May 24, 2021

McGUFF
COMPOUNDING
PHARMACY
SERVICES, INC.

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. FDA-2021-N-0357; Pharmacy Compounding Advisory Committee; Notice of Meeting; Establishment of a Public Docket; Request for Comments.

We are providing quality and stability information on the bulk drug substances, methylcobalamin and choline chloride, that are scheduled to be reviewed on June 9th, 2021, by the Pharmacy Compounding Advisory Committee for inclusion on the 503A approved list for pharmacy compounding. We submit for review the pharmacy's qualification files of methylcobalamin and choline chloride. These files detail the pharmacy's rationale and acceptance criteria on the acquisition and use of these bulk drug substances. We have created a qualification protocol which we believe will verify the integrity and quality of the bulk drug substance as it reaches our facility. Each lot of the compounded sterile injection is assayed for sterility and endotoxin along other Quality Control testing prior to being dispensed to the patient.

If there are any questions to the information we have provided, we are available to answer them.

Sincerely,

Si Pham, Pharm.D.
Pharmacist-in-Charge

McGUFF
COMPOUNDING
PHARMACY
SERVICES

2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704-6929

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THIS DOCUMENT CONTAINS CONFIDENTIAL INFORMATION

DOCKET NO. FDA-2021-N-0357

1. Purpose

To evaluate the use of Methylcobalmin bulk drug substance by McGuff Compounding Pharmacy Services, Inc. (MCPS) for sterile compounding.

2. Scope

This qualification protocol applies to the screening/testing of incoming methylcobalmin bulk drug substances prior to release for sterile compounding use.

3. Background

Usage of "non-pharmaceutical" grade material to compound sterile injection preparations for human use has raised concerns by the FDA. A review of federal compounding regulations as well as USP guidelines reveals no official definition of "pharmaceutical grade" for bulk drug substances. Currently, methylcobalamin is also on the FDA's Category 1 list under evaluation for 503A compounding (see Appendix A). It is the pharmacy's goal to verify that any incoming methycobalamin API used for sterile compounding meets applicable USP chapters on impurities. Notably, the pharmacy wants to ensure the pharmacy's source of methylcobalamin meets USP standards as they pertain to endotoxin, residual solvent, and heavy metal impurities for lead, mercury, arsenic and cadmium.

The following material qualification plan shall be used to establish additional quality assurance beyond information supplied by the certificate of analysis accompanying each received manufacturer lot of methylcobalamin powder by MCPS.

4. References

FRM-0050 Component History Form

FRM-0138 Pharmacy Raw Material Specification & Inspection Requirements Form

5. Materials and Equipment

310-0055 Methylcobalamin powder, CAS # 13422-55-4

6. Definitions

6.1. United States Pharmacopeia (USP): A reference compendium of standardized drugs and other articles published by The United States Pharmacopeia.

7. Sample Size and Preparation

Not applicable

8. Qualification Method

8.1. Applicable USP Chapters

As part of the material qualification plan, each incoming manufacturer lot of methylcobalamin bulk drug substance will be screened as noted below and per Methylcobalamin Raw Material Acceptance Criteria form (see Appendix A). The raw material acceptance criteria form will be attached to the corresponding methylcobalamin PRMSIR form (FRM-0138). Information on the supplier's certificate of analysis (CoA) or documented statement may be used to satisfy specifications noted below.

1) USP <85> Bacterial Endotoxin Tests
    a. Screening per PRMSIR current addendum form titled, "Endotoxin Screening Requirement," for PN# 310-0055, Methylcobalamin (see Appendix B)

2) USP <232> Elemental Impurities – Limits (for injections)
    a. Screening of each received manufacturer lot of methylcobalamin bulk drug substance for the following elemental impurities:
        i. Arsenic
        ii. Lead
        iii. Mercury
        iv. Cadmium

3) USP <467> Residual Solvents

4) USP <1229.3> Bioburden

5) Verification of vendor's any specifications noted on the CoA as deemed necessary

All test results will be included as part of the Component History Form (FRM-0050) for the received lot of methylcobalamin.

8.2. Manufacturer/Vendor Audit

As part of the qualification process, MCPS will perform an initial audit of the manufacturer/supplier of methylcobalamin bulk drug substance to verify the vendor is in good cGMP standing with the FDA as a registered drug establishment or chemical supplier. Such an audit may include a remote survey of the vendor's cGMP standing or, if practical, an onsite inspection. MCPS's Quality Systems will review the vendor's cGMP profile annually. All audits/reviews will be documented and filed according to MCPS's quality systems procedures.

9. Appendices:

Appendix A: FDA Category 1 List for 503A Compounders

Appendix B: Methylcobalamin, Raw Material Acceptance Specifications

Appendix B: Endotoxin Screening Requirement

Appendix C: Vendor Audit/Qualification

Appendix A

FDA Category 1 List for 503A Compounders

Updated July 1, 2020

503A Category 1 – Bulk Drug Substances Under Evaluation

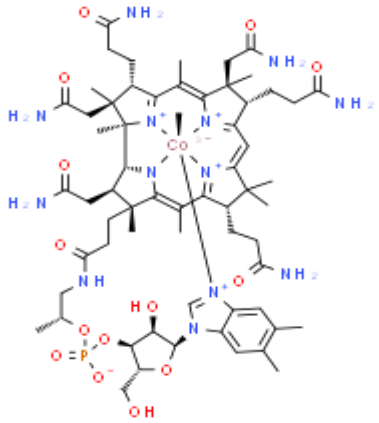
- 7 Keto Dehydroepiandrosterone
- Acetyl L Carnitine/Acetyl-L- carnitine Hydrochloride
- Alanyl-L-Glutamine
- Aloe Vera/ Aloe Vera 200:1 Freeze Dried
- Alpha Lipoic Acid
- Artemisia/Artemisinin
- Astragalus Extract 10:1
- Boswellia
- Choline Chloride
- Chondroitin Sulfate
- Chrysin
- Coenzyme Q10
- Creatine Monohydrate
- Curcumin
- Deoxy-D-Glucose
- Dichloroacetate
- Diindolylmethane
- Dimercapto-1- propanesulfonic acid (DMPS)
- EGCg
- Ferric Subsulfate
- Glutaraldehyde
- Glutathione
- Glycolic Acid
- Glycyrrhizin
- Kojic Acid
- L-Citrulline
- Melatonin
- Methylcobalamin
- Methylsulfonylmethane (MSM)
- Nettle leaf (Urtica dioica subsp. dioica leaf)
- Nicotinamide Adenine Dinucleotide (NAD)
- Nicotinamide Adenine Dinucleotide Disodium Reduced (NADH)
- Pregnenolone
- Pyridoxal 5-Phosphate Monohydrate
- Pyruvic Acid
- Quercetin/Quercetin Dihydrate
- Quinacrine Hydrochloride (except for intrauterine administration)
- Resveratrol
- Ribose (D)
- Rubidium Chloride
- Tea tree oil (Melaleuca alternifolia leaf oil)
- Trichloroacetic Acid
- Ubiquinol 30% Powder
- Vanadium
- Vasoactive Intestinal Peptide

Appendix B

Methylcobalamin, Raw Material Acceptance Specifications

Confidential
McGuff Compounding Pharmacy Services, Inc.

Methylcobalamin, Raw Material Acceptance Specifications – Potency & Impurities
CAS # 13422-55-4



$C_{63}H_{91}CoN_{13}O_{14}P$
M.W. 1344.38

Characteristics	Test Method / Standard	Specifications
Assay	USP <621> Chromatography	98.0 – 101.0% dried basis
Residual Solvents	USP <467>	All residual solvents used in manufacturer's process must be below USP <467> limits
As, Pb, Cd, Hg Elemental Impurities	USP <232>	Arsenic ≤ 1.5 ug/gm, Lead ≤ 0.5 ug/gm, Cadmium ≤ 0.2 ug/gm, and Mercury ≤ 0.3 ug/gm
Endotoxin	USP <85> Gel Clot	≤ 7 EU/mg
Bioburden	USP <1229.3>	< 10 CFU per container type

Appendix C

Endotoxin Screening Requirement

Raw Material Description: Methylcobalamin
McGuff CPS PN#: 310-0055

Procedure

1. All **new** incoming lots will follow the sampling plan as specified below:

Container sizes ≤ 1 Kilogram

# of Containers Received	# of Endotoxin Test Sample(s) To Be Collected
1	1 sample (0.05 gm)
2	2 samples (0.05 gm each container)
3 or more	3 samples (0.05 gm, 3 separate containers)

Container sizes > 1 Kilogram

# of Containers Received	# of Endotoxin Test Samples To Be Collected
1	3 samples (0.05 gm top, middle, & bottom layers of container)
2	6 samples (0.05 gm top, middle, & bottom layers of each container)
3 or more	9 samples (0.05 gm, top, middle, & bottom layers of 3 separate random containers)

2. All received containers will be physically quarantined for further processing pending the QC test results of the endotoxin screening. Material sampled and is pending lab test results will have a "QUARANTINE" label applied to the exterior of the container.
    a. If endotoxin assay sample(s) passes specification, then process the quarantined material for release per receiving procedure.
    b. If endotoxin assay sample(s) fails specification, then the designated personnel (e.g. pharmacist) will initiate a Material Review Board investigation for proper disposition of the received material.

Appendix C

Vendor Audit / Qualification

Supplier/Manufacturer Checklists

INTERQUIM, S.A	YES	NO
Does the establishment have a current FDA registration as a drug establishment?	√	
Is there any import alert to the establishment in the past 12 months?		√
Did the establishment receive any FDA 483 or warning letter in the past 12 months?		√
Miscellaneous		
Copy of supporting documents of Quality Systems and/or cGMP practice	√	

Drug Establishments Current Registration Site

New Search (default.cfm)

Search Results for **Interquim**

CSVExcel

Filter:

Firm Name	FDA Establishment Identifier	DUNS	Business Operations	Address	Expiration Date
Interquim, S.A.	3002807304	460009442	API MANUFACTURE;	C/ Joan Buscalla 10, Sant Cugat del Valles (BA), 08173, Spain (ESP)	12/31/2021
Interquim, S.A. de C.V.	3005170172	816148399	API MANUFACTURE;	Guillermo Marconi No. 16, Fracc. Parque Industrial Cuamatla, Cuautitlan Izcalli, Estado de Mexico 54730, Mexico (MEX)	12/31/2021

Showing 1 to 2 of 2 entries

Previous1Next

Data Current through: Wednesday, May 5, 2021

Return to Drug Firm Annual Registration Status Home Page (default.cfm)

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McGuff Compounding Pharmacy Services, Inc.
Stability Study Report Form

Description: Methylcobalamin 5 mg/mL, MD Part Number: 390-1000, 390-3000

Assigned BUD: 180 days Storage Conditions: RT 15 – 30 deg C (59 – 86 deg F) – Protect From Light

Study Approved by: Si Pham, Pharm.D., Pharmacist-in-Charge

Data / Results Table

Characteristic	Test Method	Result at T_0	Result at T_{BUD}
		See specific lot test result	See specific lot test result
Appearance, Seal	Visual	Lot 16H4871 Passed	Lot 16H4871 Passed
Appearance, Vial	Visual	Lot 16H4871 Passed	Lot 16H4871 Passed
Appearance, Product	Visual	Lot 16H4871 Dark red	Lot 16H4871 Dark red
Foreign Matter, Visible Particulate Subvisible Particulate	M370-0004 or per Current USP <790>	Lot 16H4871 No visible ppt	Lot 16H4871 No visible ppt
Foreign Matter, Subvisible Particulate	Current USP <788>	Lot 20B1971 passed	NA
pH [6 – 8]	McGuff Method # M370-0001	7.4 Lot 16H4871	7.0 Lot 16H4871
Assay [4.5 – 5.5 mg/mL]	[HPLC – Stability-indicating-assay] Record method	5.1 mg/mL Baseline - SIA Lot 20B1971	5.2 mg/mL Day 180 - SIA Lot 20B1971
Assay [4.5 – 5.5 mg/mL]	[HPLC - Stability-indicating-assay] Record method	5.2 mg/mL Lot 20A3751 - Baseline	5.3 mg/mL Lot 20A3751 post 7 days exposed to 50 deg C
Sterility	Current USP Chapter <71> or M370-0011	Sterile Lot 16H4871	Sterile Lot 16H4871
Or, in lieu of Sterility Container/Closure Integrity	M370-0080 or BTS Method # CM413 (dye ingress) or M370-0021 / TM-0019 (seal integrity)	N/A	Lot 16H4871 passed CCI
Sterility test [B/F] Method Suitability Test	Current USP Chapter <71>	Lot 16H4872 passed	NA
Endotoxin test [I/E] Method Suitability Test	Current USP Chapter <85>	Lot 16D3932 passed	NA
Preservative Effectiveness (for Multi Dose Vials)	Antimicrobial Effectiveness current USP <51>	Lot 16H4871 meets USP	N/A
Preservative concentration (for Multi Dose Vials) Benzyl alcohol [0.72 - 1.08%]	Current USP Chapter <341>	0.99 % Lot 16H4871	0.86 % Lot 16H4871
CHR for Study Lot Attached?		On file	

CONCLUSION: The assigned BUD x is / □ is not supported by the data gathered above.
(Check One)

Notes: Stability-Indicating-Assay [SIA] was conducted with lot 20B1971to confirm real-time potency of original study, lot 16H4871. The data from lots 16D3932, 16H4871, 16H4872, 20A3751 was used as predicate whenever appropriate to support BUD for 5 mg/mL, MD or PF 390-3103, 390-3105. Stability Study conducted and Report prepared by Doug Tran, Pharm.D.

Reviewed and Approved by P.I. C.: S Pham 10-22-20
Sign and Date

Si Pham, Pharm.D., Pharmacist-in-Charge

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: Methylcobalamin 5mg/mL, 10mL

Part Number: 390-3000

Lot Number: 15B0191

Results

Test	Specification/Limit	Result
Inhibition / Enhancement Testing Bacterial Endotoxin: Interfering factors test *06/11/15	No interfering factors at dilution tested	No interfering factors a dilution of 1:100

Report Conclusion

The sample indicates no interfering factors in the inhibition / enhancement for BET gel clot method when tested at a dilution below the maximum valid dilution. The sample was tested at a 1:100 dilution. The result shows no interfering factors at the dilution tested at 1:100. The sample has passed the interfering factors test section in USP <85>.
*Completion date of testing

Signature and Date: _____ 11-17-17
Laboratories Supervisor or designee

QA Signature and Date: _____ 11/17/17
Quality Assurance

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: Methylcobalamin 5mg/mL, 30mL

Part Number: 390-1000

Lot Number: 16D3932

Results

Test	Specification/Limit	Result
Inhibition / Enhancement Testing Bacterial Endotoxin: Interfering factors test *05/09/16	No interfering factors at dilution tested	No interfering factors a dilution of 1:100

Report Conclusion

The sample indicates no interfering factors in the inhibition / enhancement for BET gel clot method when tested at a dilution below the maximum valid dilution. The sample was tested at a 1:100 dilution. The result shows no interfering factors at the dilution tested at 1:100. The sample has passed the interfering factors test section in USP <85>.
*Completion date of testing

Signature and Date: 11·16·17
Laboratories Supervisor or designee

QA Signature and Date: 11/16/17
Quality Assurance

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: Methylcobalamin 5mg/mL, 30mL

Part Number: 390-1000

Lot Number: 16H4872

Results

Test	Specification/Limit	Result	Completion Date
Bacteriostasis / Fungistasis Testing	Visible growth of microorganisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated.	Growth	03/12/18

Report Conclusion

Sample described above indicates no bacteriostasis / fungistasis properties per membrane filtration test at 5 x 100mL rinse of Fluid A.

Signature and Date: 11·28·18
Laboratories Supervisor or designee

QA Signature and Date: 11/28/18
Quality Assurance

McGUFF
COMPOUNDING PHARMACY SERVICES INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704.
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: Methylcobalamin 5mg/mL, 30mL

Part Number: 390-1000

Lot Number: 16H4872

Results

Test	Specification/Limit	Result
Endotoxin *09/16/16	< 70 EU/mL	< 3 EU/mL
Sterility *09/22/16	No Growth / Growth 14 Day Incubation	No Growth

*Completion date of testing

Note: This is initial testing for this prep/lot, 16H4872:022717

[Doug T]

Signature and Date: 11.16.17
Laboratories Supervisor or designee

QA Signature and Date: 11/16/17
Quality Assurance

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: Methylcobalamin 5mg/mL, 10mL

Part Number: 390-3000

Lot Number: 16H4871

Results

Test	Specification/Limit	Result
Endotoxin Initial *09/16/16	< 70 EU/mL	< 3 EU/mL
Sterility Initial *09/22/16	No Growth / Growth 14 Day Incubation	No Growth
Sterility BUD *05/19/17	No Growth / Growth 14 Day Incubation	No Growth

*Completion date of testing

Note: This is initial testing for this prep/lot, 16H4871:022717.

Note: This is Sterility test post BUD for this prep/lot, 16H4871:022717. [Doug T]

Signature and Date: 11.16.17
Laboratories Supervisor or designee

QA Signature and Date: 11/16/17
Quality Assurance

LABORATORY REPORT

McGuff CPS, Inc. - 00107
2921 West MacArthur Blvd., Ste. 142
Santa Ana, CA 92704
Tel: 714-438-0536
Fax: 714-438-0520
Email:

Client #: E00107
Sample: Methylcobalamin
Conc.: 5mg/mL
Lot #: 16H4872:022717FOIL
Sample ID #: 2016-16320-01
Date Rec'd: 09/12/2016

Chemistry Tests:	Date	Reported	Measured	Potency
Benzyl Alcohol	09/22/2016	0.900 %	0.990 %	110 %
Methylcobalamin	09/22/2016	5.00 mg/mL	5.050 mg/mL	101 %

Notes: Initial Assay Results

Potency: Potency is determined via USP <621> HPLC, USP<851> Spectrophotometry, and specific monograph testing procedures.

