 0.5 ppm
Lead (ICP-MS), ppm	< LOQ (1.0)	< LOQ (1.0)	< LOQ (1.0)	<= 1.0 ppm
Mercury (ICP-MS), ppm	< LOQ (1.5)	< LOQ (1.5)	< LOQ (1.5)	<= 1.5 ppm
Assay & Related Compounds (HPLC)				
Identity retention time HPLC	conforms to reference	conforms to reference	conforms to reference	conforms to reference
Assay Mefolinate, acid as is, %	95.2	95.4	94.3	>= 91.0 %*
4-Aminobenzoylglutamic acid (ABGA), %	0.06	0.09	0.06	<= 0.5 %
Hydroxymethyl-THFA (HOMeTHFA), %	0.11	0.29	0.11	<= 1.0 %
Mefox, %	0.02	0.02	0.02	<= 1.0 %
Tetrahydrofolic acid (THFA), %	0.07	0.07	0.10	<= 0.5 %
7,8-Dihydrofolic acid (DHFA), %	0.01	0.01	0.01	<= 0.5 %
Folic acid (FA) , %	< LOQ (0.01)	< LOQ (0.01)	< LOQ (0.01)	<= 0.5 %
Methylenetetrahydrofolic acid (CH2THFA), %	0.02	0.02	0.02	<= 0.5 %
Methyltetrahydropteroic acid (MeTHPA), %	0.11	0.12	0.13	<= 0.5 %
Dimethyl-THFA (DiMeTHFA), %	0.05	0.05	0.06	<= 0.15 %

Batch	**ESY0008-XX**	**ESY0009-XX**	**ESY0010-XX**	**Specification**
Inspection Lot	**890000066482**	**890000066484**	**890000066483**	
Sum of all related compounds, %	0.62	0.85	0.65	<= 2.5 %
Diasteriomeric Purity (HPLC) (6R)-Mefolinate	0.3	0.3	0.3	<= 1.0 % area
Microbial Enumeration Tests				
Microbial Count (TAMC), CFU/g	< LOQ (10)	< LOQ (10)	< LOQ (10)	<= 100 CFU/g
Microbial Count (TYMC),CFU/g	< LOQ (10)	< LOQ (10)	< LOQ (10)	<= 100 CFU/g
Specified Microorganisms (SMO)				
Escherichia coli [in.house test]	absent in 1 g	absent in 1 g	absent in 1 g	absent in 1 g

*Arcofolin® specific acceptance criteria

**at time of CoA issuing “report result”. IR Reference is now being established and specification updated to “conform to reference”

Certificate of Analysis

Material No.	5.54176.2900
Material Description	Arcofolin®, (6S)-5-Methyltetrahydrofolic acid monosodium salt
Molecular Formula	$C_{20}H_{24}N_7NaO_6$
Molecular Weight	481.44 g/mol
Batch No.	ESY0008-XX
Inspection Lot No.	890000066482
Retest Date	31 March 2019 (Retest period of CoA expired)
Storage Conditions	+2°C to +8°C
Manufacturing Date	13 March 2018
Batch Size	21.11 Kilogram

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Print Date: 05/11/2020

SAFC®

Certificate of Analysis

Inspection Lot No. 890000066482

Test	Specification	Result
Appearance		
- Appearance color	white to yellow or beige	beige
- Appearance texture	powder	powder
Identification		
- IR-spectrum (ATR)	ok	ok
Water (KF, Coulometric)		
- Water content	NMT 1.0%	0.2 %
Residual solvents (GC)		
- Ethanol	NMT 0.5%	0.06 %
- 2-Propanol	NMT 0.5%	<LOQ (0.03%)
IC (Cations)		
- Identity retention time Sodium	conforms to reference	conforms to reference
- Assay Sodium	4.0 to 5.0 %	4.5%
Elemental Impurities		
- Assay Boron (ICP-OES)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Platinum (ICP-MS)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Arsenic (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
- Assay Cadmium (ICP-MS)	NMT 0.5 ppm	<LOQ (0.5 ppm)
- Assay Lead (ICP-MS)	NMT 1.0 ppm	<LOQ (1.0 ppm)
- Assay Mercury (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
Assay & Related Compounds (HPLC)		
- Identity retention time HPLC	conforms to reference	conforms to reference
- Assay Mefolinate (acid as is)	NLT 91.0%	95.2%
- 4-Aminobenzoylglutamic acid (ABGA)	NMT 0.5%	0.06%
- Hydroxymethyl-THFA (HOMeTHFA)	NMT 1.0%	0.11%
- Mefox	NMT 1.0%	0.02%
- Tetrahydrofolic acid (THFA)	NMT 0.5%	0.07%
- 7,8-Dihydrofolic acid (DHFA)	NMT 0.5%	0.01%
- Folic acid (FA)	NMT 0.5%	<LOQ (0.01%)
- Methylenetetrahydrofolic acid (CH2THFA)	NMT 0.5%	0.02%
- Methyltetrahydropteroic acid (MeTHPA)	NMT 0.5%	0.11%
- Dimethyl-THFA (DiMeTHFA)	NMT 0.15%	0.05%
- Sum of related compounds	NMT 2.5%	0.62%

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Certificate of Analysis

Inspection Lot No. 890000066482

Test	Specification	Result
Diastereomeric Purity (HPLC)		
- (6R)-Mefolinate	NMT 1.0% area	0.3% area
Microbial enumeration Test		
- Total Aerobic Microbial Count (TAMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
- Total Combined Yeasts and Molds Count (TYMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
Absence of Specified Microorganisms		
Escherichia coli	absent in 1 g	absent in 1 g

The abbreviation LOQ represents the Limit of Quantitation or, if applicable, the Reporting Threshold. The Limit of Detection is abbreviated as LOD.

Merck & Cie

Schaffhausen, 05 November 2020

Dr. Timo Huxel
Quality Services

Jean-Pierre Knapp
Quality Services

Certificate of Analysis

Inspection Lot No. 890000066482

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Certificate of Analysis

Material No.	5.54176.2900
Material Description	Arcofolin®, (6S)-5-Methyltetrahydrofolic acid monosodium salt
Molecular Formula	$C_{20}H_{24}N_7NaO_6$
Molecular Weight	481.44 g/mol
Batch No.	ESY0009-XX
Inspection Lot No.	890000066484
Retest Date	31 March 2019 (Retest period of CoA expired)
Storage Conditions	+2°C to +8°C
Manufacturing Date	13 March 2018
Batch Size	25.35 Kilogram

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Certificate of Analysis

Inspection Lot No. 890000066484

Test	Specification	Result
Appearance		
- Appearance color	white to yellow or beige	beige
- Appearance texture	powder	powder
Identification		
- IR-spectrum (ATR)	ok	ok
Water (KF, Coulometric)		
- Water content	NMT 1.0%	0.2 %
Residual solvents (GC)		
- Ethanol	NMT 0.5%	0.07 %
- 2-Propanol	NMT 0.5%	<LOQ (0.03%)
IC (Cations)		
- Identity retention time Sodium	conforms to reference	conforms to reference
- Assay Sodium	4.0 to 5.0 %	4.5%
Elemental Impurities		
- Assay Boron (ICP-OES)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Platinum (ICP-MS)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Arsenic (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
- Assay Cadmium (ICP-MS)	NMT 0.5 ppm	<LOQ (0.5 ppm)
- Assay Lead (ICP-MS)	NMT 1.0 ppm	<LOQ (1.0 ppm)
- Assay Mercury (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
Assay & Related Compounds (HPLC)		
- Identity retention time HPLC	conforms to reference	conforms to reference
- Assay Mefolinate (acid as is)	NLT 91.0%	95.4%
- 4-Aminobenzoylglutamic acid (ABGA)	NMT 0.5%	0.09%
- Hydroxymethyl-THFA (HOMeTHFA)	NMT 1.0%	0.29%
- Mefox	NMT 1.0%	0.02%
- Tetrahydrofolic acid (THFA)	NMT 0.5%	0.07%
- 7,8-Dihydrofolic acid (DHFA)	NMT 0.5%	0.01%
- Folic acid (FA)	NMT 0.5%	<LOQ (0.01%)
- Methylenetetrahydrofolic acid (CH2THFA)	NMT 0.5%	0.02%
- Methyltetrahydropteroic acid (MeTHPA)	NMT 0.5%	0.12%
- Dimethyl-THFA (DiMeTHFA)	NMT 0.15%	0.05%
- Sum of related compounds	NMT 2.5%	0.85%

Certificate of Analysis

Inspection Lot No. 890000066484

Test	Specification	Result
Diastereomeric Purity (HPLC)		
- (6R)-Mefolinate	NMT 1.0% area	0.3% area
Microbial enumeration Test		
- Total Aerobic Microbial Count (TAMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
- Total Combined Yeasts and Molds Count (TYMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
Absence of Specified Microorganisms		
Escherichia coli	absent in 1 g	absent in 1 g

The abbreviation LOQ represents the Limit of Quantitation or, if applicable, the Reporting Threshold. The Limit of Detection is abbreviated as LOD.

Merck & Cie

Schaffhausen, 05 November 2020

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Quality Services

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Certificate of Analysis

Inspection Lot No. 890000066484

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Certificate of Analysis

Material No.	5.54176.2900
Material Description	Arcofolin®, (6S)-5-Methyltetrahydrofolic acid monosodium salt
Molecular Formula	$C_{20}H_{24}N_7NaO_6$
Molecular Weight	481.44 g/mol
Batch No.	ESY0010-XX
Inspection Lot No.	890000066483
Retest Date	31 March 2019 (Retest period of CoA expired)
Storage Conditions	+2°C to +8°C
Manufacturing Date	13 March 2018
Batch Size	23.37 Kilogram

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Print Date: 05/11/2020

Certificate of Analysis

Inspection Lot No. 890000066483

Test	Specification	Result
Appearance		
- Appearance color	white to yellow or beige	beige
- Appearance texture	powder	powder
Identification		
- IR-spectrum (ATR)	ok	ok
Water (KF, Coulometric)		
- Water content	NMT 1.0%	0.2 %
Residual solvents (GC)		
- Ethanol	NMT 0.5%	0.06 %
- 2-Propanol	NMT 0.5%	<LOQ (0.03%)
IC (Cations)		
- Identity retention time Sodium	conforms to reference	conforms to reference
- Assay Sodium	4.0 to 5.0 %	4.7%
Elemental Impurities		
- Assay Boron (ICP-OES)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Platinum (ICP-MS)	NMT 10 ppm	<LOQ (5 ppm)
- Assay Arsenic (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
- Assay Cadmium (ICP-MS)	NMT 0.5 ppm	<LOQ (0.5 ppm)
- Assay Lead (ICP-MS)	NMT 1.0 ppm	<LOQ (1.0 ppm)
- Assay Mercury (ICP-MS)	NMT 1.5 ppm	<LOQ (1.5 ppm)
Assay & Related Compounds (HPLC)		
- Identity retention time HPLC	conforms to reference	conforms to reference
- Assay Mefolinate (acid as is)	NLT 91.0%	94.3%
- 4-Aminobenzoylglutamic acid (ABGA)	NMT 0.5%	0.06%
- Hydroxymethyl-THFA (HOMeTHFA)	NMT 1.0%	0.11%
- Mefox	NMT 1.0%	0.02%
- Tetrahydrofolic acid (THFA)	NMT 0.5%	0.10%
- 7,8-Dihydrofolic acid (DHFA)	NMT 0.5%	0.01%
- Folic acid (FA)	NMT 0.5%	<LOQ (0.01%)
- Methylenetetrahydrofolic acid (CH2THFA)	NMT 0.5%	0.02%
- Methyltetrahydropteroic acid (MeTHPA)	NMT 0.5%	0.13%
- Dimethyl-THFA (DiMeTHFA)	NMT 0.15%	0.06%
- Sum of related compounds	NMT 2.5%	0.65%

Certificate of Analysis

Inspection Lot No. 890000066483

Test	Specification	Result
Diastereomeric Purity (HPLC)		
- (6R)-Mefolinate	NMT 1.0% area	0.3% area
Microbial enumeration Test		
- Total Aerobic Microbial Count (TAMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
- Total Combined Yeasts and Molds Count (TYMC)	NMT 100 CFU/g	<LOQ (10 CFU/g)
Absence of Specified Microorganisms		
Escherichia coli	absent in 1 g	absent in 1 g

The abbreviation LOQ represents the Limit of Quantitation or, if applicable, the Reporting Threshold. The Limit of Detection is abbreviated as LOD.