Respectfully submitted,
EAGLE ANALYTICAL SERVICES INC.

Glenda Lampkin, Quality Assurance Supervisor

ACCREDITED
Cert#: 3980.01

LABORATORY REPORT

McGuff CPS, Inc. - 00107
2921 West MacArthur Blvd., Ste. 142
Santa Ana, CA 92704
Tel: 714-438-0536
Fax: 714-438-0520
Email:

Client #: E00107
Sample: Methylcobalamin 5 mg/ml MD
Conc.: 5 mg/ml, 0.9%
Lot #: 16H4871:022717FOIL
Sample ID #: 2017-08926-01
Date Rec'd: 05/12/2017

Chemistry Tests:	***Date***	***Reported***	***Measured***	***Potency***
Benzyl Alcohol	05/15/2017	0.900 %	0.858 %	95.3 %
Methylcobalamin	05/15/2017	5.00 mg/mL	5.260 mg/mL	105 %

Notes: Post BUD Assay Results

Potency: Potency is determined via USP <621> HPLC, USP<851> Spectrophotometry, and specific monograph testing procedures.

Respectfully submitted,
EAGLE ANALYTICAL SERVICES INC.

Glenda Lampkin, Quality Assurance Manager

BioScreen® Testing Services, Inc.
3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

MICROBIOLOGICAL REPORT

McGuff Compounding Pharmacy Services, Inc.
Attn: Doug Tran
2921 West MacArthur Blvd., Suite 142
Santa Ana, CA 92704

Page 1 of 2

Report Date: 04/19/17
Date Received: 02/14/17
Date Completed: 04/19/17
Project #: 985344
P.O. #: DT021217A/DT021217B
Reference #: 10742-045; 10720-153

SAMPLE DESCRIPTION

ACCESSION #	SAMPLE:	LOT #:	BATCH #:	QTY:
985344	Methylcobalamin 5mg/mL, MD,	16H4871:022717	16H487	6x10mL

TEST PERFORMED:	BTS METHOD #	REFERENCE:
Validation Test for Antimicrobial Preservative Effectiveness Test	M115.R13	United States Pharmacopeia **39,** <51>
Antimicrobial Effectiveness Test (Category 1)	M101.R14	United States Pharmacopeia **39,** <51>

PLATING MEDIA:
Microbial Content Test Agar (Bacteria)
Sabouraud Dextrose Agar (Yeast and Mold)

ANTIMICROBIAL PRESERVATIVE EFFECTIVENESS VALIDATION TEST

Test Microorganism		Diluent	Dilution	Inoculum CFU/plate	Microbial Recovery CFU/plate	Percent Recovery
Aspergillus brasiliensis	(mold)	DNB	1:10	20	24	120
Aspergillus brasiliensis	(mold)	DNB	1:100	20	24	120
Aspergillus brasiliensis	(mold)	DNB	1:1000	20	25	125
Candida albicans	(yeast)	DNB	1:10	116	82	71
Candida albicans	(yeast)	DNB	1:100	116	95	82
Candida albicans	(yeast)	DNB	1:1000	116	97	84
Escherichia coli	(bacteria)	DNB	1:10	131	90	69
Escherichia coli	(bacteria)	DNB	1:100	131	109	83
Escherichia coli	(bacteria)	DNB	1:1000	131	136	104
Pseudomonas aeruginosa	(bacteria)	DNB	1:10	108	112	104
Pseudomonas aeruginosa	(bacteria)	DNB	1:100	108	123	114
Pseudomonas aeruginosa	(bacteria)	DNB	1:1000	108	132	122
Staphylococcus aureus	(bacteria)	DNB	1:10	141	158	112
Staphylococcus aureus	(bacteria)	DNB	1:100	141	170	121
Staphylococcus aureus	(bacteria)	DNB	1:1000	141	173	123

CFU = Colony Forming Units
DNB = D/E Neutralizing Broth

CONCLUSION:
The antimicrobial preservative properties present in the sample can be neutralized under the test conditions described below:

	DILUENT	DILUTION
Aspergillus brasiliensis	DNB	1:10
Candida albicans	DNB	1:10
Escherichia coli	DNB	1:10
Pseudomonas aeruginosa	DNB	1:10
Staphylococcus aureus	DNB	1:10

ANTIMICROBIAL PRESERVATIVE EFFECTIVENESS TEST

RESULTS:

TABLE SUMMARY

MICROORGANISM		INITIAL INOCULUM/gm	COLONY FORMING UNITS/gm 7 DAYS	14 DAYS	28 DAYS
Aspergillus brasiliensis	(mold)	5.1E5	2.6E4	<10	<10
Candida albicans	(yeast)	1.3E5	<10	<10	<10
Escherichia coli	(bacteria)	9.1E5	<10	<10	<10
Pseudomonas aeruginosa	(bacteria)	9.1E5	<10	<10	<10
Staphylococcus aureus	(bacteria)	1.3E6	<10	<10	<10

Note: Numbers in the report such as 2.3E5 are an alternate exponential format for 2.3×10^5.

LOG REDUCTION FROM INITIAL INOCULUM

	7 DAYS	14 DAYS	28 DAYS
Aspergillus brasiliensis	1.3	4.7	4.7
Candida albicans	4.1	4.1	4.1
Escherichia coli	5.0	5.0	5.0
Pseudomonas aeruginosa	5.0	5.0	5.0
Staphylococcus aureus	5.1	5.1	5.1

CONCLUSION:
The sample described above meets the current USP Category 1 Criteria of Acceptance for the Antimicrobial Preservative Effectiveness Test.

Jessica Balderas
Microbiology Project Coordinator

Dustin Tran
QA Analyst I

ARL BIO PHARMA
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Certificate Of Analysis

CLIENT: McGuff Compounding Pharmacy Services, Inc.
2921 West MacArthur Blvd., Ste. 142
Santa Ana, CA 92704

ARL #: 496634-01
LOT #: 16H4871
DESCRIPTION: Methylcobalamin 5mg/mL MD
DATE RECEIVED: 10/11/2018
STORAGE: 20°C to 25°C (68°F to 77°F)
CONTAINER: Five 30 mL amber vials w/30 mL each in a clear bag

Test	Test Method	Limits	Results	Date Tested
Container Closure	AMIN-1821	Pass/Fail	Pass	10/24/2018

Client Comment From Web Submission: vacuum/dye ingress method followed by a spectrophotometric examination
Formulation ID: 390-3000
Testing performed using AMIN-1821, a non-validated method, is for non-cGMP purposes only

R Wheeler

10/26/2018

Richard Wheeler - Data Reviewer Chemist II

Date Reported

Results reported above relate only to the sample that was tested.

COMPOUNDER'S INTERNATIONAL
ANALYTICAL LABORATORY
Better Quality Through Quality Testing

Stability Indicating Assay (SIA) Method Development and Validation

CONFIDENTIAL REPORT

for

McGuff Compounding Pharmacy Services, Inc.
2921 W MacArthur Blvd, Ste 142
Santa Ana, CA 92704

Methylcobalamin (MCB) Injectable Formulation

Compounder's International Analytical Laboratory
4760 Castleton Way, Suite A
Castle Rock, CO 80109
Toll Free in the US: (800) 788-9922
Or Local Phone: (303) 471-8015
Fax: (303) 569-6101

COMPOUNDER'S INTERNATIONAL
ANALYTICAL LABORATORY
Better Quality Through Quality Testing

Executive Summary

February 14, 2020

This report provides information covering the equipment, chemistries, standards, procedures, and results of the development and validation of the stability indicating method for testing the stability of McGuff Compounding Pharmacy Services, Inc. formulation of methylcobalamin (MCB) injectable solution.

We conclude from this data that the method, which was developed and illustrated here, is stability indicating and validated to provide accurate, precise assays for the MCB injectable formulation, free of interference from process impurities, inactive or excipient ingredients, and degradation products.

Ronald Sutton

Director

Contents

Overview

Scope

The goal of this project is to determine if the method used to quantify the potency of compounded injectable formulation of methylcobalamin (referred to as MCB) received from McGuff Compounding Pharmacy Services, Inc. is valid as a stability indicating method (SIM). The term stability indicating method can be used interchangeably with stability indicating assay (SIA). The sample lot: 20A0351, was used for forced degradation and method validation study, while the placebo lot: 20A1161, was used to verify the accuracy of the method. Table 1 summarizes sample information including CIAL sample IDs along with active and inactive ingredients.

Table 1: Description of sample(s) used for validation study.

CIAL Tracking # / Customer Lot #	Sample Description	Active Ingredient(s)	Inactive Ingredients
Mcg 011720 3.1 / 20A0351	Validation Sample	Methylcobalamin (5 mg/mL)	Sodium chloride, benzyl alcohol, sodium acetate, glacial acetic acid, and sterile water for injection
Mcg 011720 3.2 / 20A1161	Placebo Sample	N/A	Sodium chloride, benzyl alcohol, sodium acetate, glacial acetic acid, and sterile water for injection

Introduction

At the start of the validation, the sample was forcibly degraded with 80ºC heat, UV light radiation, 30% hydrogen peroxide, 1 N HCl, and 1 N KOH. These stressed samples were checked regularly using an ultra-high performance liquid chromatography (UHPLC) for breakdown and possible interferences. The goal was to achieve at least 10% degradation if possible. Once this was achieved, the method was again checked for interferences using the technique of spiking with standards along with spectral analysis. The final step was to validate the method parameters. The validation specifications and a summary of results are shown in Table 2.

In order for the potency of stability samples to be considered valid, any stability samples must be analyzed on the validated SIA and must contain the same active and inactive ingredients as the validation lot which underwent the forced degradation. Active or inactive ingredients may be removed from the formulation and concentrations may be changed, but no new compounds can be added. If these conditions are met, the stability data collected on these samples using the validated SIA, will be considered valid and stability indicating. The removal of ingredients or change in concentration, will not affect our ability to quantify the active(s), but these changes may affect the product stability, thus a new stability study or bracketed study is recommended.

Table 2: Stability indicating assay validation specifications and summary of results.

Validation Parameter	Acceptance Criteria	Result
Stability Indicating Assay	Recovery of standard spiked into degraded samples not greater than 105%. Any possible interference from degradation product must be less than 3%.	Pass
Specificity	Retention time and UV Spectra match reference standard. No interference from formulation, impurities, or degradation products.	Pass
Accuracy	Average Recovery of Standard between 95 – 105%	Pass
Precision	% RSD for replicates < 3%	Pass
Detection Limit	Documented	Pass
Quantitation Limit	Documented	Pass
Linearity	Documented	Pass
Range	Documented	Pass
Robustness	Documented	Pass
System Suitability	Documented	Pass

Experimental

Reagents, Chemicals, and Instrumentation

A methylcobalamin analytical reference standard was purchased from USP. The standard was within expiration or re-test date. High purity 18.2 MΩ water was prepared using an EMD Millipore Milli-Q Integral 5 water purification system. HPLC grade or better solvents were used to dilute standards, samples and/or in the preparation of buffers. Forced degradation was performed using 30% hydrogen peroxide, 1 N hydrochloric acid, and 1 N potassium hydroxide.

The UHPLC instrument consisted of the Waters Acquity Quaternary Solvent Manager, Acquity Sample Manager, Acquity Temperature Controlled Column Compartment, and Acquity Photodiode Array (PDA) Detector. Heat degradation was performed at 80ºC using a Thermo Scientific vacuum oven model 3618-5 at ambient pressure. UV light degradation was performed using Waters instrument at a wavelength of 254 nm, with samples stored in quartz vials. Other equipment used was a Branson Ultrasonic Water Bath, Mettler Toledo XP 26 Microbalance capable of reading down to 1.0 µg, 1 mL precision syringes, 1 mL Gilson Microman positive-displacement pipette, and Pall 0.2 µm GHP Membrane Filters. All equipment was calibrated, qualified, and within recertification dates.

Results and Discussion

Development and Optimization of Method

At CIAL, the standard practice for developing methods is as follows. First research is conducted on the structures of the active(s) and available method(s) for these active ingredient(s). An initial UHPLC method is then developed, using certified reference standards, which would assay for the active(s). After receipt of the sample from the customer, the sample is diluted, chromatographically analyzed, and checked for possible interferences based upon visual inspection as well as spectral overlay analysis. Any interferences found are evaluated and the method is optimized for resolution, sensitivity, accuracy, and precision.

The chromatographic method and sample preparation was optimized for the analysis of methylcobalamin. Depicted in Figure 1 is the chromatogram of a methylcobalamin standard prepared at the target concentration and analyzed on the MCB UHPLC method. The corresponding UV spectrum for the methylcobalamin standard is shown in Figure 2. To ensure specificity, both the retention time and UV spectrum of the samples were compared against those of the reference standard. A typical chromatogram of the sample analyzed on the MCB UHPLC method is shown in Figure 3. A chromatogram of the methylcobalamin placebo is depicted in Figure 4.

Figure 1: Chromatogram of a methylcobalamin standard analyzed on the MCB UHPLC method.

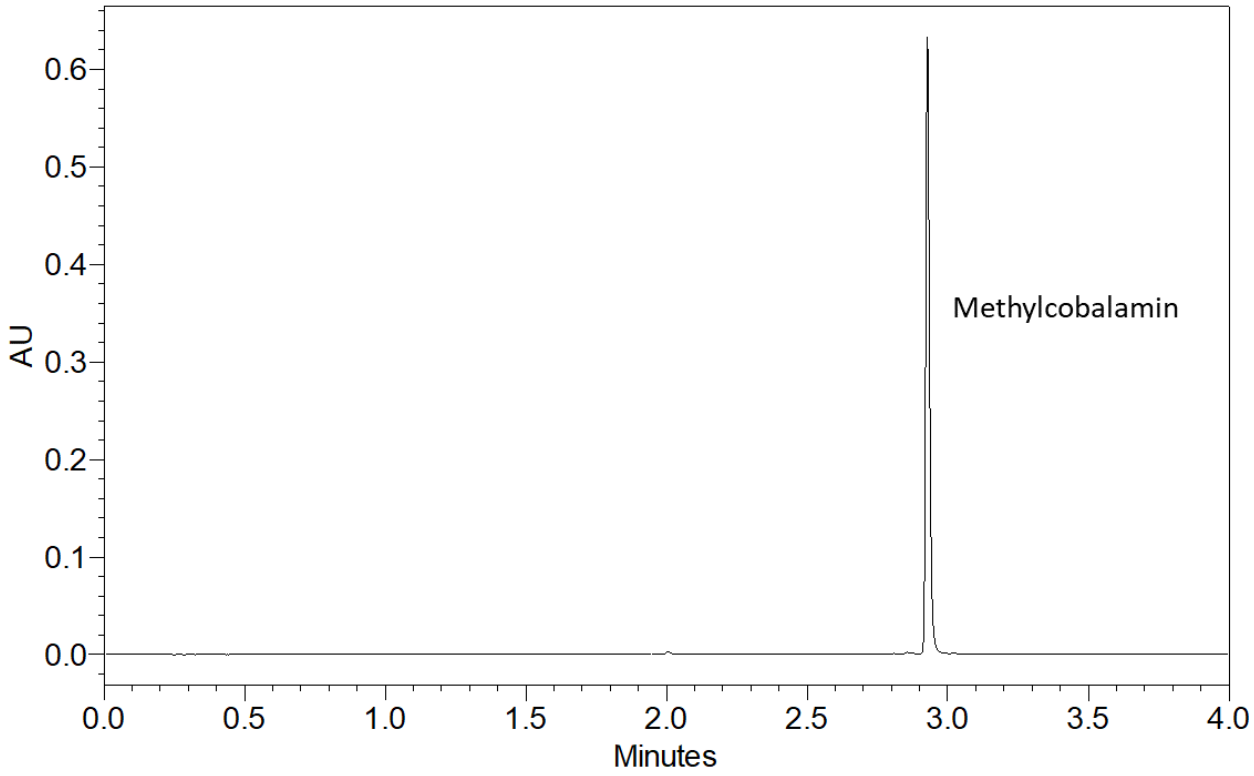


Figure 2: Extracted UV spectrum of a methylcobalamin standard analyzed on the MCB UHPLC method.

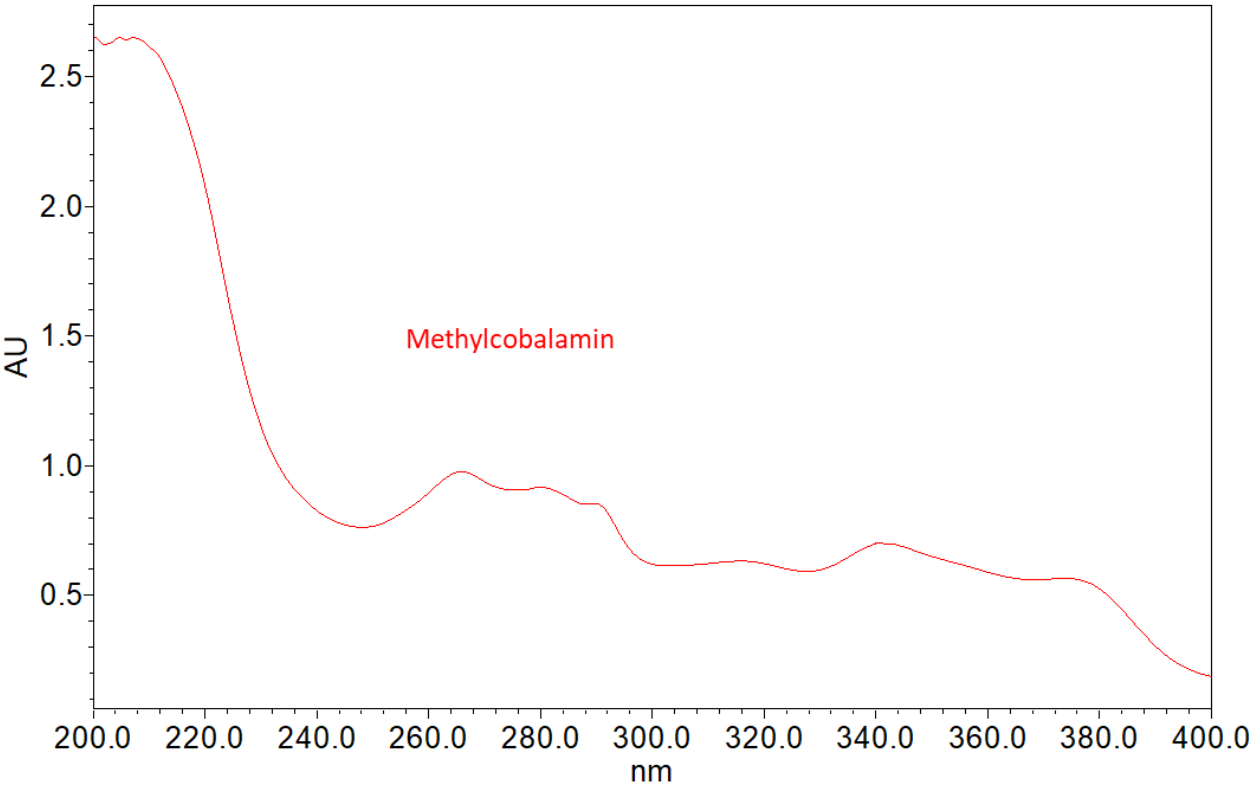


Figure 3: Typical MCB UHPLC method chromatogram of the prepared sample.

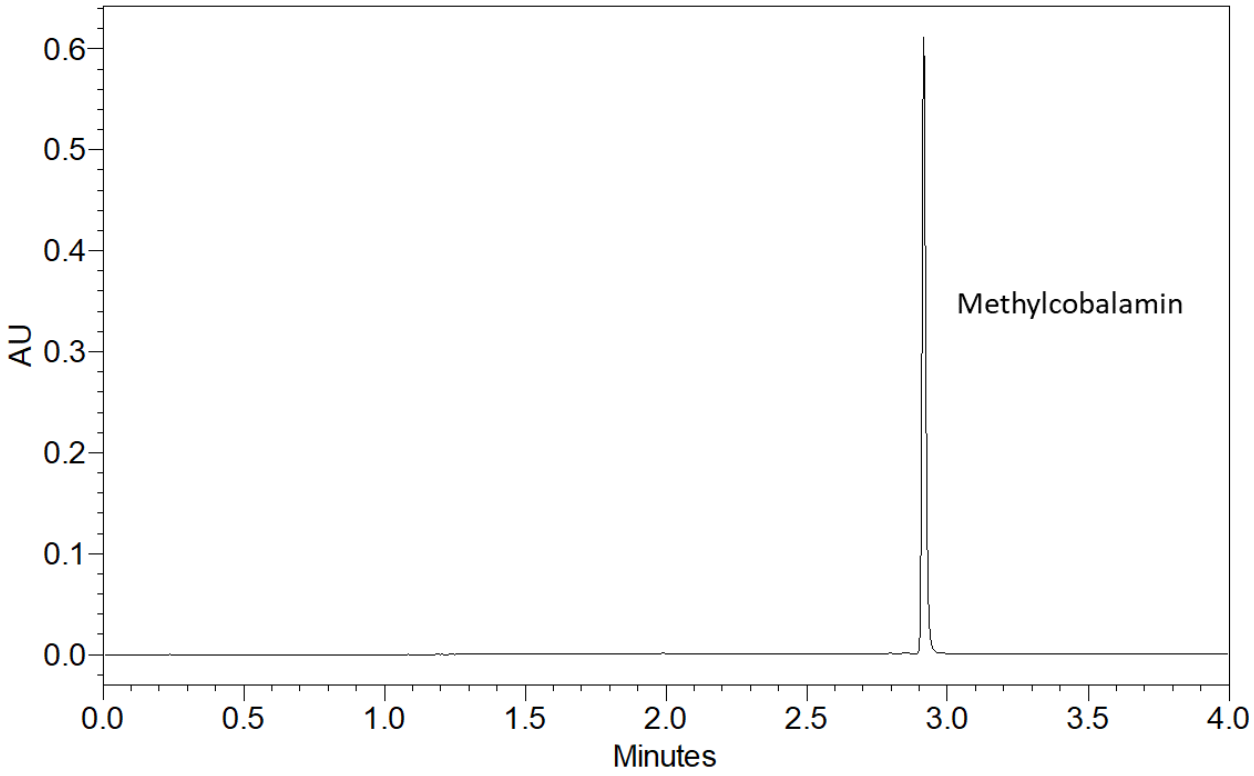
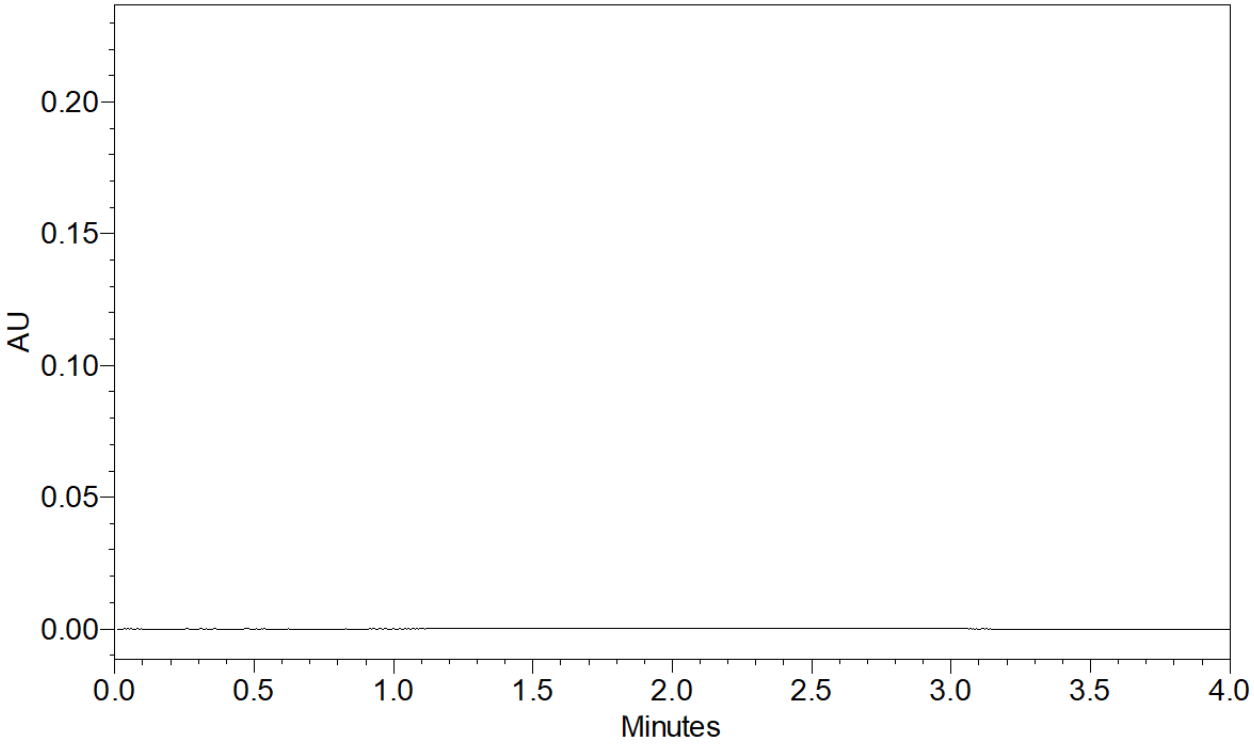


Figure 4: MCB UHPLC method chromatogram of the placebo, prepared at the target concentration of the method.



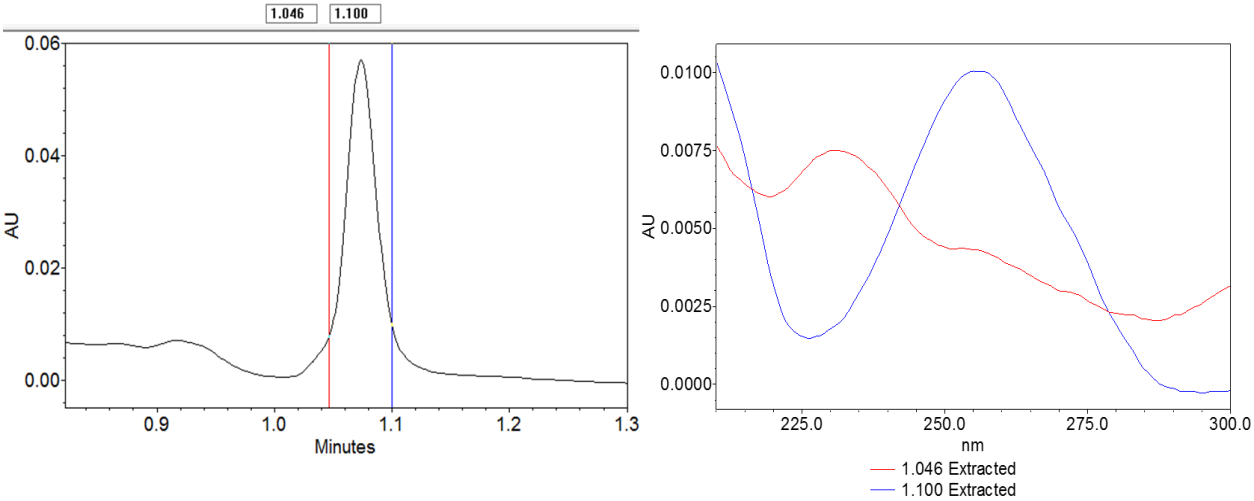
Forced Degradation

Forced degradation was performed on the sample to obtain data on any potential degradant that might interfere with the MCB UHPLC method and ultimately the method's stability indicating ability. Samples exposed to 80˚C heat and UV light were individually prepared and placed into an amber serum vial and quartz vial, respectively, and sealed with a rubber and aluminum crimp cap. Samples exposed to chemical degradation where individually prepared with the addition of the corresponding chemical (1 N HCl, 1 N KOH, and 30% H_2O_2). Ideally, there would have been 10-50% degradation of each active to simulate what might actually occur during a stability study. Minimally, 5-10% loss would mimic the end of the stability study when there may be a 10% loss compared to the starting potency value. Highly stable compounds might not break down at all, thus indicating they would not be expected to break down during the actual stability study.

After being forcibly degraded, each peak was checked for purity to make sure no degradation peak would interfere with accurate quantitation. The purity of the chromatographic peaks were verified using a two part procedure. The first part of the procedure is a mass balance technique called percent recovery, which is completed by spiking the degraded sample with a known amount of standard and then calculating the percent recovered by the assay. The second part is done by overlaying the UV spectrum of the analyte peak at the leading and trailing edges. If the UV spectra from leading and trailing edges overlaid match, the peak could be considered pure and therefore can be quantified accurately. The comparison of the UV spectra is more accurate than just spiking with standards and checking for recovery because a small interference (<5%) can be determined by UV spectra comparison, but easily overlooked as instrument error with the spiking technique.

For illustration purposes, an unknown peak was found in an unrelated method and contains a very small amount of interference. This illustration is shown in Figure 5 to demonstrate the high degree of sensitivity this technique has for detecting interference, even when the interference is estimated to be only 1-2%. Note, that even a small amount of impurity resulted in a significant spectral variance. When all peaks have proven to be pure by UV spectra comparison and the percent recovery is within specification, then the method is considered to be stability indicating.

Figure 5: Illustration of UV spectra comparison showing an impure peak obtained from an unrelated analysis.



Forced Degradation Results

The amount of degradation observed during the degradation study is summarized in Table 3. Samples were exposed to degradation conditions for fifteen minutes to twenty-four hours. Moderate degradation was observed under all degradation conditions; with UV light and 80˚C heat exposure observed to produce the most degradation in the shortest amount of time. An overlay of the UHPLC chromatograms of the samples which underwent chemical forced degradation is shown in Figure 6, while the heat and light forced degradation chromatograms are shown in Figure 7. The slight peak shift in the sample peak, from the sample exposed to oxidic conditions, is due to the sample being analyzed on a different day. The retention specificity of the sample analyte peak correlates with methylcobalamin standard calibrated for that sample sequence. As shown, each of the peaks for methylcobalamin are well separated from degradant or preservative peaks.

Table 3: Percent degradation of sample from forced degradation: 10% or more is desired.

Active Component	% Degraded in 80°C Heat for 2 hours	% Degraded in UV Light for 15 minutes	% Degraded in Peroxide for 1 day	% Degraded in Acid for 5 hours	% Degraded in Base for 5 hours
Methylcobalamin	37%	21%	31%	16%	15%

The first procedure to determine peak purity was accomplished by spiking the degraded samples with a known amount of methylcobalamin standard. After analyzing the spiked and non-spiked samples, the percent recovery was calculated and the results are shown in Table 4. Ideally, there should be a 100% mass balance recovery of the amount of standard spiked into the forced degradation samples. If the recovery is greater than 100%, it may indicate there is some other compound underneath the peak(s) producing an interference; if less than 100%, it would indicate no interference, but some loss of recovery due to degradation of the standard. A reasonable error of 5% should be factored in to account for method variability. Note that no percent recovery exceeds 100%, thus illustrating that no interference was observed in the degraded samples.

Table 4: Percent recovery of standard spiked into degraded samples (peak purity checked by mass balance).

Active Component	% Recovery in 80°C Heat after 2 hours	% Recovery in UV Light after 15 minutes	% Recovery in Peroxide after 1 day	% Recovery in Acid after 5 hours	% Recovery in Base after 5 hours
Methylcobalamin	99%	98%	96%	96%	99%

The second procedure to determine peak purity was accomplished by overlaying the UV spectra of the analyte peaks specifically at the leading and trailing edges of the peak chromatogram. Figure 8 is an example of this technique, showing a chromatogram of the methylcobalamin peak with vertical lines on leading and trailing edges of the peak where the UV spectra was extracted and overlaid. Figure 8a and c are the chromatogram and corresponding UV spectra of the sample post fifteen minute light degradation and Figure 8b and d are the chromatogram and corresponding UV spectra of the sample post five hour acid degradation. The results of the UV spectral analysis for leading and trailing edges of the active peaks in the chromatograms are in Table 5.

Table 5: Results of the UV spectral analysis of leading and trailing edges of the active(s) in the chromatograms to determine peak purity of degraded samples. No interferences are present when the UV spectra match.

Active Component	Degraded in 80°C Heat	Degraded in UV Light	Degraded in Peroxide	Degraded in Acid	Degraded in Base
Methylcobalamin	No Interference	No Interference	No Interference	No Interference	No Interference

Figure 6: Overlay of three MCB UHPLC method chromatograms after samples underwent chemical forced degradation for five hours up to one day in (a) peroxide, (b) acid, and (c) base.

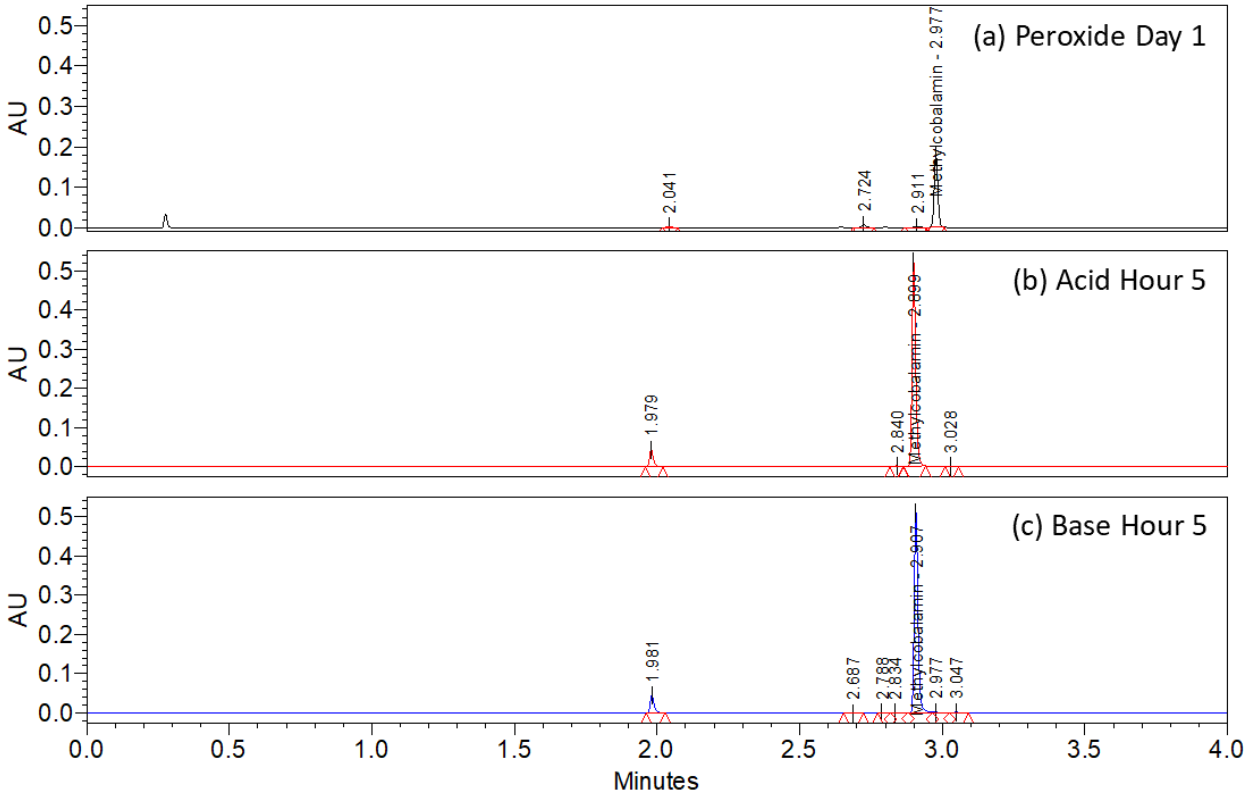


Figure 7: Overlay of two MCB UHPLC method chromatograms after samples underwent (a) heat and (b) UV light forced degradation for fifteen minutes to two hours.