Merck & Cie

Schaffhausen, 05 November 2020

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Quality Services

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Certificate of Analysis

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CERTIFICATE OF GMP COMPLIANCE

We certify herewith

that the company **Merck & Cie, Weisshausmatte, 6460 Altdorf UR,** Authorisation No. 511206-102638264 with its site **Merck & Cie, Im Laternenacker 5, 8200 Schaffhausen, Switzerland,** Site No. 1003730 has been duly authorised to perform the manufacturing activities according to the table below;

that the company is keeping the required level for Good Manufacturing Practices for Medicinal Products (GMP) according to the Swiss regulations in force. These regulations are in accordance with the requirements for good practices in the manufacture and quality control of the Pharmaceutical Inspection Convention/Co-operation Scheme (PIC/S) as well as with The Good Manufacturing Practice requirements referred to in the Agreement of Mutual Recognition between the European Union/Canada and Switzerland;

that the manufacturing plant of the company is subject to official periodic inspections; the last regular inspection was conducted on **26.04.2019** (dd.mm.yyyy).

No.	*Operation*	*Scope**
3	**MANUFACTURE OF ACTIVE SUBSTANCES**	
3.1	**Manufacture of active substance by chemical synthesis**	
3.1.1	Manufacture of active substance intermediates	-
3.1.2	Manufacture of crude active substance	-
3.1.3	Salt formation / Purification steps: Complexation, crystallization, re-crystallization	-
3.5	**General finishing steps**	
3.5.1	Physical processing steps: drying, milling, filling	-
3.5.2	Primary packaging	-
3.5.3	Secondary packaging	-
3.6	**Quality control testing of medicinal products**	
3.6.1	Physical / Chemical testing	-
3.6.2	Microbiological: testing (excluding sterility testing)	-
3.7	**Other activities: Manufacturing of non-sterile APIs for clincal trials.**	
3.8	List of active substances: Zinc Histidine Dihydrate Vernakalant HCL Calcium Folinate Hydrate Calcium Levofolinate Hydrate Levomefolate Calcium Morphine HCL Tepotinib unmicronized	-

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* Scope of authorisation:

H/V	Human and veterinary medicinal products, without investigational products
V	Veterinary medicinal products only, without investigational products
I	Human investigational medicinal products
–	Not specified

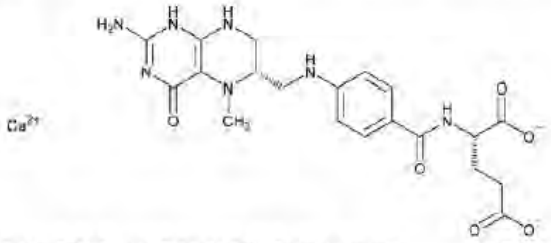
Berne, **18.09.2020** (dd.mm.yyyy)
No. GMP-CH-1001361

Swissmedic, Swiss Agency for Therapeutic Products

Marianne Baumann

Appendix B. Metafolin® USP-NF Monograph

Calcium L-5-Methyltetrahydrofolate



$C_{20}H_{23}CaN_7O_6 \cdot xH_2O$ $C_{20}H_{23}CaN_7O_6$(anhydrous) 497.52

N-[4-[[(2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-(6*S*)-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, calcium salt (1:1);
N-(4-[[((6*S*)-2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]-benzoyl)-L-glutamic acid, calcium salt (1:1)
[151533-22-1].

DEFINITION

Calcium L-5-Methyltetrahydrofolate contains NLT 95.0% and NMT 102.0% of calcium 5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$), the sum of the L- and D-diastereoisomers, calculated on the anhydrous and solvent-free basis, of which NMT 1.0% corresponds to calcium D-5-methyltetrahydrofolate.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests (197), *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

[Note—If the spectra obtained show differences, dissolve the substance to be examined and the USP Calcium DL-5-Methyltetrahydrofolate RS separately in the minimum quantity of water, and add dropwise sufficient acetone to produce a precipitate. Allow to stand for 15 min, centrifuge to collect the precipitate, wash the precipitate twice with a minimum quantity of acetone, and dry. Record new spectra using the residues.]

• **B. Identification Tests—General, *Calcium* (191):** A 5-mg/mL solution meets the requirements.

• **C. HPLC:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. It complies with the acceptance criteria of the test for *Enantiomeric Purity*.

ASSAY

• **Procedure**

Buffer: 7.8 g/L of sodium dihydrogen phosphate dihydrate in water

Solution A: Adjust the *Buffer* with 32% (w/v) sodium hydroxide solution to a pH of 6.5.

Solution B: Methanol and *Buffer* (35:65). Adjust with 32% (w/v) sodium hydroxide solution to a pH of 8.0.

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
	100	0
	45	55
17	0	100
24	0	100
24.01	100	0
33	100	0

[Note—After analysis the column should be flushed and stored in a mixture of methanol and water (85:15).]

System suitability solution: Transfer 25 mg of USP Folic Acid RS and 25 mg of USP 4-Aminobenzoylglutamic Acid RS to a 100-mL volumetric flask. Add about 15 mg each of sodium hydrogen carbonate and sodium carbonate to the flask, add sufficient water, sonicate to dissolve, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask containing 50 mg of USP Calcium DL-5-Methyltetrahydrofolate RS, dissolve, and dilute with water to volume.

[NOTE—The following Standard and Sample solutions must be injected immediately after preparation and injected only once.]

Standard solution: 0.5 mg/mL of USP Calcium DL-5-Methyltetrahydrofolate RS in water

Sample solution: 0.5 mg/mL of Calcium L-5-Methyltetrahydrofolate in water

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 32°

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the *System suitability solution* the relative retention times of the component peaks are listed in *Table 2*. The L- and D-isomers of 5-methyltetrahydrofolate co-elute as a single peak. The 4a-hydroxy-5-methyltetrahydrofolic acid, 5-methyltetrahydropteroic acid, and dimethyltetrahydrofolic acid are included as minor components in USP Calcium DL-5-Methyltetrahydrofolate RS.]

Suitability requirements

Resolution: *System suitability solution*

NLT 6 between 4-aminobenzoylglutamic acid and 4a-hydroxy-5-methyltetrahydrofolic acid

NLT 8 between folic acid and 5-methyltetrahydrofolic acid

NLT 15 between 5-methyltetrahydrofolic acid and dimethyltetrahydrofolic acid

Relative standard deviation: Prepare three separate *Standard solutions*, and inject each immediately and only one time. NMT 2.0%; peak response factor from three injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcium 5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$), the sum of the L- and D-diastereoisomers, in the portion of Calcium L-5-Methyltetrahydrofolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Calcium DL-5-Methyltetrahydrofolate RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcium L-5-Methyltetra hydrofolate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **CHLORIDE**

Sample: 300 mg

Blank: Mix 1 mL of nitric acid with 75 mL of water.

Titrimetric system

(See *Titrimetry ⟨541⟩*.)

Mode: Direct titration

Titrant: 0.025 M silver nitrate VS

End-point detection: Potentiometric

Analysis: Dissolve the *Sample* in 75 mL of water (heat to maximum of 40°), add 1 mL of nitric acid, and titrate with the *Titrant*. Perform a *Blank* determination, and make any necessary correction.

Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = [(V_S - V_B) \times M \times F/W] \times 100$$

V_S = volume of *Titrant* consumed by the *Sample* (mL)

V_B = volume of *Titrant* consumed by the *Blank* (mL)

M = actual molarity of the *Titrant* (mmol/mL)

F = equivalency factor, 35.45 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: NMT 0.5%

- **Elemental Impurities—Procedures (233)**

Acceptance criteria

Boron: NMT 50 µg/g

Platinum: NMT 10 µg/g

Arsenic: NMT 1.5 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 1.0 µg/g

Mercury: NMT 1.5 µg/g

- **Residual Solvents (467)**

Acceptance criteria

Ethanol: NMT 0.5%

2-Propanol: NMT 0.5%

[Note—For acceptance criteria for any other residual solvents, see *Residual Solvents (467)*.]

- **Related Compounds**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, System suitability, and **Suitability requirements:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The impurities are listed in *Table 2*.]

Calculate the percentage of each impurity, as free acid, in the portion of Calcium L-5-Methyltetrahydrofolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the corresponding impurity from the *Sample solution*

r_S = peak response of the principal peak from the *Standard solution*

C_S = concentration of USP Calcium DL-5-Methyltetrahydrofolate RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcium L-5-Methyltetra hydrofolate in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity peak (see *Table 2*)

M_{r1} = molecular weight of L-5-methyltetrahydrofolic acid, 459.46

M_{r2} = molecular weight of calcium L-5-methyltetra hydrofolate, 497.52

Acceptance criteria

[Note—Disregard any impurity peak less than 0.05%.]

Individual impurities: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Aminobenzoylglutamic acid[a]	0.29	0.91	0.5
4a-Hydroxy-5-methyl tetrahydrofolic acid[b]	0.37	1.09	1.0
(6*R*)-Mefox[c,d]	0.49	1.05	—
(6*S*)-Mefox[c,d]	0.50	1.05	1.0 (sum of 6*R* and 6*S*)
Tetrahydrofolic acid[e]	0.65	1.00[k]	0.5
7,8-Dihydrofolic acid[f]	0.83	0.95	0.5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Folic acid[a]	0.85	0.83	0.5
5,10-Methylenetetrahydrofolic acid[b]	0.88	1.00[x]	0.5
5-Methyltetrahydropteroic acid[c]	1.10	0.67	0.5
Dimethyltetrahydrofolic acid[d]	1.25	1.00[x]	0.15
Total impurities	—	—	2.5

[a] *N*-(4-Aminobenzoyl)-L-glutamic acid.
[b] *N*-[4-({[(6*S*)-2-Amino-4a-hydroxy-5-methyl-4-oxo-1,4,4a,5,6,7,8,8a-octahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamic acid.
[c] 2-Amino-8-methyl-4,9-dioxo-7-methyl-*p*-aminobenzoyl-glutamate-6,7,8,9-tetrahydro-4*H*-pyrazino-(1,2-a)-*s*-triazine.
[d] Report the impurity Mefox as the sum of 6*S*- and 6*R*-Mefox.
[e] *N*-[4-({[(*S*)-2-Amino-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]meth yl}amino)benzoyl]-L-glutamic acid.
[f] *N*-(4-[[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino)benzoyl)-L-glutamic acid.
[g] *N*-(4-[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid.
[h] *N*-(4-(3-Amino-1-oxo-5,6,6a,7-tetrahydroimidazo[1,5-*f*]pteridin-8(1*H*,4*H*,9*H*)-yl)benzyl)-L-glutamic acid.
[i] (*S*)-4-[[(2-Amino-5-methyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino)benzoic acid.
[j] *N*-[4-({[(*S*)-5-Methyl-2-(methylamino)-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamic acid.
[x] Estimated factor.

• **Enantiomeric Purity**

Buffer: 4.54 g/L of sodium dihydrogen phosphate dihydrate in water

Mobile phase: Acetonitrile and *Buffer* (3:97). Adjust with 32% (w/v) sodium hydroxide to a pH of 6.8.

Standard solution: 0.5 mg/mL of USP Calcium D-5-Methyltetrahydrofolate RS in water

Sample solution: 0.5 mg/mL of Calcium L-5-Methyltetrahydrofolate in water

System suitability solution: Transfer 1.0 mL of *Standard solution* to a 50-mL volumetric flask, and dilute with *Sample solution* to volume.

Chromatographic system

(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 15-cm; 5-µm packing L79

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times of L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate are about 1 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.5 between L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate

Analysis

Sample: *Sample solution*

Calculate the percentage of D-5-methyltetrahydrofolate in the portion of Calcium L-5-Methyltetrahydrofolate taken:

$$\text{Result} = [(r_D/(r_D + r_L) \times 100]$$

r_D = peak response of D-5-methyltetrahydrofolate from the *Sample solution*

r_L = peak response of L-5-methyltetrahydrofolate from the *Sample solution*

Acceptance criteria: NMT 1.0% of D-5-methyltetra hydrofolate

SPECIFIC TESTS

• **Calcium**

Sample: 250 mg

Blank: 150 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue

Titrimetric system

(See *Titrimetry (541)*.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with the *Titrant* until the solution is deep blue in color. Perform a *Blank* determination, and make any necessary correction. Calculate the percentage of calcium (Ca) in the *Sample* taken:

$$\text{Result} = [(V_S - V_B) \times M \times F/W] \times 100$$

V_S = volume of *Titrant* consumed by the *Sample* (mL)

V_B = volume of *Titrant* consumed by the *Blank* (mL)

M = actual molarity of the *Titrant* (mmol/mL)

F = equivalency factor, 40.08 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: 7.0%–8.5% on the anhydrous and solvent-free basis

- **Water Determination,** *Method Ic* ⟨921⟩

Sample: Transfer 40 mg of Calcium L-5-Methyltetrahydrofolate to a 20-mL headspace vial, and cap tightly. Heat the vial in a suitable Karl Fischer oven at 250°.

Analysis: The released and evaporated water is transferred into the titration-cell in a stream of dry nitrogen at a flow rate of about 40 mL/min as directed in *Water Determination, Method Ic* ⟨921⟩.

Acceptance criteria: 6.0%–17.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in a tight container, in a cool and dry place.
- **USP Reference Standards** ⟨11⟩

USP 4-Aminobenzoylglutamic Acid RS

N-(4-Aminobenzoyl)-L-glutamic acid.

$C_{12}H_{14}N_2O_5$ 266.25

USP Calcium L-5-Methyltetrahydrofolate RS

N[4-[[(2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, calcium salt (1:1).

$C_{20}H_{23}CaN_7O_6$ 497.52

USP Folic Acid RS

Auxiliary Information– Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CALCIUM L-5-METHYLTETRAHYDROFOLATE	Natalia Davydova Scientific Liaison	NBDS2015 Non-botanical Dietary Supplements 2015

Chromatographic Database Information: ...tographic Database

Most Recently Appeared
Pharmacopeial Forum: Volume ...

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Appendix C. Stability Study

Stability Report

Stability of Arcofolin® Bulk Powder

Document Number

B-08-32-223

Version 3

Scope	Merck Schaffhausen
Purpose	Discussion of Arcofolin® stability data derived from stability studies so far. A conclusion with regard to retest period and recommended storage condition is given.

Drafted:
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Table of Contents

1 Summary

Arcofolin®, also known as Monosodium (6S)-5-Methyltetrahydrofolate, is an essential B vitamin which is used for nutritional applications.

The aim of this report is to justify the current recommendations for the retest period and storage conditions for Arcofolin®, and to suggest future options for storage and retest period.[1]

Presently, the re-test period for Arcofolin® is 24 months at the recommended storage condition of 5°C. The re-test period was originally set based on 12 months stability data of 3 production batches together with 24 months data of a laboratory scale batch [1]. Furthermore, some parts of the 24 months stability data of the 3 production batches were already discussed for an extension of the retest period of the latter batches [2].

In this report the stability data of 3 production batches over 36 months are presented and discussed.

Based on the presented real time data it is concluded that:

- The current re-test period for Arcofolin® of 12 months at the recommended storage condition of 5°C is confirmed.
- Due to the excellent stability both at 5°C and 25°C/60% RH an extension of the re-test period up to 36 months at 5°C would be justified.
- Moreover, due to the excellent stability both at 25°C/60% RH (36 months) and 40°C/75% RH (12 months), a change of the re-test period to 36 months is also supported for a recommended storage condition of 25°C.

2 Stability Testing Program

The follow-up stability testing conditions are presented in this report:

- -20°C ± 5°C
- 5°C ± 3°C (currently recommend long-term condition)
- 25°C ± 2°C / 60% ± 5% RH (current accelerated condition)
- 40°C ± 2°C / 75% ± 5% RH (additional accelerated condition)

2.1 Packaging

Samples were portioned into polyethylene bags as primary packaging. Heat sealed aluminium composite foil was used as secondary packaging. Steel drums were used as outer packaging.

2.2 Included batches

The stability studies were started in April 2018.

Batch	Manu-factured	-20°C, Months completed (total)	5°C, Months completed (total)	25°C/60%RH, Months completed (total)	40°C/75%RH, Months completed (total)
ESY0008-XX	03/2018	36 (60)	36 (60)	36(60)	12 (12)
ESY0009-XX	03/2018	36 (60)	36 (60)	36(60)	12 (12)
ESY0010-XX	03/2018	36 (60)	36 (60)	36(60)	12 (12)

[1] The stability data of the laboratory scale batch EUY-0001-A, included in version 01 of this document [1], are not further evaluated in this report.

2.3 Parameters & Specifications

The following parameters are presented in this report. Specifications were taken from the testing instruction for Arcofolin® A-08-24-736.

Parameter	Specification
Water	Not more than 1.0% w/w
Assay free acid (MeTHFA)	Not less than 91.0% w/w
ABGA	Not more than 0.5% w/w
HOMeTHFA	Not more than 1.0% w/w
Mefox	Not more than 1.0% w/w
THFA	Not more than 0.5% w/w
DHFA	Not more than 0.5% w/w
FA	Not more than 0.5% w/w
CH2THFA	Not more than 0.5% w/w
MeTHPA	Not more than 0.5% w/w
DiMeTHFA	Not more than 0.15% w/w
Sum of related compounds	Not more than 2.5% w/w

Further parameters, which were tested but are not discussed in this report are "Appearance" and "TAMC/TYMC" (microbiological tests). No trends or OOS were observed for these parameters.

3 Statistics

Scatter plots were generated with software R and R Studio (Version 4.0.2 and Version 1.3.1056).

4 Results

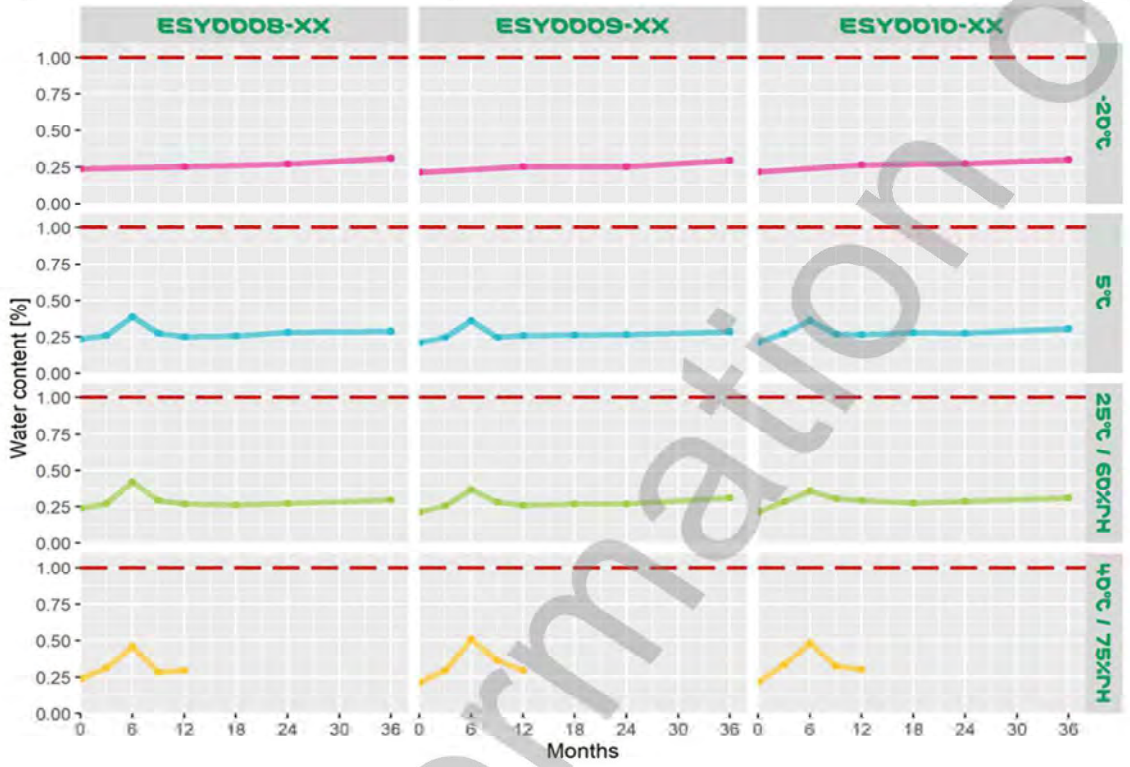
All results are presented as scatter plots for graphic representation. In general the progression of the stability plots is not uncommon and similar curves are described in current literature[2].

[2] Waterman, K.C.; Understanding and Predicting Pharmaceutical Product Shelf-Life. In: Handbook of Stability Testing in Pharmaceutical Development; Huynh-Ba, K., Eds.; Springer: New York, 2009; pp 115-135.

4.1 Water content

Under all investigated storage conditions no significant change of the water content was observed. Over the whole stability testing period the water content remained within the limits of specification for all batches.

Figure 1: Water content vs storage time

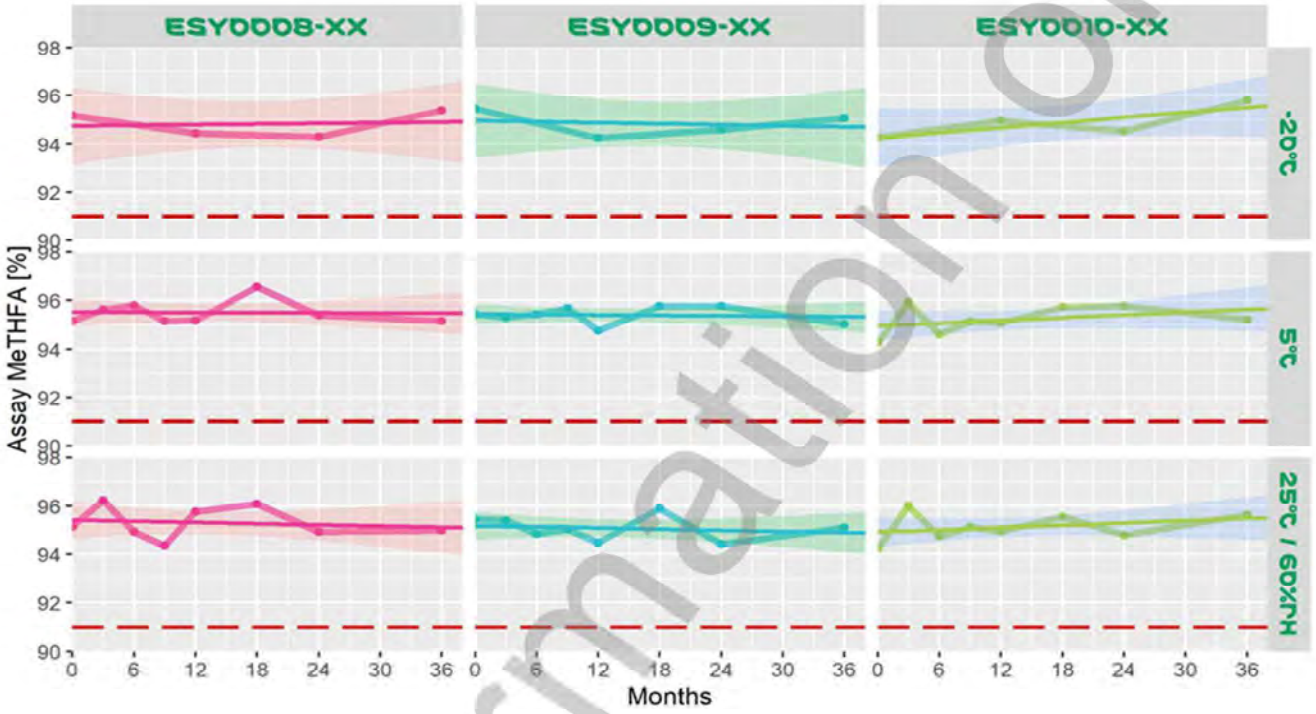


Scatterplot of all batches at all available storage conditions. Content of water [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted line.