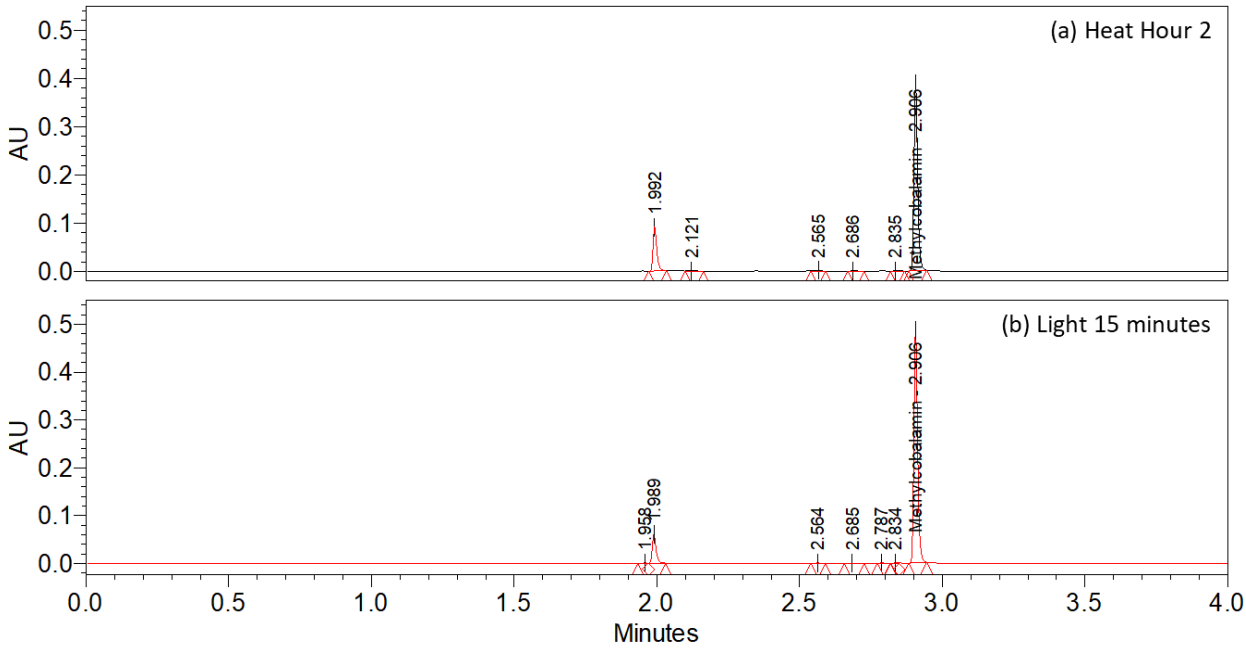
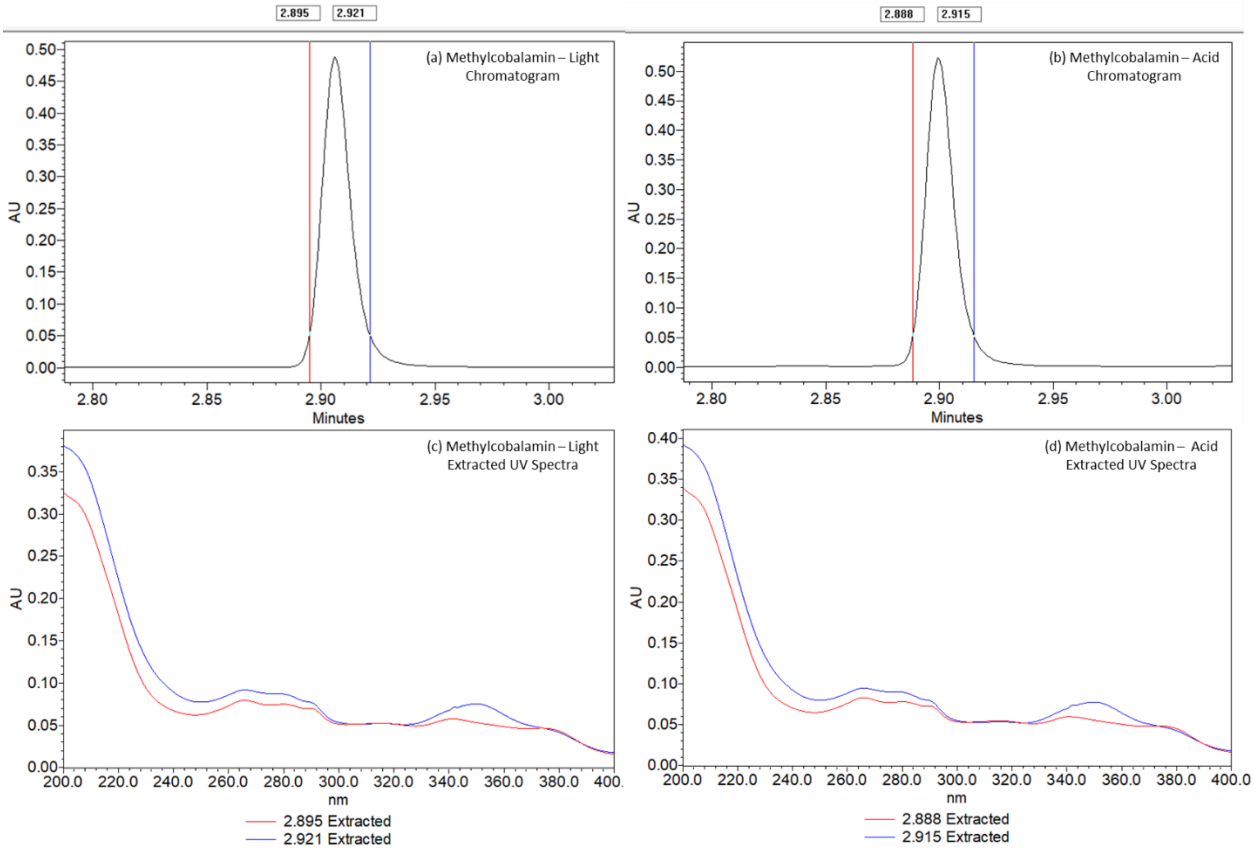


Figure 8: MCB UHPLC method chromatogram and corresponding extracted UV spectra of methylcobalamin after fifteen minutes of degradation in UV light (a and c) and after five hour in acid (b and d). Vertical lines indicate points on the peak where the UV spectrum were taken to illustrate peak purity.



Method Validation

According to USP <1225>, "Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications." It is important to validate the established stability indicating method used to measure the potency of the compounded formulation, so that we can be confident in the data. The following laboratory tests are recommended to meet USP criteria with the ones in *italics* required:

1. *Specificity*
2. *Accuracy*
3. *Precision (Repeatability)*
4. Ruggedness and Robustness
5. *Linearity, LOQ, LOD, Range*
6. System Suitability

Specificity

The most important part of the assay is to ensure that it is specific to the compound being analyzed and there are no interferences that could cause an artificially high/low result. The USP, using the ICH definition, defines specificity as, "The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components." As shown previously, in the forced degradation results, this criterion was met by matching retention time and UV spectrum of a standard(s) with the sample(s). Furthermore, percent recovery demonstrated that a known amount could accurately be recovered from the degraded matrix. It was determined that this method identified the active ingredients properly and no impurities, degradation products, or matrix components interfered with the peaks of interest.

Accuracy

The second most important part of the assay is to make sure that the value being reported by the method matches the true value. The accuracy of the method was assessed by spiking an analytical standard into the sample matrix at four different levels and injected in triplicate. The percent recovery was then calculated, along with the %RSD and 95% confidence interval. The results are shown below in Table 6. All recoveries were within the 95-105% limitations.

Table 6: Accuracy of the MCB UHPLC method at four different levels shown by percent recovery of certified reference standard.

Accuracy Name	Active Component	% of Nominal Conc.	%RSD n = 3 inj.	Average +/- 95% Confidence Interval, n = 3 inj.
Accuracy A:	Methylcobalamin	49%	0.17%	99.1% +/- 0.43%
Accuracy B:	Methylcobalamin	100%	0.10%	100.9% +/- 0.25%
Accuracy C:	Methylcobalamin	119%	0.12%	99.6% +/- 0.29%
Accuracy D:	Methylcobalamin	148%	0.12%	98.9% +/- 0.29%

Precision

In order to ensure the results are repeatable, the precision of the method is evaluated. This is referred to as inter-assay precision. Samples were prepared according to the method procedure, but diluted to three different concentration levels and prepared in triplicate. Each sample was injected three times. Table 7 shows the summary of the precision for the quantitation of the actives using the SIA method. The percent relative standard deviation and 95% confidence interval were calculated on the averages of the three injections of different sample preparations. From these reported values, we can conclude the method is precise and highly reliable for the determination of potency values.

Table 7: Precision of the MCB UHPLC method at three different dilution levels and prepared in triplicate.

Active Component	Concentration Level	%RSD n = 3 samples with 3 inj. each	Average +/- 95% Confidence Interval, n = 3 samples with 3 inj. each
Methylcobalamin	50%	0.75%	102.9% +/- 1.93%
Methylcobalamin	100%	0.34%	103.4% +/- 0.88%
Methylcobalamin	250%	0.21%	104.4% +/- 0.53%

Ruggedness

A second criteria to evaluate the repeatability of the method is called ruggedness or intermediate precision. The objective is to verify that the same laboratory will provide similar results on different days, with different analysts, equipment, and/or columns. The validated method was tested for ruggedness by analyzing it on three different UHPLC systems with three different analysts. Each Acquity UHPLC instrument was equipped similarly with gradient pumping capabilities, automatic injection systems, column heating compartments, photodiode array detectors, and computer integration systems, but were different models with different internal volumes, which could potentially make a difference in retention times of the peaks and in their resolution. The same type of column was used, but they were from different lots. Each analyst made their own mobile phase using the same solvents from the same lots. Acceptable reproducibility was achieved between systems and the results are summarized in Table 8.

Table 8: Method Ruggedness of the MCB UHPLC method using three different instruments to quantitate potency of the validation sample prepared at the target concentration.

Instrument	Active Component	Average of % Label Claim n = 3 inj.	%RSD n = 3 inj.	%RSD n = 3 samples with 3 inj. each	Average +/- 95% Confidence Interval, n = 3
Instrument A	Methylcobalamin	101.8%	0.11%	0.31%	101.4% +/- 0.77%
Instrument B	Methylcobalamin	101.2%	0.06%		
Instrument C	Methylcobalamin	101.3%	0.21%		

Method Robustness

During the course of the method development and validation study the method was evaluated for robustness. Method robustness is the ability for the analytical procedure to remain unaffected by small variations of the method parameters. To test the robustness of the method, small deliberate changes in the analytical procedure were made and the %RSD of the calculated potency was calculated for the variables. The method remains unaffected by the change if the %RSD is less than 3%. Table 9 provides the summary of the all of the changes and variables evaluated with corresponding %RSD of calculated potency. The effects of using glass vials, plastic vials, or filtering was tested on the standard solution. For the subsequent tests the sample prepared at the 100% level was used. These tests include varying injection volume, processing the data at higher and lower wavelengths, varying the method of mixing in sample preparation, and determining the effect increasing or decreasing the buffer concentration. As shown, none of the changes evaluated affect the method performance.

Table 9: Effect of small variations of method parameters to determine the robustness of the MCB UHPLC method.

Method Change Description	Variables Evaluated	Methylcobalamin %RSD of Method Changes
Effect of Plastic Vials or Filtering	Glass Vials, Plastic Vials, Filtered into Glass Vial	0.5%
Effect of Injection Volume	0.5 µL, 1.0 µL, and 2 µL	1.0%
Effect of Processing Wavelength	Wavelength Varied	0.1%
Effect of Mixing Method	Vortex and Sonicated up to Three Minutes	0.6%
Effect of Buffer Concentration	+/- 20% Buffer concentration	0.22%

Linearity, Range, LOD and LOQ

The parameters, linearity, range, limit of detection (LOD), and limit of quantitation (LOQ) are used to further evaluate the accuracy and sensitivity of the method. These results are provided below in Table 10. Range provides the suitable lower and upper limits of concentration where the SIA method is most accurate. The data collected within this range should display a linear response relationship. The coefficient of determination (R^2) value of 0.999 or greater indicate the method is linear within the given range, therefore providing accurate quantification for such samples. The LOD is the concentration at which the presence of an active ingredient can be detected but not accurately quantified, while the LOQ is the lowest concentration that the active ingredient can be accurately quantified. The plot of the data used to obtain the equation of line and coefficient of determination is shown in Figure 9.

Table 10: Sensitivity and linearity for stability indicating method validation of the MCB UHPLC method.

Active Component	Lower Limit of Detection (mg/mL)	Lower Limit of Quantitation (mg/mL)	Range Plotted (mg/mL)	Coefficient of Determination R^2 value	Equation of Line
Methylcobalamin	0.00002	0.002	0.002 to 0.4	0.999870	y = 2665820x - 3768

Another way to look at the linear range of each method is to plot the percent recovery of the diluted standard versus the concentration of the diluted standard. Parallel horizontal lines are drawn on the plot to correspond to 95% and 105% recovery. Where the data exceeds these limits, the method is no longer linear. This data is plotted in Figure 10 which shows that methylcobalamin is linear across a very broad range. At the lowest concentrations the data of each injection is more scattered because it is at or below the limit of quantitation. While it is expected at concentrations higher than 0.4 mg/mL for methylcobalamin the method would significantly deviate from linearity.

Figure 9: Linearity plot used to calculate the equation of the line and coefficient of determination (R^2) of the MCB UHPLC method.

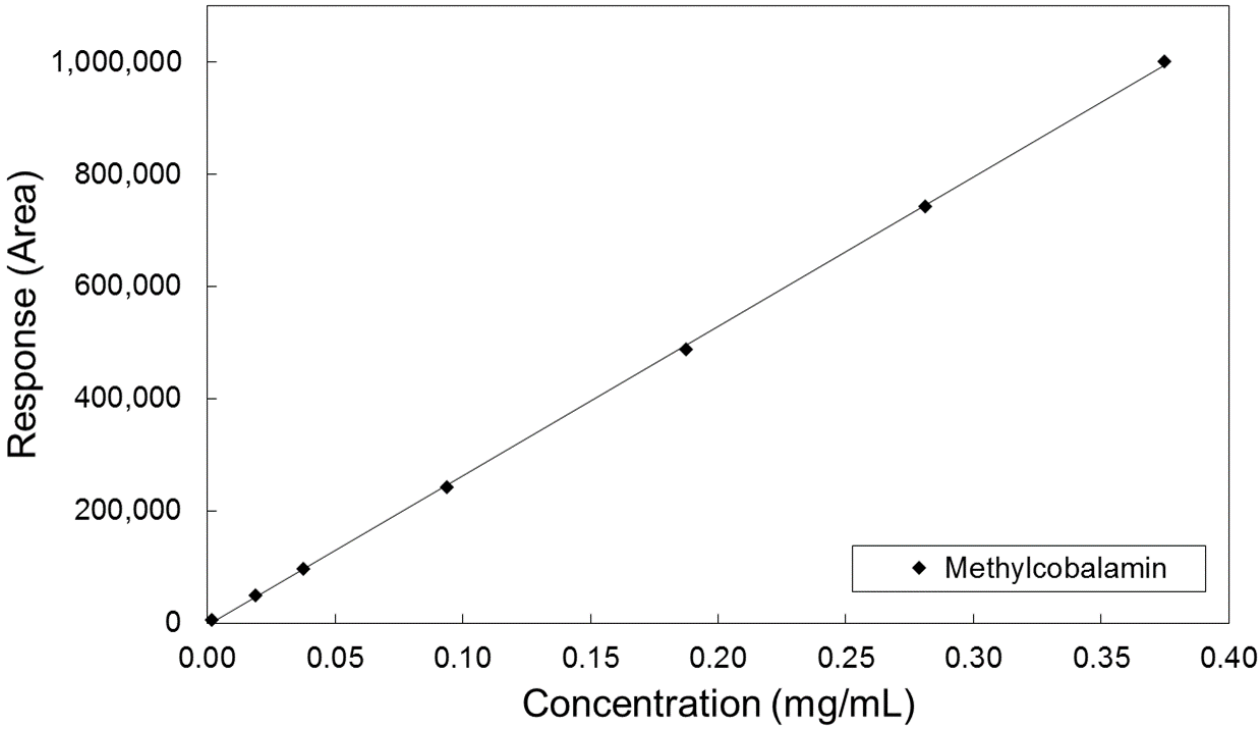
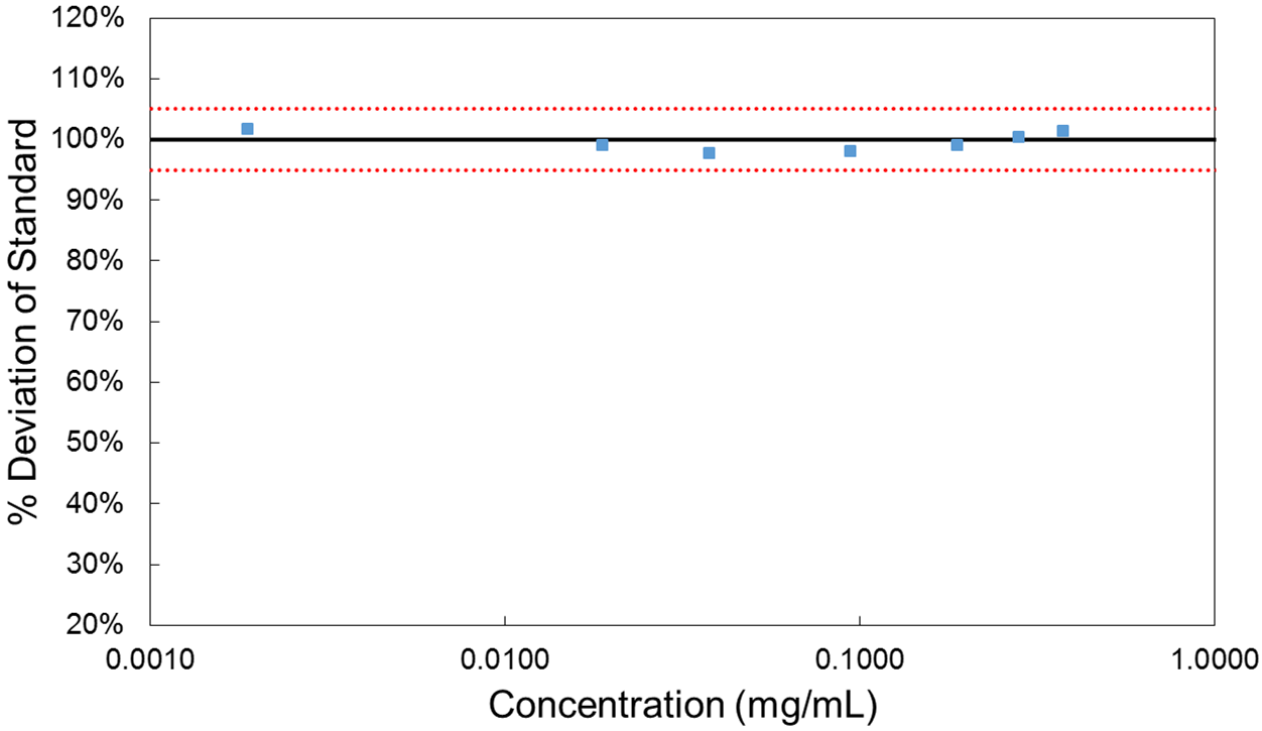