4.2 HPLC – Assay

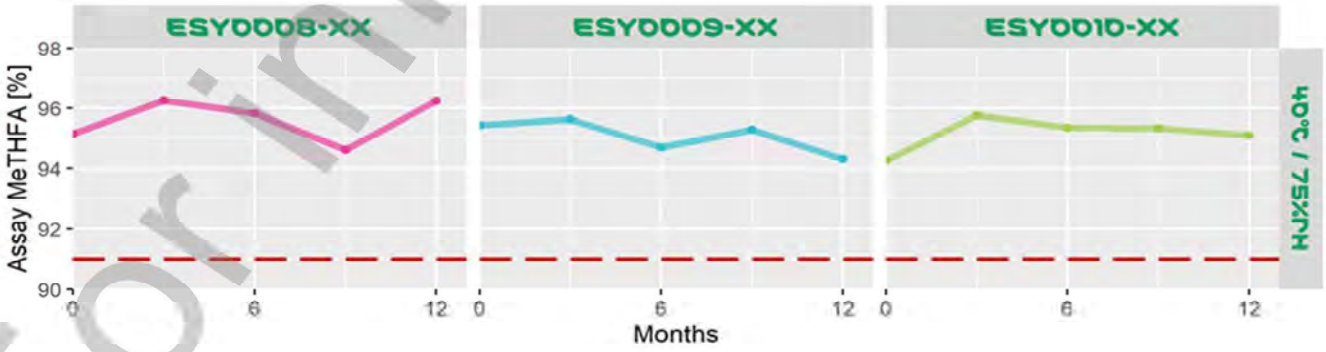
The storage conditions -20°C, 5°C and 25°C/60% RH were investigated and thereby, no significant change of the assay of MeTHFA was observed and the specification limit (not less than 91.0% w/w) was met for any time point and storage condition.

Figure 2: Assay of MeTHFA vs storage time



Scatterplot of all available samples stored at -20°C, 5°C and 25°C/60% RH including linear regression and confidence interval at 90% probability. The assay of MeTHFA [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted lines.

Figure 3: Assay of MeTHFA at accelerated conditions vs storage time



Scatterplot of samples stored at accelerated conditions (40°C/75% RH) for 12 months. The assay of MeTHFA [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted lines.

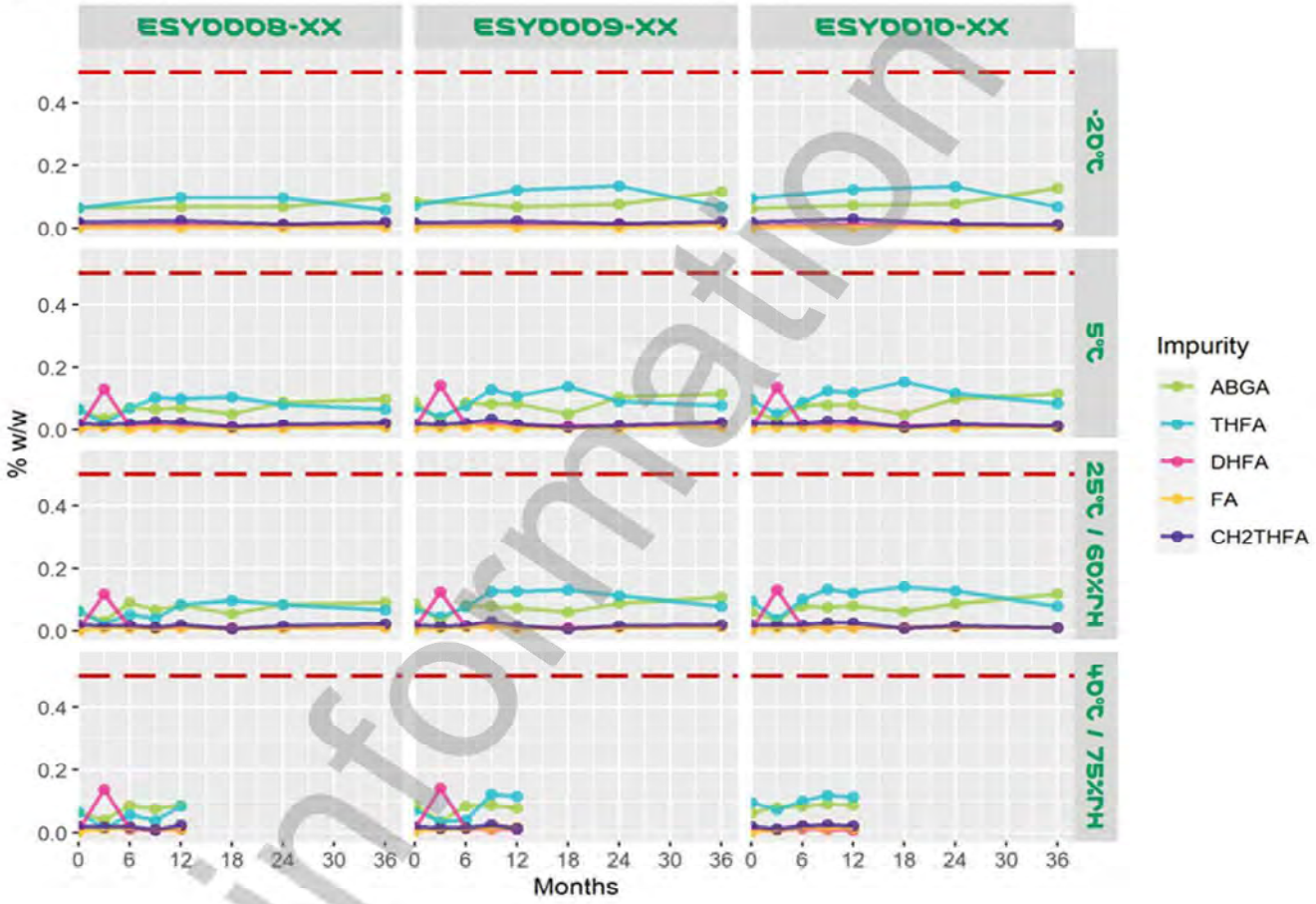
4.3 HPLC – Related Compounds (RC)

Under all investigated storage conditions no significant change of the concentration of the related compounds were observed for all three batches.

All results were below the relevant specification limits (chapter 2.2).

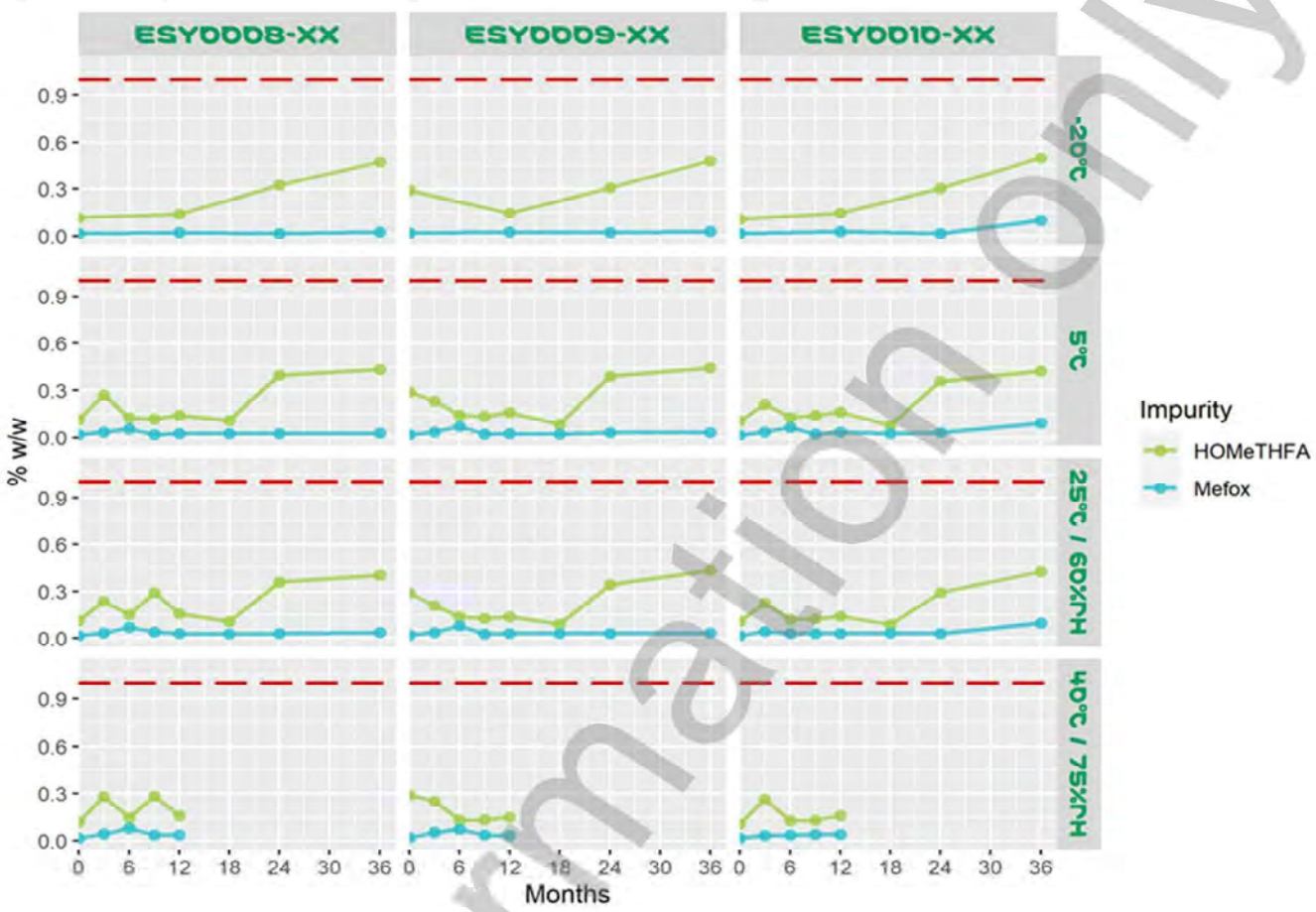
No significant increase of any of the related compounds could be observed not even at accelerated conditions.