Figure 10: Plot of the percent recovery of a methylcobalamin standard diluted at wide range of concentrations for the MCB UHPLC method. Parallel horizontal lines correspond to 95% and 105% recovery.



System Suitability

The chromatographic separation obtained on the UHPLC system was checked for its ability to accurately quantify the samples being tested. For six replicate standard injections, the %RSD of the area was calculated along with the average peak retention (k'), peak symmetry (USP tailing), and efficiency (theoretical plates). For a typical sample, the average peak resolution was calculated to account for any inactive or impurities present in a sample. These system suitability results are shown in Table 11 and all are within USP/ICH recommended limits.

The %RSD of the area of the six standard injections is less than the target of <2.0% and indicates the method meets injection repeatability requirements. The target of >2 k' retention indicates the peaks are very well retained on the column and could be expected to provide ideal separation from possible breakdown products during the stability study. In general, as retention increases, resolution will improve. The peak symmetry (referred to as USP tailing) of <2.0 indicates the peaks have very little tailing or fronting, therefore being symmetrical and having a reduced possibility of interference during the assay, which provides for increased confidence in accuracy and precision. The efficiency value or USP plate count is a measure of peak sharpness. Our value of theoretical plates indicates the peaks are very sharp and therefore are able to provide better separations with reduced likelihood of interferences during the study. The target of >1.5 resolution would indicate the peak was at least baseline separated from its neighboring peak. The resolution values that were obtained on the sample indicate the peaks were well separated and therefore would be accurately quantified from any impurities present in the formulation.

Table 11: System Suitability results of the MCB UHPLC method using six standard injections. USP resolution was calculated on a sample prepared at the target concentration.

	Six Replicate Standard Injections				Sample Diluted to Method Target
Active Component	%RSD Area (Target < 2.0)	Retention k' (Target >2.0)	USP Tailing (Target <2.0)	Efficiency USP Plate Count (Target >2000)	USP Resolution on Sample (Target >1.5)
Methylcobalamin	0.09%	10.719	1.256	237592	141.8

Physical Characteristics Results

The container used for the compounded MCB injectable formulation for the forced degradation and SIA study were 30 mL amber glass serum container with rubber stopper and aluminum crimp cap. The injectable formulation was a transparent dark red liquid and did not change noticeably over the course of the validation testing. A picture of the sample is shown in Figure 11.

Figure 11: Customer sample container used for forced degradation and SIA testing (left) and the placebo (right).

Conclusion

The data within this report demonstrates the method used to quantify the potency has been validated. The method was shown to be stability indicating through forced degradation of the sample along with other validation parameters. The SIA can be used for a variety of samples prepared with this formulation. For the compounded methylcobalamin (MCB) injectable formulation received from McGuff Compounding Pharmacy Services, Inc., please reference the appropriate time point stability study for beyond use dating. All samples containing these active and inactive ingredients received from McGuff Compounding Pharmacy Services, Inc. will be analyzed using the validated SIA for any category of testing.

Compounder's International Analytical Laboratory
Better Quality Through Quality Testing

4760 Castleton Way
Castle Rock, CO 80109
800-788-9922 Toll Free
303-471-8015 Phone
303-569-6101 Fax
lab@compounderslab.com

Certificate of Analysis

McGuff Compounding Pharmacy Services, Inc.
2921 W. MacArthur Blvd., Suite 142
Santa Ana, CA 92704

Formulation: Methylcobalamin MD, Injection
Batch Size: Not Disclosed
Storage Temp: Room Temp

Date Received: Monday, March 9th 2020
CIAL Tracking #: MCG 030920 3.1
Customer Lot Number #: 20B1971:081220

Amount Received: 15 Vial(s) , 15.00 mL
Condition of Sample: Good

Potency Time Point Testing

Time Point/ Scheduled	Test Date	Active(s)	Label Claim	Test Method	Acceptance Criteria	Results: Amount Found	Results: % Label Claim	Pass/Fail
Baseline	03/10/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.075 mg/mL	101.5%	Pass
	Comments: *Meets USP potency requirements*							
Day 30								
	04/10/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.149 mg/mL	103.0%	Pass
Day 60								
	05/08/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.101 mg/mL	102.0%	Pass
Day 90								
	06/12/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.138 mg/mL	102.8%	Pass
Day 120								
	07/08/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.202 mg/mL	104.0%	Pass
Day 180								
	09/11/2020	Methylcobalamin	5 mg/mL	UHPLC	90.0-110.0%	5.189 mg/mL	103.8%	Pass

Special Testing

Test Date	Test	Test Method	Acceptance Criteria	Results	Pass/Fail
03/16/2020	Particulates	<788><789>	<788><789>	See Attached Report	Pass
Pass - 330 ,â• 10 ¬µm ,â§ 6000/container AND 13 ,â• 25 ¬µm ,â§ 600/container					

Laura Wester - Quality Approval

Wednesday, September 16th 2020
Date Signed

CONFIDENTIAL **McGuff Compounding Pharmacy Services, Inc.**		
Document Type	**Qualification Protocol**	Addendum #
Study #	**QD213004**	N/A
Title	**Qualification of Pharmacy Compounding Component: Basis for Use of Choline Chloride**	**Pages 1 of 9**

1. Purpose

To evaluate the use of Choline Chloride bulk drug substance by McGuff Compounding Pharmacy Services, Inc. (MCPS) for sterile compounding.

2. Scope

This qualification protocol applies to the screening/testing of incoming Choline Chloride bulk drug substances prior to release for sterile compounding use.

3. Background

Choline Chloride is a bulk drug substance that is listed on the Food & Drug Administration's (FDA) "Category 1" list (see Appendix A) which permits Choline Chloride to be use for compounding while the FDA evaluates and finalizes the final list of bulk drug substances that may be used for compounding by 503A pharmacies. Choline Chloride bulk drug substance has been identified as a concern for not meeting "pharmaceutical" standards since it typically available as a USP dietary supplement. USP and federal compounding guidance do not have a definition or specifications for what constitutes as pharmaceutical grade for bulk drug substances. In response to the FDA's concerns, MCPS implemented a screening program for the bulk drug substances of concern to ensure various impurities are within the limits specified by USP standards.

The following material qualification plan shall be used to establish ongoing quality assurance for each incoming manufacturer lot of Choline Chloride received and beyond information supplied by the supplier's certificate of analysis.

4. References

FRM-0050 Component History Form

FRM-0138 Pharmacy Raw Material Specification & Inspection Requirements Form

5. Materials and Equipment

310-0056 Choline Chloride, CAS # 67-48-1

6. Definitions

6.1. United States Pharmacopeia (USP): A reference compendium of standardized drugs and other articles published by The United States Pharmacopeia.

7. Sample Size and Preparation

Not applicable

8. Qualification Method

8.1. Applicable USP Chapters

As part of the material qualification plan, each incoming supplier lot of Choline Chloride bulk drug substance will be screened as noted below and per Choline Chloride Raw Material Acceptance Criteria form (see Appendix B). The raw material acceptance criteria form will be attached to the corresponding Choline Chloride PRMSIR form (FRM-0138). Information on the supplier's certificate of analysis (CoA) or documented statement may be used to satisfy specifications noted below.

1) USP <85> Bacterial Endotoxin Tests
    a. Screening per PRMSIR current addendum form titled, "Endotoxin Screening Requirement," for PN# 310-0056, Choline Chloride (see Appendix C)

2) USP <232> Elemental Impurities – Limits (for injections)
    a. Screening of each received manufacturer lot of Choline Chloride bulk drug substance for the following elemental impurities:
        i. Arsenic
        ii. Lead
        iii. Mercury
        iv. Cadmium

3) USP <467> Residual Solvents

4) USP <1229.3> Bioburden

5) Verification of vendor's any specifications noted on the CoA as deemed necessary

All test results will be included as part of the Component History Form (FRM-0050) for the received lot of Choline Chloride.

8.2. Manufacturer/Vendor Audit

As part of the qualification process, MCPS will perform an audit of the manufacturer/supplier of Choline Chloride bulk drug substance to verify the vendor is in good cGMP standing with the FDA as a registered drug establishment or chemical supplier. Such an audit may include a remote survey of the vendor's cGMP standing or, if practical, an onsite inspection. MCPS's Quality Systems will review the vendor's cGMP profile annually. All audits/reviews will be documented and filed according to MCPS's quality systems procedures.

9. Appendices:

Appendix A: FDA Category 1 List for 503A Compounders

Appendix B: Choline Chloride, Raw Material Acceptance Specifications

Appendix C: Endotoxin Screening Requirement

Appendix D: Vendor Audit/Qualification

Appendix A

FDA Category 1 List for 503A Compounders

Updated July 1, 2020

503A Category 1 – Bulk Drug Substances Under Evaluation

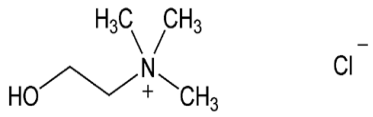
- 7 Keto Dehydroepiandrosterone
- Acetyl L Carnitine/Acetyl-L- carnitine Hydrochloride
- Alanyl-L-Glutamine
- Aloe Vera/ Aloe Vera 200:1 Freeze Dried
- Alpha Lipoic Acid
- Artemisia/Artemisinin
- Astragalus Extract 10:1
- Boswellia
- Choline Chloride
- Chondroitin Sulfate
- Chrysin
- Coenzyme Q10
- Creatine Monohydrate
- Curcumin
- Deoxy-D-Glucose
- Dichloroacetate
- Diindolylmethane
- Dimercapto-1- propanesulfonic acid (DMPS)
- EGCg
- Ferric Subsulfate
- Glutaraldehyde
- Glutathione
- Glycolic Acid
- Glycyrrhizin
- Kojic Acid
- L-Citrulline
- Melatonin
- Methylcobalamin
- Methylsulfonylmethane (MSM)
- Nettle leaf (Urtica dioica subsp. dioica leaf)
- Nicotinamide Adenine Dinucleotide (NAD)
- Nicotinamide Adenine Dinucleotide Disodium Reduced (NADH)
- Pregnenolone
- Pyridoxal 5-Phosphate Monohydrate
- Pyruvic Acid
- Quercetin/Quercetin Dihydrate
- Quinacrine Hydrochloride (except for intrauterine administration)
- Resveratrol
- Ribose (D)
- Rubidium Chloride
- Tea tree oil (Melaleuca alternifolia leaf oil)
- Trichloroacetic Acid
- Ubiquinol 30% Powder
- Vanadium
- Vasoactive Intestinal Peptide

Appendix B

Choline Chloride, Raw Material Acceptance Specifications

Confidential
McGuff Compounding Pharmacy Services, Inc.

Choline Chloride, Raw Material Acceptance Specifications – Potency & Impurities
CAS # 67-48-1



$C_5H_{14}ClNO$
M.W. 139.62

Characteristics	Test Method	Specifications
Assay (anhydrous)	USP Monograph	99.0 – 100.5%
1,4 Dioxane	USP Monograph	10 ppm
Residual Solvents	USP <467>	All residual solvents used in manufacturer's process must be below USP <467> limits
As, Pb, Cd, Hg Elemental Impurities	USP <232>	Arsenic ≤ 1.5 ug/gm, Lead ≤ 0.5 ug/gm, Cadmium ≤ 0.2 ug/gm, and Mercury ≤ 0.3 ug/gm
Endotoxin	USP <85> Gel Clot	≤ 0.093 EU/mg
Bioburden	USP <1229.3> Monitoring of Bioburden	< 10 CFU per container type

Appendix C

Endotoxin Screening Requirement

Raw Material Description: Choline Chloride
McGuff CPS PN#: 310-0056

Procedure

1. All **new** incoming lots will follow the sampling plan as specified below:

Container sizes ≤ 1 Kilogram

# of Containers Received	# of Endotoxin Test Sample(s) To Be Collected
1	1 sample (1.5 gm)
2	2 samples (1.5 gm each container)
3 or more	3 samples (1.5 gm, 3 separate containers)

Container sizes > 1 Kilogram

# of Containers Received	# of Endotoxin Test Samples To Be Collected
1	3 samples (1.5 gm top, middle, & bottom layers of container)
2	6 samples (1.5 gm top, middle, & bottom layers of each container)
3 or more	9 samples (1.5 gm, top, middle, & bottom layers of 3 separate random containers)

2. All received containers will be physically quarantined for further processing pending the QC test results of the endotoxin screening. Material sampled and is pending lab test results will have a "QUARANTINE" label applied to the exterior of the container.
    a. If endotoxin assay sample(s) passes specification, then process the quarantined material for release per receiving procedure.
    b. If endotoxin assay sample(s) fails specification, then the designated personnel (e.g. pharmacist) will initiate a Material Review Board investigation for proper disposition of the received material.

Appendix D

Vendor Audit / Qualification

Supplier/Manufacturer Checklists

May 21, 2021

MERCK HEALTHCARE KGaA	YES	NO
Does the establishment have a current FDA registration as a drug establishment?	√	
Is there any import alert to the establishment in the past 12 months?		√
Did the establishment receive any FDA 483 or warning letter in the past 12 months?		√
Miscellaneous		

Drug Establishments Current Registration Site

New Search (default.cfm)

Search Results for **Merck**

CSVExcel

Filter:

Firm Name	FDA Establishment Identifier	DUNS	Business Operations	Address	Expiration Date
Merck & Cie	3002806918	485528488	ANALYSIS; API MANUFACTURE; LABEL; PACK;	Im Laternenacker 5, Schaffhausen, 8200, Switzerland (CHE)	12/31/2021
Merck & Cie	3004369363	480016906	ANALYSIS; API MANUFACTURE; LABEL; PACK;	Weisshausmatte, Altdorf, 6460, Switzerland (CHE)	12/31/2021
Merck Biodevelopment	3009563840	260400248	ANALYSIS; API MANUFACTURE;	1 rue Jacques Monod, Martillac, Gironde 33650, France (FRA)	12/31/2021
Merck Healthcare KGaA	3016570199	314174946	ANALYSIS; LABEL; MANUFACTURE; PACK;	Frankfurter Str. 250, Darmstadt, 64293, Germany (DEU)	12/31/2021

McGuff Compounding Pharmacy Services, Inc.
Stability Study Report Form

Description: _Methionine-Inositol-Choline CL 25/50/50 mg/mL MD_ Part Number: 390-0645__

Assigned BUD: _90 days_ Storage Conditions: _RT 15 – 30 deg C (59 – 86 deg F)

Study Approved by: ___Si Pham, Pharm.D., Pharmacist-in-Charge_____

Data / Results Table			
Characteristic	Test Method	Result at T_0	Result at T_{BUD}
		See specific lot test results	Post BUD or See specific lot test results
Appearance, Seal	Visual	Lot 15D2181 Passed	Lot 15D2181 Passed
Appearance, Vial	Visual	Lot 15D2181 Passed	Lot 15D2181 Passed
Appearance, Product	Visual	Lot 15D2181 Clear, colorless	Lot 15D2181 Clear, colorless
Foreign Matter, Visible Particulate	M370-0004 or per Current USP <790>	Lot 15D2181 No visible ppt	Lot 15D2181 No visible ppt
pH [4 -7]	McGuff Method # M370-0001	Lot 15D2181 5.6	Lot 15D2181 5.7
Assay [M = 22.5 – 27.5 mg/mL I =45 – 55 mg/mL C = 45 -55 mg/mL]	[HPLC] Record method	Lot 15D2181 M = 22.7 mg/mL I = 48.2 mg/mL C = 49.9 mg/mL	Lot 15D2181 M = 24.7 mg/mL I = 47.9 mg/mL C = 47.9 mg/mL
Sterility	Current USP Chapter <71> or M370-0011	Lot 15D2181 Sterile Initial	Lot 15D2181 Sterile BUD
Or, in lieu of Sterility Container/Closure Integrity	M370-0080 or BTS Method # CM413 (dye ingress) or M370-0021 / TM-0019 (seal integrity)	Lot 18J0091 Passed CCI [Initial]	Lot 17M3741 Passed CCI [BUD]
Endotoxin test [I/E] Method Suitability Test	Current USP Chapter <85>	Lot 15A2481 Passed	NA
Sterility test [B/F] Method Suitability Test	Current USP Chapter <71>	Lot 14F2221 Passed	NA
Preservative [AET] Effectiveness (for Multi Dose Vials)	Antimicrobial Effectiveness Current USP <51>	Lot 15D2181 Passed	NA
Preservative concentration (for Multi Dose Vials) [BA 80 – 120% of label claim, USP]	Current USP Chapter <341>	Lot 15D2181 1.14 % Initial	Lot 15D2181 1.11 % BUD
CHR for Study Lot Attached?		On file	

CONCLUSION: The assigned BUD x is / □ is not supported by the data gathered above.
(Check One)

Notes: Data from lots 14F2221, 15A2481, 17M3741, 18J0091 were used whenever appropriate to support BUD for formulation 390-0645. Stability Study conducted and Report prepared by Doug Tran, Pharm.D.

Reviewed and Approved by P.I. C.: S Pham 05-24-21
Sign and Date

Si Pham, Pharm.D. Pharmacist-in-Charge

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: M.I.C. + Supplements, 30mL

Part Number: 390-2566

Lot Number: 14F2221

Results

Test	Specification/Limit	Result
Bacteriostasis / Fungistasis Testing *01/13/15	Visible growth of microorganisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated.	Growth

Report Conclusion

Sample described above indicates no bacteriostasis / fungistasis properties per membrane filtration test at 5 x 100mL rinse of Fluid A.
Note: M.I.C.+ Supplements (2566) as of Aug 2014, formerly Custom Lipotrovite (2566) 30mL
*Completion date of testing

Signature and Date: 11·03·17
Laboratories Supervisor or designee

QA Signature and Date: 11/03/17
Quality Assurance

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: M.I.C. 30mL

Part Number: 390-0645

Lot Number: 15A2481

Results

Test	Specification/Limit	Result
Inhibition / Enhancement Testing Bacterial Endotoxin: Interfering factors test *02/19/15	No interfering factors at dilution tested	No interfering factors a dilution of 1:100

Report Conclusion

The sample indicates no interfering factors in the inhibition / enhancement for BET gel clot method when tested at a dilution below the maximum valid dilution. The sample was tested at a 1:100 dilution. The result shows no interfering factors at the dilution tested at 1:100. The sample has passed the interfering factors test section in USP <85>.
*Completion date of testing

Signature and Date: 11·03·17
Laboratories Supervisor or designee

QA Signature and Date: 11/03/17
Quality Assurance

McGUFF
PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: M.I.C. 30mL

Part Number: 390-0645

Lot Number: 15D2181

Results

Test	Specification/Limit	Result
Endotoxin *04/28/15	< 11.67 EU/mL	< 3 EU/mL
Sterility *05/05/15	No Growth / Growth 14 Day Incubation	No Growth
Sterility BUD *11/03/15	No Growth / Growth 14 Day Incubation	No Growth

*Completion date of testing

Signature and Date: 11.03.17
Laboratories Supervisor or designee

QA Signature and Date: 11/03/17
Quality Assurance

EAGLE ANALYTICAL SERVICES
A PCCA COMPANY

LABORATORY REPORT

6/18/2015

Doug Tran, Pharm.D.
McGuff CPS, Inc.
2921 West MacArthur Blvd., Ste. 142
Santa Ana, CA 92704
Tel:
Fax:

Client #: E00107
Sample: **M.I.C MD 25/50/50**
22.5/27.5MG/ML
Lot #: 15D2181:101415
Sample ID #: 357889
Date Rec'd: 6/9/2015

LABORATORY TEST RESULTS

Microbiological Tests:	**Date**	**Measured**	**Result**
Bacterial Endotoxin USP <85>	--		
Eagle Sterility Test	--		
Rapid ScanRDI Microbial Detection	--		

Chemical Tests:	**Date**	**Reported**	**Measured**	**Potency**
Benzyl Alcohol	6/18/2015	- --	1.14 %	- --
Choline Chloride	6/18/2015	- --	49.9 mg/mL	- --
Inositol	6/18/2015	- --	48.2 mg/mL	- --
Methionine	6/18/2015	- --	22.7 mg/mL	- --

Notes: These are the Initial Assay Results for lot 15D2181. [2015June19 DT]

USP <795> states: "…compound preparations are to be prepared to ensure that each preparation shall contain not less than 90% and not more than 110% of the theoretically calculated and labeled quantity of an active ingredient…". Potency is determinations follow USP <621> HPLC, USP<851> Spectrophotometry, and specific monograph testing procedures.

Respectfully submitted,
EAGLE ANALYTICAL SERVICES LTD.

William J. Zolner, Ph.D., Chief Scientific Officer

EAGLE ANALYTICAL SERVICES
A PCCA COMPANY

LABORATORY REPORT

10/28/2015

Doug Tran, Pharm.D.
McGuff CPS, Inc.
2921 West MacArthur Blvd., Ste. 142
Santa Ana, CA 92704
Tel:
Fax:

Client #: E00107
Sample: **M.I.C MD 25/50/50**
25/50/50/0.8%-1.2%
Lot #: 15D2181:101415BUD
Sample ID #: 368246
Date Rec'd: 10/21/2015

LABORATORY TEST RESULTS

Microbiological Tests:	**Date**	**Measured**	**Result**
Bacterial Endotoxin USP <85>	--		
Eagle Sterility Test	--		
Rapid ScanRDI Microbial Detection	--		

Chemical Tests:	**Date**	**Reported**	**Measured**	**Potency**
Benzyl Alcohol	10/28/2015	-	1.11 %	-
Choline Chloride	10/28/2015	50 mg/mL	47.9 mg/mL	95.8 %_
Inositol	10/28/2015	50 mg/mL	47.9 mg/mL	95.8 %_
Methionine	10/28/2015	25 mg/mL	24.7 mg/mL	98.8 %_

Notes: The BUD for this Lot 15D2181 is Oct 14, 2015. These are the Assay Results 14 days post the BUD. [2015Oct29 DT]

USP <795> states: "…compound preparations are to be prepared to ensure that each preparation shall contain not less than 90% and not more than 110% of the theoretically calculated and labeled quantity of an active ingredient…". Potency is determinations follow USP <621> HPLC, USP<851> Spectrophotometry, and specific monograph testing procedures.

Respectfully submitted,
EAGLE ANALYTICAL SERVICES LTD.

William J. Zolner, Ph.D., Chief Scientific Officer

BioScreen® Testing Services, Inc.

3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043 • Fax(310) 370-3642
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

MICROBIOLOGICAL REPORT

McGuff Compounding Pharmacy Services, Inc.
Attn: Doug Tran
2921 West MacArthur Blvd., Suite 142
Santa Ana, CA 92704

Page 1 of 2

Report Date: 09/02/15
Date Received: 06/09/15
Date Completed: 08/25/15
Project #: 903630
P.O. #: 19318
Reference #: 9575-003; 9489-057

SAMPLE DESCRIPTION

ACCESSION #	SAMPLE:	LOT #:	BATCH #:	QTY:
903630	M.I.C. 25/50/50 Multiple Dose, 390-0645	15D2181:101415	15D218	4x30mL

TEST PERFORMED:	BTS METHOD #	REFERENCE:
Validation Test for Antimicrobial Preservative Effectiveness Test	M115.R13	United States Pharmacopeia **38,** <51>
Antimicrobial Effectiveness Test (Category 1)	M101.R14	United States Pharmacopeia **38,** <51>

PLATING MEDIA:
Microbial Content Test Agar (Bacteria)
Sabouraud Dextrose Agar (Yeast and Mold)

ANTIMICROBIAL PRESERVATIVE EFFECTIVENESS VALIDATION TEST

Test Microorganism		Diluent	Dilution	Inoculum CFU/plate	Microbial Recovery CFU/plate	Percent Recovery
Aspergillus brasiliensis	(mold)	DNB	1:10	18	17	94
Aspergillus brasiliensis	(mold)	DNB	1:100	18	17	94
Aspergillus brasiliensis	(mold)	DNB	1:1000	18	17	94
Candida albicans	(yeast)	DNB	1:10	126	130	103
Candida albicans	(yeast)	DNB	1:100	126	139	110
Candida albicans	(yeast)	DNB	1:1000	126	137	109
Escherichia coli	(bacteria)	DNB	1:10	122	145	119
Escherichia coli	(bacteria)	DNB	1:100	122	135	111
Escherichia coli	(bacteria)	DNB	1:1000	122	108	89
Pseudomonas aeruginosa	(bacteria)	DNB	1:10	116	148	128
Pseudomonas aeruginosa	(bacteria)	DNB	1:100	116	146	126
Pseudomonas aeruginosa	(bacteria)	DNB	1:1000	116	112	97
Staphylococcus aureus	(bacteria)	DNB	1:10	87	89	102
Staphylococcus aureus	(bacteria)	DNB	1:100	87	73	84
Staphylococcus aureus	(bacteria)	DNB	1:1000	87	84	97

CFU = Colony Forming Units
DNB = D/E Neutralizing Broth

CONCLUSION:
The antimicrobial preservative properties present in the sample can be neutralized under the test conditions described below:

	DILUENT	DILUTION
Aspergillus brasiliensis	DNB	1:10
Candida albicans	DNB	1:10
Escherichia coli	DNB	1:10
Pseudomonas aeruginosa	DNB	1:10
Staphylococcus aureus	DNB	1:10

ANTIMICROBIAL PRESERVATIVE EFFECTIVENESS TEST

RESULTS:

TABLE SUMMARY

MICROORGANISM		INITIAL INOCULUM/mL	COLONY FORMING UNITS/mL 7 DAYS	14 DAYS	28 DAYS
Aspergillus brasiliensis	(mold)	3.0E5	110	<10	<10
Candida albicans	(yeast)	1.9E5	<10	<10	<10
Escherichia coli	(bacteria)	1.0E6	<10	<10	<10
Pseudomonas aeruginosa	(bacteria)	1.2E6	<10	<10	<10
Staphylococcus aureus	(bacteria)	7.1E5	<10	<10	<10

Note: Numbers in the report such as 2.3E5 are an alternate exponential format for 2.3×10^5.

LOG REDUCTION FROM INITIAL INOCULUM

	7 DAYS	14 DAYS	28 DAYS
Aspergillus brasiliensis	3.4	4.5	4.5
Candida albicans	4.3	4.3	4.3
Escherichia coli	5.0	5.0	5.0
Pseudomonas aeruginosa	5.1	5.1	5.1
Staphylococcus aureus	4.9	4.9	4.9

CONCLUSION:
The sample described above meets the current USP Category 1 Criteria of Acceptance for the Antimicrobial Preservative Effectiveness Test.

Smitha John
Microbiology Supervisor

Rose Holbrook
Quality Assurance Specialist II

McGUFF PHARMACEUTICALS, INC.

2921 W. MacArthur Blvd.
Suite 141
Santa Ana, CA 92704
Phone 714-918-7277

Certificate of Analysis / Report

Sample Information

Customer: McGuff COMPOUNDING PHARMACY SERVICES INC
2921 W. MacArthur Blvd.
Suite 142
Santa Ana, CA 92704
Phone 877-444-1133

Description: M.I.C 30mL

Part Number: 390-0645

Batch Number: 18J0091 (SPL18092019)

Results

Test	Specification/Limit	Result	Completion Day
Container Closure Integrity	1. Negative control must show no spectrophotometric absorbance equal to or greater than the absorbance of the 0.5 ppm Eosin Y LOD solution. 2. Positive control must show spectrophotometric absorbance greater than 0.5 ppm Eosin Y LOD solution. 3. Sample must show spectrophotometric absorbance less than the LOD	1. Negative control: 0.057< 0.094 PASS 2. Positive control: 3.204 > 0.094 PASS 3. Sample: 0.056 < 0.094 PASS	10/03/18

Note: This Lot 18J0091's BUD is Mar 30, 2019. This is Initial CCI testing.

Signature and Date: 10/03/18
Laboratories Supervisor or designee

QA Signature and Date: 10/03/18
Quality Assurance

COMPOUNDER'S INTERNATIONAL
ANALYTICAL LABORATORY
Better Quality Through Quality Testing

Stability Indicating Assay (SIA) Development and Validation

Validation Number: VAL.900.105

CONFIDENTIAL REPORT

for

McGuff Compounding Pharmacy Services, Inc.
2921 W MacAuthur Blvd, Ste 142
Santa Ana, CA 92704

L-Methionine, Inositol, and Choline Chloride (MIC) Injectable Formulation

Formula ID: 390-0645

Compounder's International Analytical Laboratory
4760 Castleton Way, Suite A
Castle Rock, CO 80109
Toll Free in the US: (800) 788-9922
Or Local Phone: (303) 471-8015
Fax: (303) 569-6101

COMPOUNDER'S INTERNATIONAL
ANALYTICAL LABORATORY
Better Quality Through Quality Testing

Executive Summary

May 20, 2021

This report provides information covering the equipment, chemistries, standards, procedures, and results of the development and validation of the stability indicating method for testing the stability of McGuff Compounding Pharmacy Services, Inc. formulation of L-methionine, inositol, and choline chloride (MIC) injectable.

We conclude from this data that the method, which was developed and illustrated here, is stability indicating and validated to provide accurate, precise assays for the MIC injectable formulation, free of interference from process impurities, inactive or excipient ingredients, and degradation products.

Ronald Sutton

Director

Contents

Overview

Scope

The goal of this project is to develop and validate a stability indicating method (SIM) to be used to quantify the potency of compounded injectable formulation of L-methionine, inositol, and choline chloride (referred to as MIC) received from McGuff Compounding Pharmacy Services, Inc. Since choline chloride dissociates in solution, the molecular weight ion for choline will be seen throughout this report. The term stability indicating method (SIM) can be used interchangeably with stability indicating assay (SIA). For this report, the more commonly used term 'stability indicating assay' or SIA will be used. After developing the SIA, the method is validated by additional testing according to the guidelines of USP general chapters <1225> and others. When a method has been verified as stability indicating and validated, it is referred to as a validated SIA. For this project, the sample lot: MIC20J3351 was used for the SIA and the additional validation testing. The placebo lot: 20M0281, was used to verify the accuracy of the method. Table 1 summarizes sample information including CIAL sample IDs along with active and inactive ingredients.

Table 1: Description of sample used for the stability indicating assay (SIA) and validation.

CIAL Tracking # / Customer Lot #	Sample Description	Active Ingredient(s)	Inactive Ingredients
Mcg 121420 1.1 / MIC20J3351	Validation Sample	L-Methionine (25 mg/mL) Inositol (50 mg/mL) Choline Chloride (50 mg/mL)	Benzyl alcohol and WFI or SWFI
Mcg 121420 1.2 / 20M0281	Placebo Sample	Not Present	Benzyl alcohol and WFI or SWFI

Introduction

At the start of this project, an initial trial method was developed using an ultra-high performance liquid chromatography (UHPLC) instrument equipped with a mass spectrometer (LC-MS). The sample was forcibly degraded under heat, light, oxidative and acid/base hydrolyzing conditions. These stressed samples were checked regularly using the trial method and checked for breakdown and possible interferences. The goal was to achieve at least 10% degradation if possible. Once this was achieved, the method was again checked for interferences using the technique of spiking the sample with standards along with mass spectral analysis. If any interference were found, a new method was developed and again checked for interferences. This process was repeated until no interference were found. The final step was to validate the SIA. The SIA and additional validation specifications along with the results specific to this project are shown in Table 2.

In order for the potency results of stability samples to be considered valid, any stability samples must be analyzed on the validated SIA and must contain the same active and inactive ingredients as the validation lot which underwent forced degradation. Active or inactive ingredients may be removed from the formulation and concentrations may be changed, but no new compounds can be added. If these conditions are met, the stability data collected on these samples using the validated SIA, would be considered valid and stability indicating. The removal of ingredients or change in concentration, should not affect the method's ability to quantify the active(s), but these changes may affect the product stability, thus a new stability study or bracketed study is recommended.

Table 2: Summary of the results for each specification for the stability indicating assay (SIA) and validation.