Figure 4: Impurities with 0.5% specification limit vs storage time



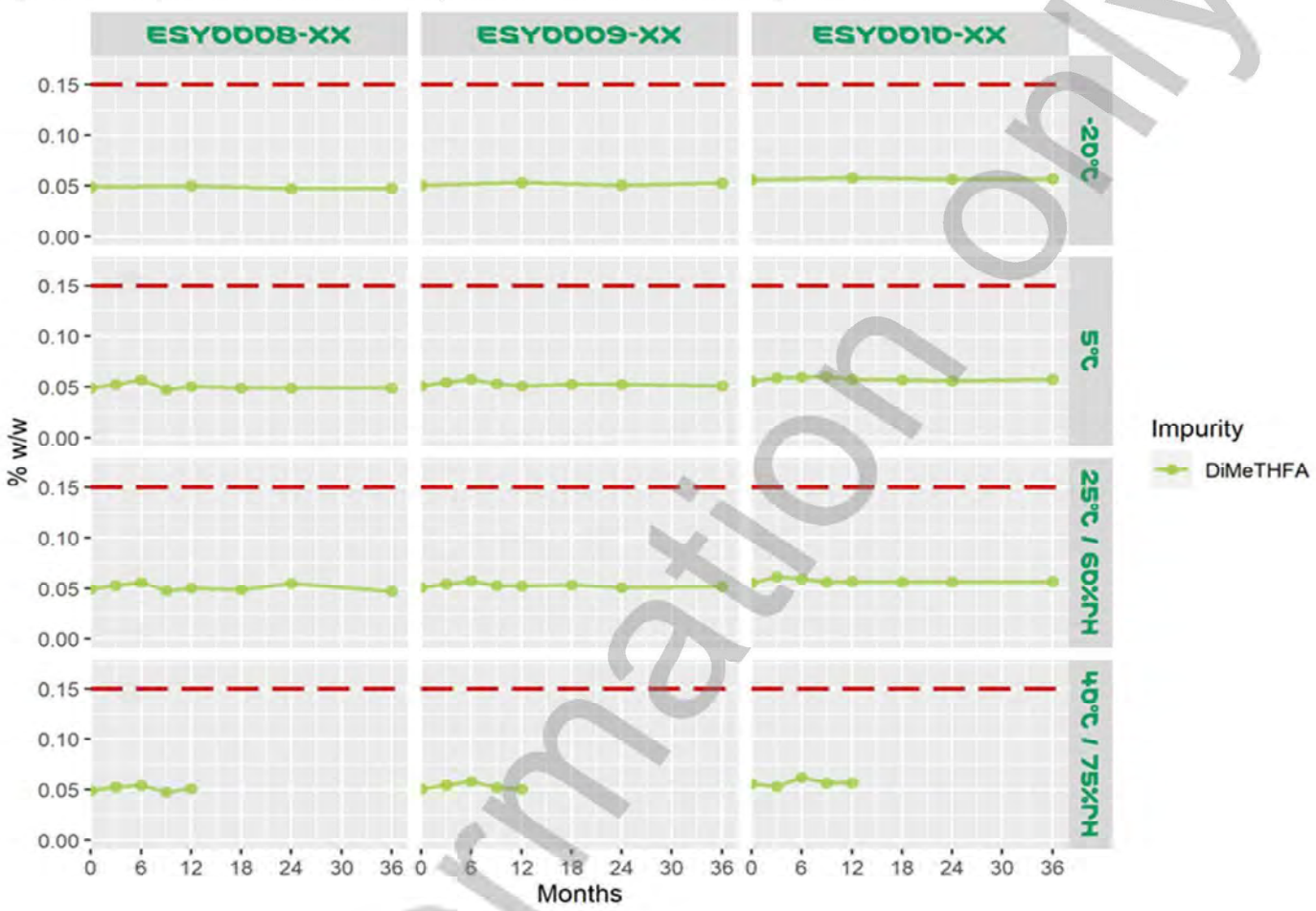
Scatterplot of related compounds with specification limit of ≤ 0.5% including all batches at all available storage conditions. Content of related compound [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted line.

Figure 5: Impurities with 1% specification limit vs storage time



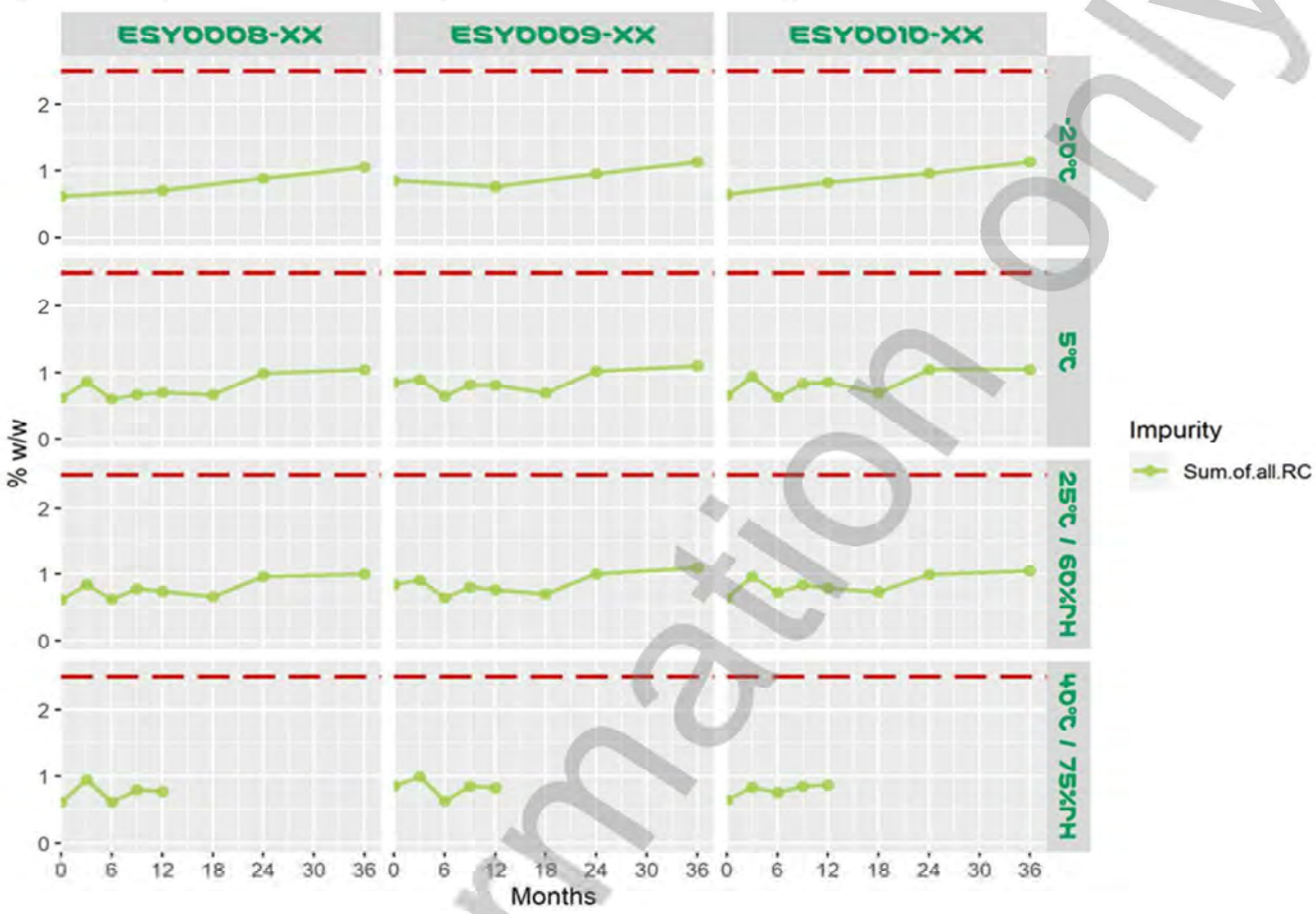
Scatterplot of related compounds with specification limit of ≤ 1.0% including all batches at all available storage conditions. Content of related compound [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted line.

Figure 6: Impurities with 0.15% specification limit vs storage time



Scatterplot of related compounds with specification limit of ≤ 0.15% including all batches at all available storage conditions. Content of related compound [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted line.

Figure 7: Impurities with 2.5% specification limit vs storage time



Scatterplot of related compounds with specification limit of ≤ 2.5% including all batches at all available storage conditions. Content of related compound [% w/w] is plotted against storage duration [months]. The specification limit is indicated by the red dotted line.

5 Discussion

Generally, it can be observed that the production batches have a comparable stability at -20°C, 5°C and 25°C/60% RH. At all three conditions, all investigated parameters such as water content, assay and related compounds remain within the specifications during the observation period (36 months) for all three production batches.

Results for samples stored at accelerated conditions (40°C/75% RH) were available for 12 months and also remained within the specified limits.

6 Conclusion

Based on the presented real time stability data it is concluded that:

- The current re-test period for Arcofolin® of 12 months at the recommended storage condition of 5°C is confirmed.
- Due to the excellent stability both at 5°C and 25°C/60% RH an extension of the re-test period up to 36 months at 5°C would be justified.
- Moreover, due to the excellent stability both at 25°C/60% RH (36 months) and 40°C/75% RH (12 months), a change of the re-test period to 36 months is also supported for a recommended storage condition of 25°C.

7 Further applicable documents

[1] Stability Report "Stability of Arcofolin™ Bulk Powder" (Doc. No. B-08-32-223, v01)
[2] Report "Extension of the Retest Period of ESY0008-XX, ESY0009-XX & ESY0010-XX" (Doc. No. B-08-35-270)

History

Version	Date	Editor	Changes
01	26.07.2019	J.P. Knapp / V. Schöwe	New document
02	08.12.2020	R. Itel	Addition of further stability data for batches ESY0008-XX, ESY0009-XX, ESY0010-XX Removal stability data for lab scale batch EUY-0001-A
03	10.05.2021	R. Itel	Update with 36 months data. Start of stability study was corrected. Stability time points were analyzed correctly.

Appendices

Appendix 1 Batch ESY0008-XX

Sodium Acrofolin; Stability testing Program

Lot Nr.: ESY0008-XX
Date of production: 03.2018
Stability start: 04.2018

-20°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000036482	complies	0.2	95.15	0.06	0.11	0.02	n.d	0.07	n.d	0.01	0.00	0.02	0.00	0.11	n.d	0.05	0.04	0.62	0.5	0.5
12	04.2019	890000082227	complies	0.3	94.43	0.07	0.14	0.02	n.d	0.10	n.d	0.01	0.00	0.03	0.01	0.11	n.d	0.05	0.03	0.70	-	-
24	04.2020	890000096479	complies	0.3	94.29	0.07	0.33	0.02	n.d	0.10	n.d	0.01	0.00	0.01	0.00	0.11	n.d	0.05	0.04	0.89	-	-
36	04.2021	890000113440	complies	0.3	95.37	0.10	0.47	0.03	n.d	0.06	0.008	0.01	0.00	0.02	0.00	0.11	n.d	0.05	0.06	1.05	-	-
48	04.2022																					
60	04.2023																					

+5°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000036482	complies	0.2	95.15	0.06	0.11	0.02	n.d	0.07	n.d	0.01	0.00	0.02	0.00	0.11	n.d	0.05	0.04	0.62	0.5	0.5
3	07.2018	890000071315	complies	0.3	95.64	0.04	0.27	0.03	n.d	0.02	n.d	0.13	0.01	0.02	0.00	0.11	n.d	0.05	0.03	0.87	-	-
6	10.2018	890000075120	complies	0.4	95.79	0.07	0.12	0.06	n.d	0.07	n.d	0.01	0.00	0.02	0.01	0.11	n.d	0.06	0.02	0.60	-	-
9	01.2019	890000078600	complies	0.3	95.16	0.07	0.12	0.02	n.d	0.10	n.d	0.02	0.01	0.03	0.01	0.11	0.009	0.05	0.04	0.67	-	-
12	04.2019	890000082228	complies	0.3	95.19	0.07	0.14	0.02	n.d	0.10	n.d	0.01	0.01	0.02	0.00	0.11	n.d	0.05	0.03	0.70	0.5	0.5
18	10.2019	890000089524	complies	0.3	96.56	0.05	0.11	0.02	n.d	0.10	n.d	0.01	0.00	0.01	0.01	0.11	n.d	0.05	0.03	0.67	-	-
24	04.2020	890000096480	complies	0.3	95.39	0.09	0.40	0.03	0.001	0.08	0.002	0.01	0.00	0.02	0.01	0.11	n.d	0.05	0.05	0.99	0.5	0.5
36	04.2021	890000113441	complies	0.3	95.15	0.10	0.43	0.03	n.d	0.06	0.009	0.0129	0.01	0.02	0.01	0.11	n.d	0.05	0.05	1.04	-	-
48	04.2022																					
60	04.2023																					