Validation Parameter	Acceptance Criteria	Result
Forced Degradation	Recovery of standard spiked into degraded samples not greater than 105%. Any possible interference from degradation product must be less than 3%.	Pass
Specificity	Retention time and mass spectra match reference standard. Three structurally relevant ions, preferably one of which is an ion representing the molecular mass of the analyte that matches the analyte standard. No interference from formulation, impurities, or degradation products.	Pass
Accuracy	Average Recovery of Standard between 95– 105%	Pass
Precision	Percent Relative Standard Deviation (% RSD) for replicates < 3%	Pass
Detection Limit	Documented	Pass

Quantitation Limit	Documented	Pass
Linearity	Documented	Pass
Range	Documented	Pass
Robustness	Documented	Pass
System Suitability	Documented	Pass

Experimental

Reagents, Chemicals, and Instrumentation

L-methionine, inositol, and choline chloride certified analytical reference standards were purchased from Sigma-Aldrich and were traceable to USP. Stable isotope labeled internal standards of L-methionine-(methyl-^{13}C,d_3) and choline chloride- (trimethyl-d_9) were purchased from Sigma-Aldrich. Another stable isotope labeled internal standard of myo-Inositol-1,2,3,4,5,6-d_6 was purchased from CDN Isotopes. The standards were within expiration or re-test dates. High purity 18.2 MΩ water was prepared using an EMD Millipore Milli-Q IQ7010 water purification system. MS grade or better methanol, acetonitrile, ammonium formate, and formic acid were used to dilute standards, samples and/or in the preparation of buffers. Forced degradation was performed using 30% hydrogen peroxide, 88% formic acid, and 28-30% ammonium hydroxide.

The LC-MS instrument consisted of an Agilent 1290 Flexible Pump, Vialsampler, Multicolumn Thermostat, and a 6135B MSD XT Single Quadrupole Mass Spectrometer. The column used was an Agilent UPLC Poroshell 120 Hilic-Z having a particle size of 2.7 µm with dimensions of 2.1 mm x 50 mm. Heat degradation was performed at 80ºC using a Thermo Scientific vacuum oven model 3618-5 at ambient pressure. UV light degradation was performed using Waters instrument at a wavelength of 254 nm, with samples stored in quartz vials. Other equipment used was a Branson Ultrasonic Water Bath, Mettler Toledo XP 26 Microbalance capable of reading down to 1.0 µg, 1 mL precision syringes, 1 mL and 100 µL Gilson Microman positive-displacement pipettors, and Pall 0.2 µm membrane filters. All equipment was calibrated, qualified, and within recertification dates.

Standard and Sample Preparation

Class A volumetric glassware was used to dissolve and dilute the standards and samples to the appropriate concentration. Individual standard stock solutions were prepared by dissolving 50 mg of L-methionine, 250 mg of inositol, or 10 mg of choline chloride into 20 or 25 mL of water and methanol, using volumetric glassware. Individual internal standard stock solutions were also prepared to an appropriate concentration in water and methanol. The standard and internal standards were diluted further to reach the target concentration by spiking 100 µL of L-methionine and choline chloride stock solutions, 500 µL of inositol stock solution, and 100 µL of each internal standard stock solution into a single 10 mL volumetric flask and diluting with water. As necessary, additional standards were made and/or diluted to achieve the desired concentration.

The sample was prepared according to the method procedure for each active. For analysis of inositol, the sample was prepared by placing 100 µL of sample and 100 µL of inositol internal standard stock into a 10 mL volumetric flask, dissolving with water, and inverting to mix. Additional dilutions were prepared from this first dilution for L-methionine and choline chloride by placing 1 mL or 100 µL, respectively, into separate 10 mL volumetric flasks with 100 µL of the appropriate internal standard stock solution, and diluting with water. After mixing thoroughly, the diluted samples were placed directly into HPLC vials. This 1:100 dilution equal to 0.5 mg/mL inositol, 1:1,000 dilution equal to 0.025 mg/mL methionine, and 1:10,000 dilution equal to 0.005 mg/mL of choline chloride and L-carnitine were used as the 100% nominal concentrations during the validation study.

Results and Discussion

Development and Optimization of Method

Mass spectrometer (MS) detectors are advantageous over UV-light detectors because they measure the mass of a species. However, the key to detection with MS is to be able to ionize the sample so it can be detected. Ions can be formed with a positive or a negative charge and as such the MS can be run in two different voltage polarity modes. Negative ion mode will only allow the negatively charged ions through the detector, while positive ion mode will only allow positively charged ions through the detector. An analyte may ionize better in a positive or negative ion mode, but in general, the presence of a positive or negative ion already in solution is indicative of

which polarity mode will be best. Neutral species in solution will add or subtract a hydrogen ion (H+) in order to ionize.

Initial development of the quantitative LC-MS method involves obtaining an analytical grade pure reference standard and either infusing it directly into the mass spectrometer or performing flow injection analysis. To begin the flow injection analysis, the standard is put into solution at a relatively high concentration, a variety of signals are observed, and some parameters are set to 'variable' in order to obtain multiple ions that are ideally characteristic of the active's structure. Initially, a scan is obtained at either the anticipated positive or negative ion mode and the scan range is set to view masses above and below the analyte's molecular weight. The standard solution is injected multiple times with this signal at various fragmentor voltages to ideally promote both ionization of the molecular weight ions (positive or negative, depending on mode) and insource fragmentation. It is ideal for three ions to be identified: the molecular weight ion and two ions of lower molecular weight. The ions of lower molecular weight are indicative of fragmentation and occur as a result of the application of higher energies which cause the molecular weight ion to fragment. Once three or more ions are obtained, further flow injections are performed in selected ion monitoring (SIM) mode, at varying fragmentor voltages, until a specific voltage is found where each ion has its highest possible response. Each ion is evaluated in the full range for rough tuning, and, for purposes of efficiency and practicality, fine tuning of each ion is limited to voltages divisible by five. Quantifying an unknown in a quantitative assay is primarily evaluated via relative responses but normalized to internal standards between the quantitation ion over both additional qualifier ions. The findings for the best available ions for each active and their relative responses to one another at the time of this report are detailed in Table 3.

Table 3: Instrument parameters in selective ion monitoring for the validated SIA.

Active	Polarity	Molecular Weight (g/mol)	Quantitation Ion	Qualifier Ion 1	Relative Response*	Qualifier Ion 2	Relative Response*
L-Methionine	Positive	149.21	150.0	104.0	16.0	133.0	15.9
Inositol	Negative	180.16	179.0	125.0	2.3	87.1	4.7
Choline	Positive	104.17	104.1	58.1	5.2	60.0	7.9

*Note that relative responses for individual ions can change for a variety of reasons including, but not limited to instrument condition, different mobile phase lots, background instrument current, relative concentrations, etc. The relative responses used to qualify an active are acquired at the time the sample is run by averaging the relative responses of the six injections of the analytical reference standard.

Another value of LC-MS testing is the potential to use stable isotope labeled internal standards, in which multiple atoms of the actives are replaced with 'heavier' versions of themselves (1H → 2H (d), 12C → 13C, 14N → 15N, etc.). When selecting isotopically labeled standards it is important that each isotope labeled standard is stable (not subject to radioactive decay) and that the total molecular weight is three or more mass units greater than the active, so that naturally occurring isotopes do not interfere. For these internal standards it is only necessary to acquire the ion that is most analogous to the quantitation ion. The same procedure to acquire the correct ion and its fragmentor voltage still applies, but is truncated. The optimum fragmentor voltage may differ from the active, however if they differ greatly, it may be necessary to evaluate if that internal standard is appropriate for the assay. The chosen stable isotope labeled active used as internal standard in this assay, as well as the ion observed is detailed in Table 4.

Table 4: Selected stable isotope labeled active used as an internal standard for the validated SIA.

Active	Polarity	Molecular Weight (g/mol)	Quantitation Ion
L-Methionine-(methyl-^{13} C, d_3)	Positive	153.22	154.0
myo-Inositol-1,2,3,4,5,6-d_6	Negative	186.19	185.1
Choline chloride-(trimethyl-d_9)	Positive	148.5	113.2

After the optimization of the MS detector conditions, the chromatographic method and sample preparation was optimized for the analysis of MIC. Depicted in Figure 1 is the total ion chromatogram (TIC) of a 0.025 mg/mL L-methionine, 0.5 mg/mL inositol, and 0.005 mg/mL choline chloride standard analyzed on the validated SIA. To ensure specificity, the retention time, quantification ion, and qualifier ions of samples were compared against these reference standards. The extracted ion chromatogram (EIC) of the internal standard is displayed in Figure 2.

Figure 1: Total ion chromatogram of standard containing 0.005 mg/mL choline chloride, 0.025 mg/mL L-methionine, and 0.5 mg/mL inositol, analyzed on the validated SIA.

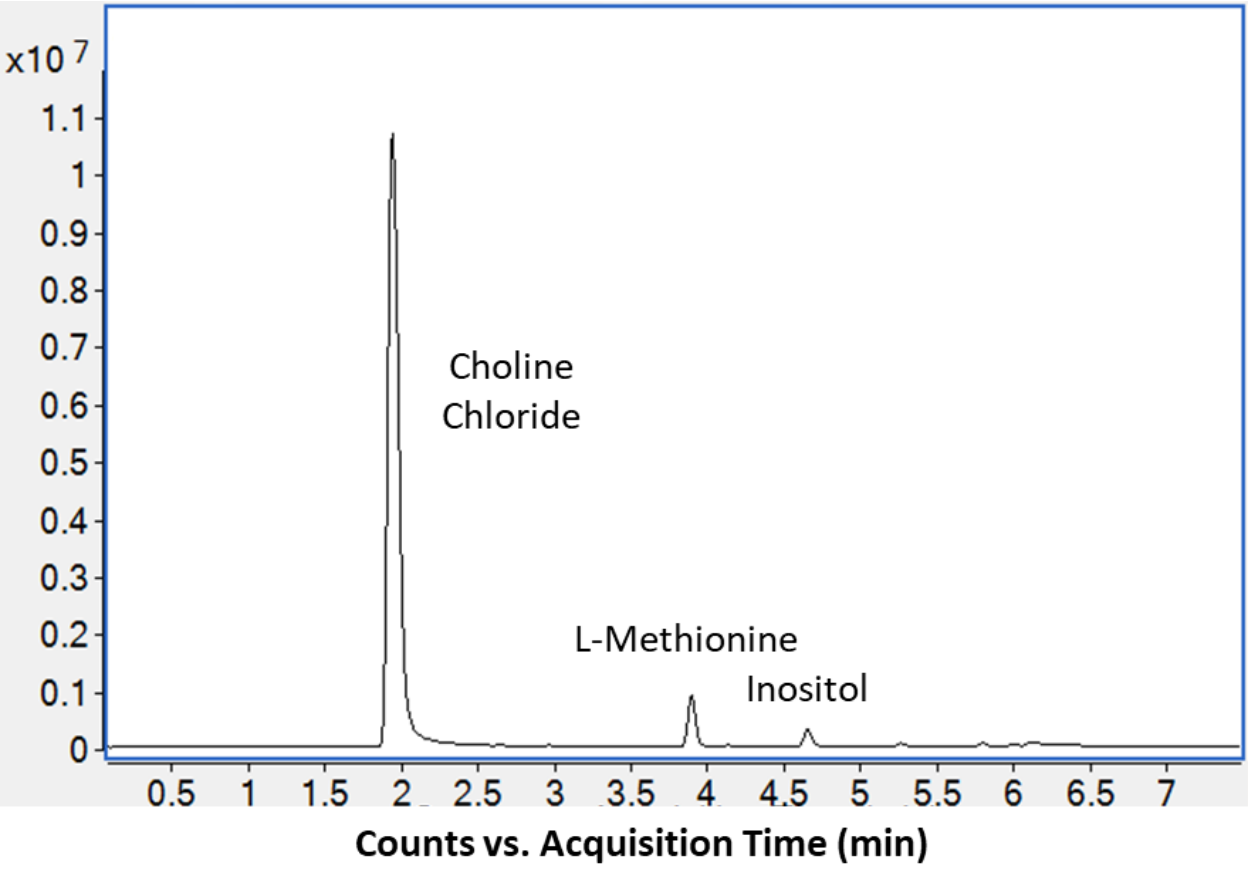
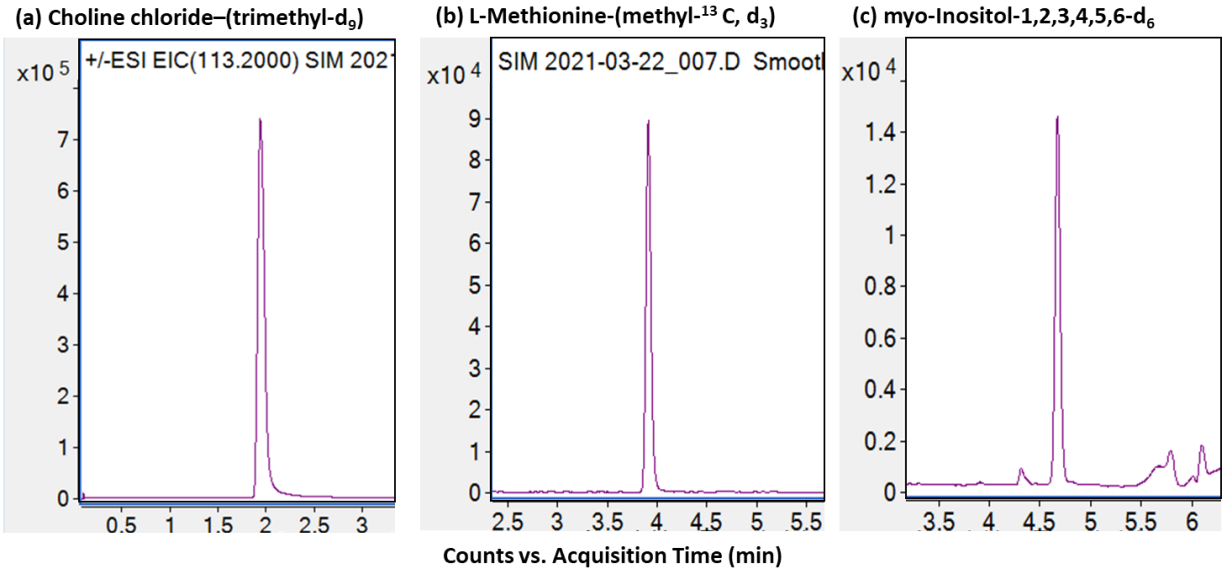
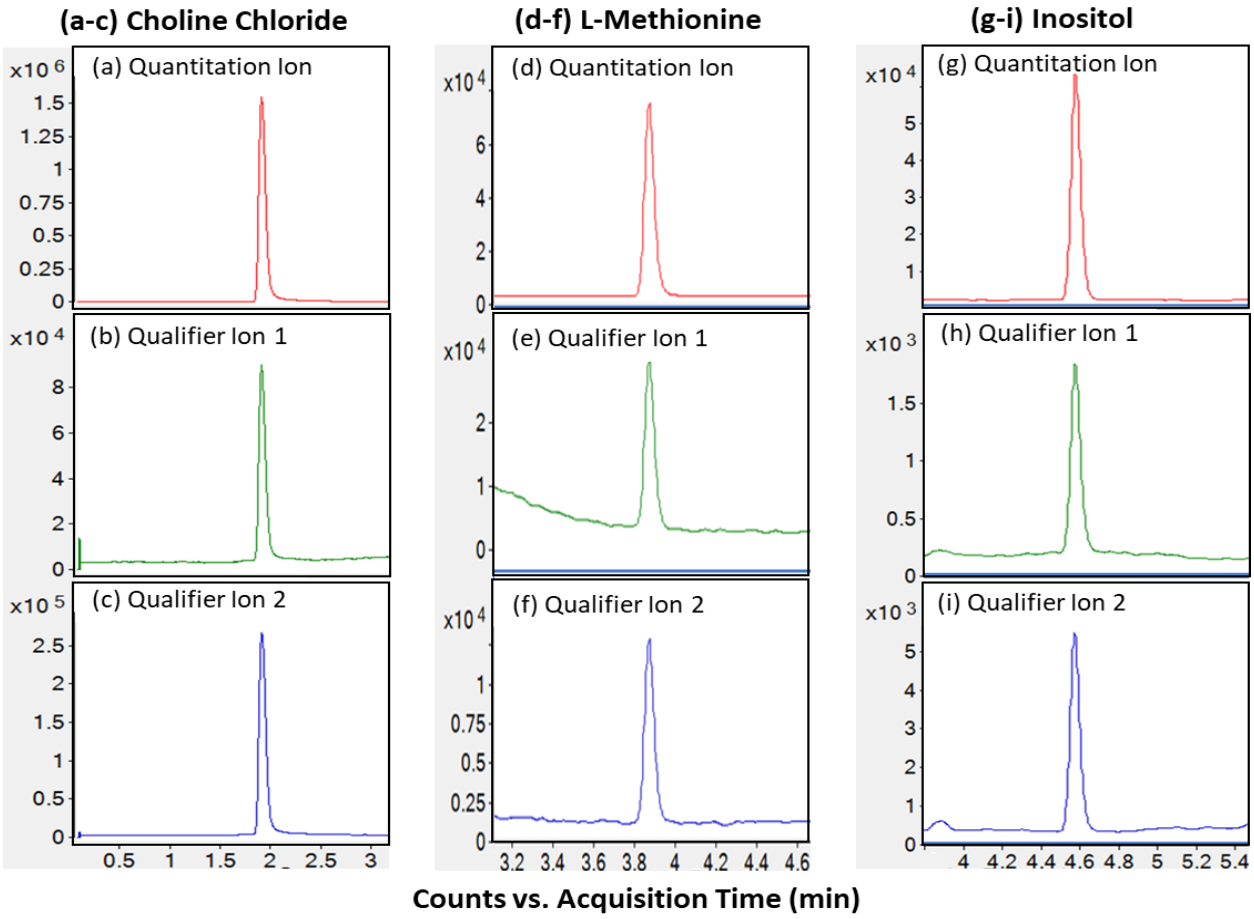


Figure 2: Extracted ion chromatogram of the internal standards containing 0.005 mg/mL choline chloride-(trimethyl-d_9), 0.025 mg/mL L-methionine-(methyl-^{13}C, d_3), and 0.5 mg/mL myo-inositol-1,2,3,4,5,6-d_6, analyzed on the validated SIA.