+25°C / 60% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000036482	complies	0.2	95.15	0.06	0.11	0.02	n.d	0.07	n.d	0.01	0.00	0.02	0.00	0.11	n.d	0.05	0.04	0.62	0.5	0.5
3	07.2018	890000071316	complies	0.3	96.23	0.03	0.23	0.03	n.d	0.03	n.d	0.12	0.01	0.02	0.00	0.12	n.d	0.05	0.03	0.85	-	-
6	10.2018	890000075121	complies	0.4	94.93	0.09	0.15	0.07	n.d	0.05	n.d	0.01	0.01	0.02	0.01	0.11	n.d	0.06	0.02	0.63	-	-
9	01.2019	890000078601	complies	0.3	94.36	0.07	0.29	0.04	n.d	0.04	n.d	0.01	0.01	0.01	0.01	0.11	n.d	0.05	0.03	0.78	-	-
12	04.2019	890000082229	complies	0.3	95.75	0.08	0.16	0.03	n.d	0.09	n.d	0.01	0.01	0.02	0.01	0.11	n.d	0.05	0.04	0.74	0.5	0.5
18	10.2019	890000089528	complies	0.3	96.08	0.06	0.11	0.03	n.d	0.10	0.007	0.01	0.01	0.01	0.01	0.11	n.d	0.05	0.03	0.67	-	-
24	04.2020	890000096483	complies	0.3	94.94	0.09	0.36	0.03	0.004	0.09	0.008	0.01	0.01	0.02	0.01	0.11	n.d	0.05	0.04	0.96	0.5	0.5
36	04.2021	890000113442	complies	0.3	94.99	0.09	0.40	0.04	n.d	0.07	0.010	0.012	0.01	0.02	0.01	0.11	n.d	0.05	0.05	1.00	-	-
48	04.2022																					
60	04.2023																					

+40°C / 75% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000036482	complies	0.2	95.15	0.06	0.11	0.02	n.d	0.07	n.d	0.01	0.00	0.02	0.00	0.11	n.d	0.05	0.04	0.62	0.5	0.5
3	07.2018	890000071317	complies	0.3	96.26	0.04	0.28	0.04	n.d	0.01	0.010	0.14	0.01	0.02	0.00	0.11	n.d	0.05	0.03	0.94	-	-
6	10.2018	890000075122	complies	0.5	95.84	0.09	0.15	0.08	n.d	0.06	n.d	0.01	0.02	0.02	0.01	0.11	n.d	0.05	0.02	0.62	-	-
9	01.2019	890000078606	complies	0.3	94.61	0.08	0.29	0.04	0.002	0.04	0.002	0.01	0.01	0.01	0.01	0.11	n.d	0.05	0.04	0.80	-	-
12	04.2019	890000082231	complies	0.3	96.24	0.09	0.16	0.04	n.d	0.08	0.005	0.01	0.01	0.02	0.01	0.12	n.d	0.05	0.03	0.77	-	-

original

Appendix 2 Batch ESY0009-XX

Sodium Acrofolin; Stability testing Program

Lot Nr. : ESY0009-XX
Date of production: 03.2018
Stability start: 04.2018

-20°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000065484	complies	0.2	95.43	0.09	0.29	0.02	n.d	0.07	n.d	0.01	0.00	0.02	n.d	0.12	n.d	0.05	0.05	0.85	0.5	0.5
12	04.2019	890000082232	complies	0.3	94.24	0.07	0.15	0.03	n.d	0.12	n.d	0.01	0.00	0.02	0.00	0.12	n.d	0.05	0.04	0.76	-	-
24	04.2020	890000095484	complies	0.3	94.57	0.08	0.31	0.02	0.001	0.14	0.006	0.01	0.00	0.02	0.00	0.12	n.d	0.05	0.05	0.95	-	-
36	04.2021	890000113444	complies	0.3	95.04	0.12	0.48	0.03	n.d	0.07	0.006	0.01	0.01	0.02	0.00	0.13	n.d	0.05	0.07	1.14	-	-
48	04.2022																					
60	04.2023																					

+5°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000065484	complies	0.2	95.43	0.09	0.29	0.02	n.d	0.07	n.d	0.01	0.00	0.02	n.d	0.12	n.d	0.05	0.05	0.85	0.5	0.5
3	07.2018	890000071319	complies	0.2	95.31	0.03	0.23	0.03	n.d	0.04	0.005	0.14	0.01	0.02	n.d	0.13	n.d	0.05	0.03	0.90	-	-
6	10.2018	890000075123	complies	0.4	95.43	0.09	0.14	0.07	n.d	0.08	n.d	0.02	0.01	0.02	0.01	0.13	n.d	0.06	0.02	0.65	-	-
9	01.2019	890000073608	complies	0.2	95.69	0.08	0.13	0.02	n.d	0.13	n.d	0.02	0.01	0.03	0.00	0.13	0.014	0.05	0.04	0.82	-	-
12	04.2019	890000082233	complies	0.3	94.75	0.08	0.16	0.03	n.d	0.11	0.008	0.01	0.01	0.02	0.00	0.12	n.d	0.05	0.04	0.81	0.5	0.5
18	10.2019	890000089529	complies	0.3	95.78	0.05	0.09	0.02	n.d	0.14	0.006	0.01	0.00	0.01	0.00	0.12	n.d	0.05	0.03	0.70	-	-
24	03.2020	890000095485	complies	0.3	95.78	0.10	0.39	0.03	n.d	0.09	0.002	0.01	0.00	0.01	0.00	0.12	n.d	0.05	0.06	1.02	0.5	0.5
36	03.2021	890000113445	complies	0.3	95.03	0.11	0.45	0.03	n.d	0.08	0.010	0.02	0.01	0.02	0.00	0.13	n.d	0.05	0.06	1.10	-	-
48	03.2022																					
60	03.2022																					

+25°C / 60% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000065484	complies	0.2	95.43	0.09	0.29	0.02	n.d	0.07	n.d	0.01	0.00	0.02	n.d	0.12	n.d	0.05	0.05	0.85	0.5	0.5
3	07.2018	890000071320	complies	0.3	95.39	0.04	0.21	0.04	n.d	0.05	0.006	0.13	0.01	0.02	n.d	0.14	n.d	0.05	0.03	0.91	-	-
6	10.2018	890000075126	complies	0.4	94.85	0.08	0.14	0.08	n.d	0.08	n.d	0.02	0.01	0.02	0.01	0.13	n.d	0.06	0.02	0.65	-	-
9	01.2019	890000073609	complies	0.3	95.05	0.08	0.13	0.03	n.d	0.13	0.004	0.02	0.02	0.03	0.01	0.13	0.014	0.05	0.04	0.81	-	-
12	04.2019	890000082234	complies	0.3	94.48	0.07	0.14	0.03	n.d	0.13	n.d	0.01	0.01	0.02	n.d	0.14	n.d	0.05	0.04	0.77	0.5	0.5
18	10.2019	890000089532	complies	0.3	95.88	0.06	0.09	0.03	n.d	0.13	n.d	0.01	0.01	0.01	0.00	0.12	n.d	0.05	0.03	0.70	-	-
24	03.2020	890000095486	complies	0.3	94.41	0.09	0.34	0.03	0.005	0.11	0.009	0.01	0.01	0.02	0.00	0.12	n.d	0.05	0.05	1.01	0.5	0.5
36	03.2021	890000113446	complies	0.3	95.11	0.11	0.43	0.04	n.d	0.08	0.009	0.01	0.01	0.02	0.01	0.13	n.d	0.05	0.06	1.10	-	-
48	03.2022																					
60	03.2022																					

+40°C / 75% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	04.2018	890000065484	complies	0.2	95.43	0.09	0.29	0.02	n.d	0.07	n.d	0.01	0.00	0.02	n.d	0.12	n.d	0.05	0.05	0.85	0.5	0.5
3	07.2018	890000071321	complies	0.3	95.63	0.04	0.25	0.05	n.d	0.03	0.012	0.14	0.01	0.02	n.d	0.13	n.d	0.05	0.03	0.99	-	-
6	10.2018	890000075125	complies	0.5	94.70	0.09	0.13	0.07	n.d	0.04	n.d	0.01	0.01	0.02	0.01	0.12	n.d	0.06	0.05	0.64	-	-
9	01.2019	890000073610	complies	0.4	95.29	0.09	0.13	0.04	0.006	0.12	0.011	0.01	0.01	0.02	0.01	0.12	n.d	0.05	0.04	0.85	-	-
12	04.2019	890000082235	complies	0.3	94.32	0.08	0.15	0.03	n.d	0.12	0.005	0.01	0.02	0.01	n.d	0.14	n.d	0.05	0.06	0.83	-	-

Sodium Acrofolin; Stability testing Program

Lot Nr. : ESY0010-XX
Date of production: 03.2018
Stability start: 04.2018

-20°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	C4.2018	8S0000036483	complies	0.2	94.26	0.06	0.11	0.02	n.d	0.10	n.d	0.01	0.00	0.02	0.00	0.13	n.d	0.03	0.04	0.65	0.5	0.5
12	C4.2019	8S0000082236	complies	0.3	94.94	0.07	0.15	0.03	n.d	0.12	n.d	0.01	0.01	0.03	0.00	0.15	n.d	0.03	0.04	0.82	-	-
24	C4.2020	8S0000096487	complies	0.3	94.51	0.08	0.30	0.02	0.001	0.13	0	0.01	0.00	0.02	0.00	0.14	n.d	0.03	0.04	0.96	-	-
36	C4.2021	8S0000113447	complies	0.3	95.79	0.13	0.50	0.10	n.d	0.07	n.d	0.01	0.01	0.01	0.01	0.14	n.d	0.03	0.03	1.13	-	-
48	C4.2022																					
60	C4.2023																					

+5°C

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	C4.2018	8S0000036483	complies	0.2	94.26	0.06	0.11	0.02	n.d	0.10	n.d	0.01	0.00	0.02	0.00	0.13	n.d	0.03	0.04	0.65	0.5	0.5
3	C7.2018	8S0000071322	complies	0.3	95.92	0.03	0.21	0.04	n.d	0.05	0.010	0.13	0.01	0.02	0.00	0.15	n.d	0.03	0.03	0.95	-	-
6	10.2018	8S0000075127	complies	0.4	94.61	0.08	0.13	0.07	n.d	0.09	n.d	0.02	0.01	0.02	0.00	0.13	n.d	0.03	0.02	0.63	-	-
9	C1.2019	8S0000078613	complies	0.3	95.14	0.08	0.14	0.02	n.d	0.12	n.d	0.01	0.01	0.03	0.00	0.14	n.d	0.03	0.04	0.84	-	-
12	C4.2019	8S0000082237	complies	0.3	95.07	0.08	0.16	0.03	n.d	0.12	n.d	0.01	0.01	0.03	0.00	0.15	n.d	0.03	0.05	0.86	0.5	0.5
18	10.2019	8S0000089534	complies	0.3	95.74	0.05	0.08	0.02	n.d	0.15	n.d	0.01	0.00	0.01	0.00	0.14	n.d	0.03	0.03	0.69	-	-
24	C4.2020	8S0000096489	complies	0.3	95.77	0.10	0.36	0.03	0.003	0.11	0.003	0.01	0.01	0.02	0.00	0.14	n.d	0.03	0.05	1.05	0.5	0.5
36	C4.2021	8S0000113448	complies	0.3	95.23	0.11	0.43	0.09	n.d	0.08	n.d	0.01	0.01	0.01	0.01	0.14	n.d	0.03	0.02	1.05	-	-
48	C4.2022																					
60	C4.2023																					

+25°C / 60% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	C4.2018	8S0000036483	complies	0.2	94.26	0.06	0.11	0.02	n.d	0.10	n.d	0.01	0.00	0.02	0.00	0.13	n.d	0.03	0.04	0.65	0.5	0.5
3	C7.2018	8S0000071323	complies	0.3	93.00	0.04	0.23	0.04	n.d	0.04	0.01	0.13	0.01	0.02	0.00	0.15	n.d	0.03	0.03	0.97	-	-
6	10.2018	8S0000075128	complies	0.4	94.74	0.08	0.12	0.03	n.d	0.10	n.d	0.01	0.01	0.02	0.01	0.14	n.d	0.03	0.04	0.73	-	-
9	C1.2019	8S0000078614	complies	0.3	95.11	0.08	0.12	0.03	n.d	0.13	0.010	0.01	0.01	0.03	0.01	0.14	n.d	0.03	0.04	0.84	-	-
12	C4.2019	8S0000082238	complies	0.3	94.96	0.08	0.14	0.03	n.d	0.12	n.d	0.01	0.01	0.03	0.00	0.15	n.d	0.03	0.04	0.80	0.5	0.5
18	10.2019	8S0000089535	complies	0.3	95.55	0.06	0.09	0.03	n.d	0.14	0.004	0.01	0.01	0.01	0.00	0.14	n.d	0.03	0.03	0.74	-	-
24	C4.2020	8S0000096490	complies	0.3	94.81	0.09	0.29	0.03	0.005	0.13	0.007	0.01	0.01	0.02	0.00	0.14	n.d	0.03	0.04	1.00	0.5	0.5
36	C4.2021	8S0000113449	complies	0.3	95.63	0.12	0.42	0.10	n.d	0.08	n.d	0.01	0.01	0.01	0.01	0.14	n.d	0.03	0.03	1.05	-	-
48	C4.2022																					
60	C4.2023																					

+40°C / 75% rF

Period (months)	Date	Inspection Lot	Appearance	Water [%]	5-MeTHFA acid as is [%]	ABGA [%]	HOMeTHFA [%]	Mefox [%]	Mefdiamid [%]	THFA [%]	Mefguan [%]	DHFA [%]	FA [%]	CH2THFA [%]	rRt 1.06 [%]	MeTHPA [%]	rRt 1.16 [%]	DiMeTHFA [%]	indiv. unk. [%]	sum total [%]	TAMC cfu/g	TYMC cfu/g
0	C4.2018	8S0000036483	complies	0.2	94.26	0.06	0.11	0.02	n.d	0.10	n.d	0.01	0.00	0.02	0.00	0.13	n.d	0.03	0.04	0.65	0.5	0.5
3	C7.2018	8S0000071324	complies	0.3	95.77	0.08	0.26	0.03	n.d	0.07	n.d	0.01	0.01	0.01	0.00	0.14	n.d	0.05	0.04	0.84	-	-
6	10.2018	8S0000075129	complies	0.5	95.34	0.09	0.13	0.04	0	0.10	n.d	0.01	0.02	0.02	0.00	0.14	n.d	0.03	0.04	0.76	-	-
9	C1.2019	8S0000078615	complies	0.3	95.34	0.09	0.13	0.04	0.003	0.12	0.008	0.01	0.01	0.03	0.00	0.14	n.d	0.03	0.04	0.86	-	-
12	C4.2019	8S0000082239	complies	0.3	95.09	0.09	0.16	0.04	0	0.11	n.d	0.01	0.01	0.02	0.00	0.15	n.d	0.03	0.05	0.87	-	-

Appendix D. PubMed Literature Searches

Search Terms	# of citations
((arco folin OR 2246974-96-7 OR monosodium l-mefolinate OR monosodium l-5-methyltetrahydrofolate OR l-methylfolate OR l-5-methyltetrahydrofolic acid, monosodium salt OR (6S) methyltetrahydrofolic acid, monosodium salt OR N- (2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo- (6S) pteridinyl) methyl amino benzoyl l glutamic acid OR 5-methyltetrahydrofolate OR L-5-MTHF OR L-5-methyltetrahydrofolate)) AND (safe* OR toxicity OR toxicol* OR acute OR chronic OR subchronic OR mutagenicity OR genotox* OR reproduc* OR development* OR terat*) Filters: English; Field: Title/Abstract	250
((arco folin OR 2246974-96-7 OR monosodium l-mefolinate OR monosodium l-5-methyltetrahydrofolate OR l-methylfolate OR l-5-methyltetrahydrofolic acid, monosodium salt OR (6S) methyltetrahydrofolic acid, monosodium salt OR N- (2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo- (6S) pteridinyl) methyl amino benzoyl l glutamic acid OR 5-methyltetrahydrofolate OR L-5-MTHF OR L-5-methyltetrahydrofolate)) AND ("randomized controlled trial"[pt] OR "controlled clinical trial"[PT] OR "Clinical Trial"[pt] OR randomized OR randomised OR "controlled trial" OR "clinical trial" OR crossover OR cross-over OR pilot) Filters: English; Field: Title/Abstract	119
((arco folin OR 2246974-96-7 OR monosodium l-mefolinate OR monosodium l-5-methyltetrahydrofolate OR l-methylfolate OR l-5-methyltetrahydrofolic acid, monosodium salt OR (6S) methyltetrahydrofolic acid, monosodium salt OR N- (2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo- (6S) pteridinyl) methyl amino benzoyl l glutamic acid OR 5-methyltetrahydrofolate OR L-5-MTHF OR L-5-methyltetrahydrofolate)) AND ("randomized controlled trial"[pt] OR "controlled clinical trial"[PT] OR "Clinical Trial"[pt] OR randomized OR randomised OR "controlled trial" OR "clinical trial" OR crossover OR cross-over OR pilot) AND infant Filters: English	14
((arco folin OR 2246974-96-7 OR monosodium l-mefolinate OR monosodium l-5-methyltetrahydrofolate OR l-methylfolate OR l-5-methyltetrahydrofolic acid, monosodium salt OR (6S) methyltetrahydrofolic acid, monosodium salt OR N- (2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo- (6S) pteridinyl) methyl amino benzoyl l glutamic acid OR 5-methyltetrahydrofolate OR L-5-MTHF OR L-5-methyltetrahydrofolate)) AND (metabolism OR metabolic OR metabolite OR absorption OR bioavailability OR pharmacokinetics OR oral OR pharmacodynamics OR RBC folate OR plasma folate OR serum folate OR urinary folate OR homocysteine) AND (folic acid OR folate) Filters: English; Field: Title/Abstract; Date limit 2017/01/01	62
5-MTHF OR methyltetrahydrofolate OR methyl folate trap OR methyl trap AND mask* vitamin B12 deficiency No filters applied	9

Searches originally conducted in December 2019 and updated in April 2020.

Appendix E. Signed GRAS Panel Statement

Report of the GRAS Panel Concerning the Generally Recognized As Safe (GRAS) Status of the Use of Arcofolin®, a Monosodium Salt of L-5-Methyltetrahydrofolic Acid, As a Source of Folate

Introduction

The undersigned, an independent panel of experts, qualified by their scientific training and experience to evaluate the safety of food and food ingredients (the "GRAS Panel"), was specially convened by Hyman, Phelps & McNamara, PC to evaluate the safety and "generally recognized as safe" ("GRAS") status of the intended uses of Arcofolin® a monosodium salt of L-5-methyltetrahydrofolic acid, as a source of folate. Arcofolin® is intended to be used as an alternative to folic acid in select uses for which folic acid is an approved added nutrient, specifically breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products. The GRAS panelists are: Drs. J. Gregory, C. Kruger, and P. Pressman.

For the purpose of this review, "safe" or "safety" means that there is "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the conditions of its intended use," as defined by the U.S. Food and Drug Administration (FDA, or the "Agency") in 21 C.F.R. § 170.3(i).

Exponent, Inc. ("Exponent") performed a search of the scientific literature, through April 2020, relevant to the safety of Arcofolin®, a monosodium salt of L-5-methyltetrahydrofolic acid (L-5-MTHF-Na), as a source of folate. Exponent summarized the results of the literature search and prepared a safety dossier, "Documentation Supporting the Generally Recognized As Safe (GRAS) Conclusion of the Use of Arcofolin®, a Monosodium Salt of L-5-Methyltetrahydrofolic acid As a Source of Folate" (April 17, 2020) for consideration by the GRAS Panel.

The GRAS Panel members critically evaluated Exponent's safety documentation (the dossier), and other available data and information that the members of the Panel believed to be pertinent to the safety of the intended use of Arcofolin® as a source of folate. In particular, the Panel reviewed the following: identity of Arcofolin®, information on the manufacture of the Arcofolin®, specifications for Arcofolin®, a review of uses of salts of L-5-MTHF, and the safety of Arcofolin® and similar salts.

On April 1 and 27, 2020, the Expert Panel convened via teleconference, and independently, jointly, and unanimously concluded that Arcofolin®, is manufactured under current good manufacturing practice (cGMP), meets appropriate food grade specifications and is safe and suitable for its intended use as an alternative to folate in select uses for which folic acid is an approved added nutrient under 21 C.F.R. § 172.345, specifically breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products. The Panel further concluded unanimously that the safety and GRAS

status of Arcofolin® is based on scientific procedures. It is also the consensus opinion of this GRAS Panel that other qualified experts would concur with these conclusions.

Summarized below are the data and information upon which the Panel's conclusions relied

Description

Arcofolin® is a monosodium salt of L-5-methyltetrahydrofolic acid (L-5-MTHF-Na) that is a source of folate. Specifications for Arcofolin® define the product as ≥ 91.0% [6S]5-MTHF, ≤ 2.5% related compounds, 4.0 – 5.0% sodium, not more than 0.5% ethanol, not more than 0.5% iso-propanol, and ≤ 1% water (with appropriate specifications to control elemental impurities and microbiological contaminants). Arcofolin® is produced under cGMP.

Intended Use

Arcofolin® is intended to be used as an alternative to folic acid for the following uses for which folic acid is an approved added nutrient: breakfast cereal; corn grits; infant formula; medical foods; food for special dietary use; and foods represented to be meal-replacement products, as specified in 21 C.F.R. § 172.345.

Estimated Daily Intake (EDI)

Arcofolin® is intended for use as substitute for folic acid in foods. Thus, the use of Arcofolin® will not increase the dietary exposure to folic acid and total folate in the U.S. population.

Safety Information

Folate, which converts to several reduced forms of folate in the body, is a naturally occurring water-soluble B vitamin found in many widely consumed foods (ODS, 2020). Folate is essential to healthy growth and development, and folate deficiency results in various adverse health outcomes. There are several folates available in foods and supplements. The chemical forms of dietary folate include folate (mono- and poly-glutamates), folic acid, L-5-MTHF salts, and L-5-FTHF (L-5-formyl-tetrahydrofolate), which is also known as L-folinic acid. Folic acid is a synthetic form of folate commonly found in dietary supplements (ODS, 2020). All folates interconvert following absorption, ultimately forming the natural diastereoisomer of 5-methyltetrahydrofolate ([6S]-5-MTHF that is herein referred to as L-5-MTHF). Since all folate forms share a common metabolic fate, namely conversion to L-5-MTHF, once absorbed, the pattern of circulating folates is indistinguishable.

The safety of Arcofolin® is supported by pivotal published studies evaluating the bioavailability of this product as well as bioavailability and safety of the compositionally equivalent salt, Metafolin®.

- Both Arcofolin® and Metafolin® are salts of L-5-MTHF (Arcofolin®: L-5-MTHF-Na and Metafolin®: L-5-MTHF-Ca), which are manufactured using similar production methods and have similar specifications. These salts are expected to completely dissociate *in vivo*

- The publicly available data indicate that the bioavailability of the L-5-MTHF-Ca is at least as high as that of folic acid and that the fate of L-5-MTHF-Ca is indistinguishable from that of other absorbed and metabolized natural folate forms (ODS, 2020). In 2004, an EFSA panel concluded that the bioavailability of L-5-MTHF-Ca is at least as high as that of folic acid. In a 2008 assessment, FSANZ similarly concluded that the available evidence supports that L-5-MTHF and folic acid are "essentially bioequivalent."

- The comparable bioavailability of Arcofolin® and Metafolin® is corroborated by findings from clinical data evaluating the pharmacokinetic response with respect to total plasma folate for each salt compared to folic acid administration.

- The IOM (1998) and EFSA (2014) both established a tolerable upper intake levels (UL) for folate from fortified foods or supplements (folic acid form) as 1 mg/day for adults. This UL was established based on the relationship of folate intake and neurological dysfunction; folate has the potential to reverse megaloblastic anemia due to vitamin B_{12} deficiency, thereby delaying the timely diagnosis and appropriate treatment of the disease and allowing the neurological dysfunction caused by B_{12} deficiency to progress. L-5-MTHF supplementation reduces the potential for masking the symptoms of vitamin B_{12} deficiency as well as the hematological manifestations of the deficiency, which formed the basis for the UL. Thus, the current UL derived for supplemental folate in the form of folic acid is conservative.

- In 2016, FDA conducted a safety review and risk assessment on folic acid and concluded that the approved uses of folic acid in foods are safe.

- Other authoritative bodies have performed risk assessments and corroborated the safety of the ingestion of L-5-MTHF-Ca in foods and supplements. JECFA considered the safety of L-5-MTHF-Ca and reported no safety concern for its proposed use in dry crystalline or microencapsulated form as an alternative to folic acid used in dietary supplements, foods for special dietary uses, and other foods (JECFA, 2005). FSANZ also concluded that the use of L-5-MTHF-Ca for the fortification of certain foods would not raise public health or safety concerns (FSANZ, 2008). In the United States, NDINs were filed for the use of L-5-MTHF (is this the Ca salt) in dietary supplements as a source of folate and the notifications were accepted for filing and acknowledged without objection by FDA. Recently, EFSA concluded that the proposed uses of L-5-MTHF-Ca as a folate source in infant formula, follow-on formula, processed cereal-based food, and baby food for infants

(<12 mo in age) and young children (12-36 mo in age) is safe and that the compound does not pose any concern for allergenicity (EFSA, 2020).

- In a series of studies published by Niederberger et al. (2019), L-5-MTHF-Ca was found to be non-genotoxic, and developmental toxicity studies of standard design did not reveal any toxic potential to fetuses or pregnant dams. A 13-week rat gavage study also published in Niederberger et al. (2019) established a NOAEL for L-5-MTHF-Ca of 400 mg/kg bw/day based on the absence of any treatment-related effects at this highest tested dose.

- Unpublished genotoxicity studies on Arcofolin® were negative, providing corroborative evidence of non-genotoxicity. Although there are reports of possible adverse effects of high folic acid intake on cancer growth, underlying mechanisms have yet to be elucidated and more research is necessary to establish a causal relationship. A review of the potential adverse effect of folic acid or biosynthesized folate on colorectal cancer risk does not offer any consistent or conclusive evidence of cause and effect and consequently does not appear to impact the conclusion on the safety of folate by the IOM, EFSA, and FDA.

- Assuming Arcofolin® will serve as an alternative to folic acid for the following uses for which folic acid is an approved added nutrient: breakfast cereal; corn grits; infant formula; medical foods; food for special dietary use; and foods represented to be meal-replacement products, as specified in 21 C.F.R. § 172.345, the 95th percentile cumulative intake is 919 µg/day among age group 51-70 years (FDA, 2016b). Using a default body weight of 60 kg, a cumulative intake of 15.3 µg/kg bw/day can be estimated, which is below the IOM established UL.

Safety Assessment

Collectively, the publicly available data support that the proposed use of Arcofolin® as a source of folate when used as an alternative to folic acid for use in breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products as specified in 21 C.F.R. § 172.345, is safe within the meaning of the Federal Food, Drug, and Cosmetic Act, i.e., and meets the standard of reasonable certainty of no harm under the conditions of intended use.

Conclusion of the GRAS Panel

We, the undersigned qualified GRAS panel members, have, both individually and collectively, critically evaluated published and unpublished data and information pertinent to the safety of the intended use of Arcofolin® as an alternative to folic acid for use in breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products as specified in 21 C.F.R. § 172.345. We unanimously conclude that the intended use of Arcofolin® manufactured in accordance with cGMP, and meeting appropriate food grade specifications, is safe and is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

May 20, 2020

Jesse F. Gregory, PhD
Professor Emeritus of Food Science and Human Nutrition
University of Florida
Gainesville, FL 32611

Date

Claire Kruger, Ph.D., DABT, CFS
Spherix Consulting Group

Date

Peter Pressman, MD, MS, FACN
The Daedalus Foundation

Date

Conclusion of the GRAS Panel

We, the undersigned qualified GRAS panel members, have, both individually and collectively, critically evaluated published and unpublished data and information pertinent to the safety of the intended use of Arcofolin® as an alternative to folic acid for use in breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products as specified in 21 C.F.R. § 172.345. We unanimously conclude that the intended use of Arcofolin® manufactured in accordance with cGMP, and meeting appropriate food grade specifications, is safe and is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

Jesse F. Gregory, PhD — Date
Professor Emeritus of Food Science and Human Nutrition
University of Florida
Gainesville, FL 32611

[redacted] — May 20, 2020
Claire Kruger, Ph.D., DABT, CFS — Date
Spherix Consulting Group

Peter Pressman, MD, MS, FACN — Date
The Daedalus Foundation

Conclusion of the GRAS Panel

We, the undersigned qualified GRAS panel members, have, both individually and collectively, critically evaluated published and unpublished data and information pertinent to the safety of the intended use of Arcofolin® as an alternative to folic acid for use in breakfast cereals, corn grits, infant formula, medical foods, food for special dietary use, and foods represented to be meal-replacement products as specified in 21 C.F.R. § 172.345. We unanimously conclude that the intended use of Arcofolin® manufactured in accordance with cGMP, and meeting appropriate food grade specifications, is safe and is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

Jesse F. Gregory, PhD
Professor Emeritus of Food Science and Human Nutrition
University of Florida
Gainesville, FL 32611

Date

Claire Kruger, Ph.D., DABT, CFS
Spherix Consulting Group

Date

Peter Pressman, MD, MS, FACN
The Daedalus Foundation

May 20, 2020
Date

1909102 000 - 2438_GRN_Merck_Arcofolin June 29 2021

Final Audit Report 2021-06-30

Created:	2021-06-29
By:	Miriam Wildt (miriam.wildt@merckgroup.com)
Status:	Signed
Transaction ID:	CBJCHBCAABAAIpeLAPsnLGD1_9jtVIushg7RdH0Y6qfR

"1909102 000 - 2438_GRN_Merck_Arcofolin June 29 2021" History

Document created by Miriam Wildt (miriam.wildt@merckgroup.com)
2021-06-29 - 3:59:37 PM GMT- IP address: 155.250.198.110

Miriam Wildt (miriam.wildt@merckgroup.com) verified identity with Adobe Sign authentication
2021-06-30 - 7:09:41 AM GMT

Document e-signed by Miriam Wildt (miriam.wildt@merckgroup.com)
Signature Date: 2021-06-30 - 7:09:41 AM GMT - Time Source: server- IP address: 155.250.198.126

Document emailed to Martin Knüsel (martin.knuesel@merckgroup.com) for signature
2021-06-30 - 7:09:44 AM GMT

Email viewed by Martin Knüsel (martin.knuesel@merckgroup.com)
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Martin Knüsel (martin.knuesel@merckgroup.com) verified identity with Adobe Sign authentication
2021-06-30 - 7:18:32 AM GMT

Document e-signed by Martin Knüsel (martin.knuesel@merckgroup.com)
Signature Date: 2021-06-30 - 7:18:32 AM GMT - Time Source: server- IP address: 155.250.255.140

Agreement completed.
2021-06-30 - 7:18:32 AM GMT

From: Nga Tran
To: Morissette, Rachel
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023
Date: Monday, December 20, 2021 10:05:40 AM
Attachments: image002.png
image004.png
image006.png
image008.png
image011.png
image013.png
image016.png
image018.png
image020.png
image022.png
image024.png
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image062.png

Dear Rachel,
Following FDA's recommendation, Merck is requesting a meeting with the Agency to discuss issue concerning HEM in January 2022 and that the Agency cease to evaluate this GRAS notice.
We look forward to further discussion with the review team.
Best regards and Happy Holidays.
Nga

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, December 20, 2021 9:49 AM
To: Nga Tran <ntran@exponent.com>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Thank you!

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Nga Tran <ntran@exponent.com>
Sent: Friday, December 17, 2021 3:52 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Dear Rachel,
I have passed this information to Merck and I probably will not hear back from them until Monday. I will follow up early next week with their decision (before you leave for vacation).
Thanks
Nga

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, December 17, 2021 12:33 PM
To: Nga Tran <ntran@exponent.com>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Dear Nga,

Thank you for getting back to us. We discussed Merck's proposal. Unfortunately, the use of QSAR and read across to support the GRAS status of HEM is not going to be sufficient to establish safety and general recognition of safety. We have significant safety concerns about this toxic and possibly carcinogenic impurity and cannot move forward with a no questions letter for the intended use of L-5-MTHF-Na with HEM used in the manufacturing. We are happy to set up a meeting after the New

Year to discuss this issue further, but at this time we are recommending that Merck request that we cease to evaluate this GRAS notice. Please let me know how Merck wishes to proceed. I will be around until next Wednesday, but then am off for Christmas until Jan. 3.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Nga Tran <ntran@exponent.com>
Sent: Friday, December 10, 2021 1:45 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Dear Rachel,

I had a chance to discuss the questions raised in your email and phone call from last week with Merck. Merck would like to request that we remove the proposed use of Arcofolin in infant formula from the current GRN 001023.

Regarding the use of HEM in the manufacturing, it is an integral part of the manufacturing process and there is no suitable alternative for Merck. As such, we would like to provide the Agency with a formal QSAR and the published tox data on the read-across as the result the formal QSAR assessment to support the safety of Merck's internal reporting threshold (< 0.05%) for HEM. Given the upcoming holiday season and December being such a short month, would it be possible for us to

provide this updated narrative for HEM in January for your review as part of the current GRN?

We appreciate your guidance.
Best,
Nga

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, November 19, 2021 2:10 PM
To: Nga Tran <ntran@exponent.com>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Thank you. Have a great weekend.

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Nga Tran <ntran@exponent.com>
Sent: Friday, November 19, 2021 2:05 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

I just accepted as well as forwarded to Mary Murphy. She will be joining the call.
Thanks
Nga

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, November 19, 2021 1:02 PM
To: Nga Tran <ntran@exponent.com>
Subject: RE: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Thank you. I scheduled our meeting for Dec. from 2-3 pm. Please let me know if you didn't receive the Zoom call-in instructions.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Nga Tran <ntran@exponent.com>
Sent: Friday, November 19, 2021 12:54 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: [EXTERNAL] RE: request for a meeting to discuss GRN 001023

Dear Rachel,
Thursday 12/2 from 1-3 works for us.

Thanks
Nga

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, November 19, 2021 12:39 PM
To: Nga Tran <ntran@exponent.com>
Subject: [EXTERNAL] request for a meeting to discuss GRN 001023

Dear Dr. Tran,

We would like to schedule a phone call to discuss GRN 0001023, as we have a number of concerns with certain data and information that were provided in the notice, as well as information that is lacking. Although this is not a complete list of our questions for this notice, the high-level concerns we would like to discuss include the following:

1. The use of hydroxyethylmorpholine (HEM) in the manufacturing process.
2. The lack of dietary exposure data to sodium L-5-MTHF (i.e., folic acid) in infants (and therefore inability to evaluate downstream issues like sodium intake).
3. The insufficient safety discussion/studies related to use by infants.

The following dates and times are currently available for our team, so please let me know if any of these 1-hour slots will work for you as soon as possible, as our calendars tend to fill up quickly. If not, I can extend later into December.

Tues. Nov. 30 from 12-2 pm
Thurs. Dec. 2 from 12-3 pm
Mon. Dec. 6 from 9-10 am, 11-12 pm, or 2-3 pm

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov


FDA
U.S. FOOD & DRUG
ADMINISTRATION


I GOT MY
COVID-19
VACCINATION