A typical chromatogram of a sample prepared according to the method procedure and analyzed on the validated SIA is shown in Figure 3. In Figure 3, the chromatograms shown from top to bottom are of the quantitation ion and the two qualifier ions specific to each analyte of interest, used for the quantitative analysis.

Figure 3: Extracted ion chromatograms of a sample prepared according to the method procedure and analyzed on the validated SIA.

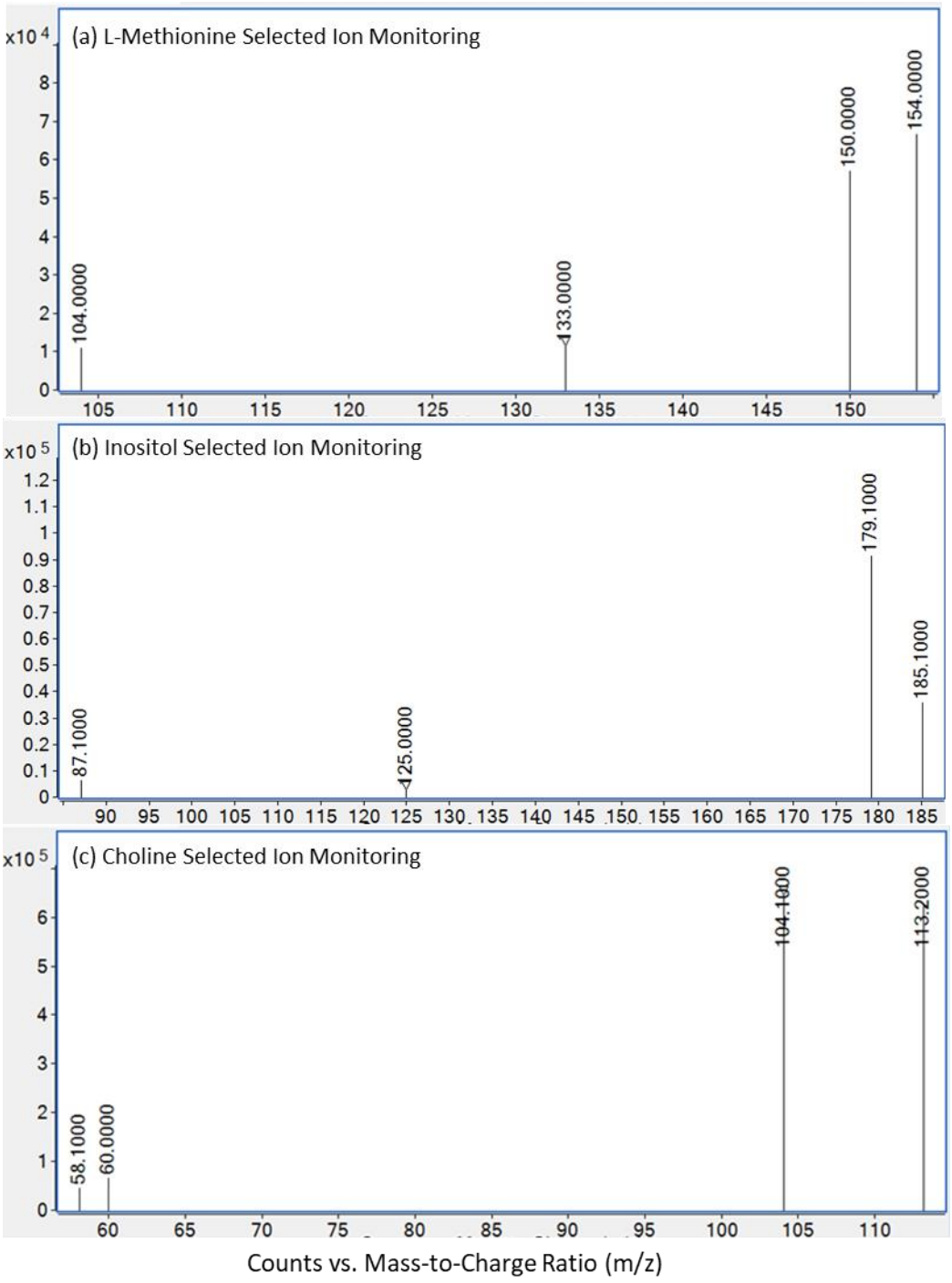


Forced Degradation

In UV based forced degradation studies, break down products can potentially interfere with accurate quantitation if they are not chromatographically resolved from the analyte of interest. In the case of LC-MS, quantitation involves analyzing ions that are specific to the analyte of interest and are indicative of its structure. Another advantage of using LC-MS is the ability to analyze a sample in SIM mode. In this mode the detector will only record a response for a species with the mass matching the selected ions of interest for each analyte (±0.13 Daltons).

To illustrate this point, Figure 4a shows the selected ion monitoring (SIM) which focuses only on the molecular weight ion, two lower weight qualifier ions, and the internal standard molecular weight ion for methionine. The SIM for inositol is show in Figure 4b, while choline chloride is shown in Figure 4c. This demonstrates the ability of the mass spectrometer to only focus on relevant information and respond only to mass data that is of interest. By monitoring the SIM counts versus time, during a chromatographic separation, the abundance of the specific mass being monitored can be quantified as a peak, as shown previously in Figure 1.

Figure 4: Mass spectrometry selected ion monitoring (SIM) of an (a) L-methionine standard peak, (b) inositol standard peak and (c) choline chloride standard peak showing the fragmentation specific to each analyte and its internal standard.



During the SIA, degradation results were obtained by quantifying L-methionine, inositol, and choline as intact molecules. If the molecules are degraded, oxidized, or changed in any way, they will not maintain the same molecular weight, and will not produce a signal for that specific mass. Other degradants or interferences can still have an effect on accurate quantitation of L-methionine, inositol, and choline chloride if they happen to coelute and either inhibit or enhance the signal for that mass. To account for this possibility, the purity of the peak was also checked by a mass balance technique called percent recovery. This is completed by spiking the degraded sample with a known amount of standard and then calculating the percent recovered by the assay. Therefore, any effects of degradants or possible interferences on quantitation can be evaluated, whether or not they can be observed.

Forced degradation was performed on the sample to obtain data on any potential degradant that might interfere with the validated method and ultimately the method's stability indicating ability. Exposure to 80˚C and UV light was performed on a portion of undiluted sample; with the sample contained in an amber serum vial and quartz vial, respectively, and secured with a rubber stopper and aluminum crimp cap. Samples exposed to chemical degradation were individually prepared by diluting the sample in water, with the appropriate amount of the corresponding chemical (30% hydrogen peroxide, 88% formic acid, and 28-30% ammonium hydroxide). All samples were diluted to the method target concentration prior to analysis. Ideally, there would have been 10-50% degradation of each active to simulate what might actually occur during a stability study. Minimally, 5-10% loss would mimic the end of the stability study when there may be a 10% loss compared to the starting potency value. Highly stable compounds might not break down at all, thus indicating they would not be expected to break down during the actual stability study.

Forced Degradation Results

The amount of degradation observed during the SIA, is summarized in Table 5. All forced degradation samples were stored at the designated conditions for a period of five days with the exception of the peroxide forced degradation sample being stored for one day. Moderate degradation was observed for L-methionine with exposure to peroxide over a period of one day. Only very slight degradation was noticed with exposure to acidic and basic conditions over a period of five days. All actives show relative stability in 80°C heat, even after five days. Negative percent degradation values are due to instrument variation and within allowable error.

Table 5: Percent degradation of sample from forced degradation: 10% or more is desired.

Active Component	% Degraded in 80°C Heat for 5 days	% Degraded in UV Light for 5 days	% Degraded in Peroxide for 1 day	% Degraded in Acid for 5 days	% Degraded in Base for 5 days
L-Methionine	0%	5%	41%	3%	3%
Inositol	0%	-1%	-3%	1%	-1%
Choline Chloride	2%	2%	-1%	6%	2%

In addition to only monitoring the specific mass of interest, the forced degraded samples were spiked with a known amount of L-methionine, inositol, and choline chloride standards. After analyzing the spiked and non-spiked samples, the percent recovery was calculated and the results are shown in Table 6. Ideally, there should be a 100% mass balance recovery of the amount of standard spiked into the forced degradation samples. If the recovery is greater than 100%, it may indicate there is some other compound underneath the peak(s) producing an interference; if less than 100%, it would indicate no interference, but some loss of recovery due to degradation of the standard. A reasonable error of 5% should be factored in to account for method variability. Note that no percent recovery exceeds 104%, thus illustrating that no interference was observed in the degraded samples.

Table 6: Percent recovery of standard spiked into degraded samples (peak purity checked by mass balance).

Active Component	% Recovery in 80°C Heat after 5 days	% Recovery in UV Light after 5 days	% Recovery in Peroxide after 1 day	% Recovery in Acid after 5 days	% Recovery in Base after 5 days
L-Methionine	100%	103%	101%	101%	100%
Inositol	101%	100%	99%	101%	99%
Choline Chloride	97%	100%	97%	104%	98%

Method Validation

According to USP general chapter <1225>, "Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications." It is important to validate the established SIA used to measure the potency of the compounded formulation, to ensure reliability of the results. While the SIA demonstrates that the method should remain unaffected by the presence of impurities or possible degredants, validation testing demonstrates the reliability, precision and accuracy of the values obtained. The following laboratory tests are recommended to meet USP criteria with the ones in *italics* required:

1. *Specificity*
2. *Accuracy*
3. *Precision and Ruggedness (Repeatability)*
4. Robustness
5. *Linearity, LOQ, LOD, Range*
6. System Suitability

Specificity

The most important part of the assay is to ensure that it is specific to the compound being analyzed and there are no interferences that could cause an artificially high/low result. The USP, using the ICH definition, defines specificity as, "The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components."

As shown previously in the forced degradation results, this criterion was met both by matching retention times, quantitation ions, and qualifier ions of both the sample and the standard. Furthermore, percent recovery demonstrated that a known amount of standard could accurately be recovered from the degraded matrix. It was determined that this method identified the active ingredients properly and no impurities, degradation products, or matrix components interfered with the peaks of interest.

Accuracy

The second most important part of the assay is to make sure that the value being reported by the method matches the true value. The accuracy of the method was assessed by spiking an analytical standard into the sample matrix (placebo, diluted to target) at three different concentration levels, prepared in triplicate. The percent recovery was then calculated, along with the percent relative standard deviation and 95% confidence interval. The results are shown below in Table 7. All recoveries were within the 95-105% limitations.

Table 7: Accuracy of the validated SIA shown by percent recovery of an analytical standard at three different concentrations.

Accuracy Name	Active Component	% of Nominal Conc.	%RSD, n = 3 samples	Average +/- 95% Confidence Interval, n = 3 samples
Accuracy A	L-Methionine	81%	1.38%	101.3% +/- 3.48%
Accuracy B	L-Methionine	101%	1.49%	100.7% +/- 3.73%
Accuracy C	L-Methionine	120%	1.37%	100.2% +/- 3.40%
Accuracy A	Inositol	80%	0.69%	99.7% +/- 1.71%
Accuracy B	Inositol	100%	0.86%	99.7% +/- 2.12%
Accuracy C	Inositol	121%	1.11%	99.9% +/- 2.76%
Accuracy A	Choline Chloride	81%	0.19%	99.6% +/- 0.48%
Accuracy B	Choline Chloride	101%	1.18%	99.4% +/- 2.92%
Accuracy C	Choline Chloride	123%	0.03%	100.9% +/- 0.06%

Precision

In order to ensure the results are repeatable, the precision of the method is evaluated. This is referred to as inter-assay precision. Samples were prepared according to the method procedure, diluted to the method target concentration, and prepared with six replicates. Table 8 shows the summary of the precision for the quantitation of the actives using the validated SIA. The percent relative standard deviation was calculated on the averages of six different sample preparations. From these reported values, we can conclude the method is precise and highly reliable for the determination of potency values.

Table 8: Precision of the validated SIA using the sample prepared at the method target concentration with six replicates.

Active Component	%RSD n = 6 samples
L-Methionine	0.82%
Inositol	0.62%
Choline Chloride	0.90%

Ruggedness

A second criteria to evaluate the repeatability of the method is called ruggedness or intermediate precision. The objective is to verify that the same laboratory will provide similar results on different days, with different analysts, equipment, and/or columns. The validated method was tested for ruggedness by analyzing a sample prepared according to the method procedure and analyzed on three different days. The same type of column was used, but may have been produced from a different lot. New mobile phase solvents and diluents were prepared as needed. Acceptable reproducibility was achieved between analyses and the results are summarized in Table 9.

Table 9: Ruggedness of the validated SIA using the sample prepared according to the method procedure and analyzed on three different days.

Analysis	Active Component	%RSD n = 3 injections	%RSD n = 3 samples with 3 injections each
Analysis A	L-Methionine	1.34%	0.73%
Analysis B	L-Methionine	1.58%	
Analysis C	L-Methionine	1.24%	
Analysis A	Inositol	2.16%	2.09%
Analysis B	Inositol	0.47%	
Analysis C	Inositol	1.66%	
Analysis A	Choline Chloride	1.57%	1.60%
Analysis B	Choline Chloride	0.43%	
Analysis C	Choline Chloride	1.60%	

Method Robustness

During the course of the method development and validation of the SIA, several variables were evaluated to determine the robustness of the method. Method robustness is the ability for the analytical procedure to remain unaffected by small variations of the method parameters. To test the robustness of the method, small deliberate changes were made in the analytical procedure and the %RSD of the potency value was calculated for each variable. The method remains unaffected by the change if the %RSD is less than 3%. By testing a large selection of variables was can better determine the extent of robustness of the validated method and complete all testing, both for this project and future testing, within those parameters. Table 10 provides the summary of the all of the variables evaluated with corresponding %RSD of the potency values. The effects of using glass vials, plastic vials, or filtering was tested on the standard solution. For the subsequent tests the sample prepared according to the method procedure was used. These tests included injection volume, varying the column temperature, determining the effect increasing or decreasing the buffer concentration, and varying the nebulizer pressure, drying gas flow, sheath gas flow, and sheath gas temperature. As shown, none of the changes evaluated affect the method performance.

Table 10: Effect of small variations of method parameters to determine the robustness of the validated SIA.

Method Change Description	Variables Evaluated	L-Methionine %RSD of Method Changes	Inositol %RSD of Method Changes	Choline Chloride %RSD of Method Changes
Effect of Plastic Vials or Filtering	Glass Vial, Plastic Vial, Filtered into Glass Vial	0.71%	0.23%	0.45%
Effect of Injection Volume	0.12 µL, 0.25 µL, and 0.5 µL	0.20%	0.42%	0.93%
Column Temperature	+/- 5˚C	1.68%	0.76%	1.07%
Buffer Concentration	Amount of Ammonium Formate and/or Formic Acid Varied	1.52%	0.47%	0.65%
Effect of Nebulizer Pressure	+/- 5 psi	1.95%	0.82%	0.32%
Effect of Drying Gas Flow	6, 7 and 8 L/min	2.62%	0.61%	1.05%
Effect of Sheath Gas Flow	11 and 12 L/min	0.85%	1.71%	0.43%
Effect of Sheath Gas Temperature	340°C and 350°C	0.86%	0.05%	0.49%

Linearity, Range, LOD and LOQ

The parameters linearity, range, limit of detection (LOD), and limit of quantitation (LOQ) are used to further evaluate the accuracy and sensitivity of the method. These results are provided below in Table 11. Range provides the suitable lower and upper limits of concentration where the validated SIA is most accurate. The data collected within this range should display a linear response relationship. A coefficient of determination (R^2) value of 0.999 or greater indicates the method is linear within the given range and therefore provides accurate quantification. The LOD is the concentration at which the presence of an active ingredient can be detected but not accurately quantified, while the LOQ is the lowest concentration that the active ingredient can be accurately quantified. The plot of the data used to obtain the equation of line and coefficient of determination is shown in Figure 5.

Table 11: Sensitivity and linearity of the validated SIA.

Active Component	Lower Limit of Detection (mg/mL)	Lower Limit of Quantitation (mg/mL)	Range Plotted (mg/mL)	Coefficient of Determination R^2 value	Equation of Line
L-Methionine	0.00009	0.003	0.003 to 0.3	0.999944	y = 35x + 0.017
Inositol	0.002	0.05	0.05 to 5.1	0.999775	y = 5x - 0.041
Choline Chloride	0.00009	0.00003	0.00003 to 0.05	0.999997	y = 174x - 0.001

Another way to look at the linear range of each method is to plot the percent recovery of the diluted standard versus the concentration of the diluted standard. Parallel horizontal lines are drawn on the plot to correspond to 95% and 105% recovery. Where the data exceeds these limits, the method is no longer linear. This data is plotted in Figure 6 and shows that L-methionine, inositol, and choline chloride are all linear across a very broad range. At the lowest concentrations the data of each injection is more scattered because it is at or below the limit of quantitation. While it is expected at concentrations higher than 5.1 mg/mL for inositol or 0.05 mg/mL for choline chloride and observed at concentrations higher than 0.3 for L-methionine the method would start to deviate from linearity.

Figure 5: Linearity plot used to calculate the equation of the line and coefficient of determination (R^2) of the validated SIA.

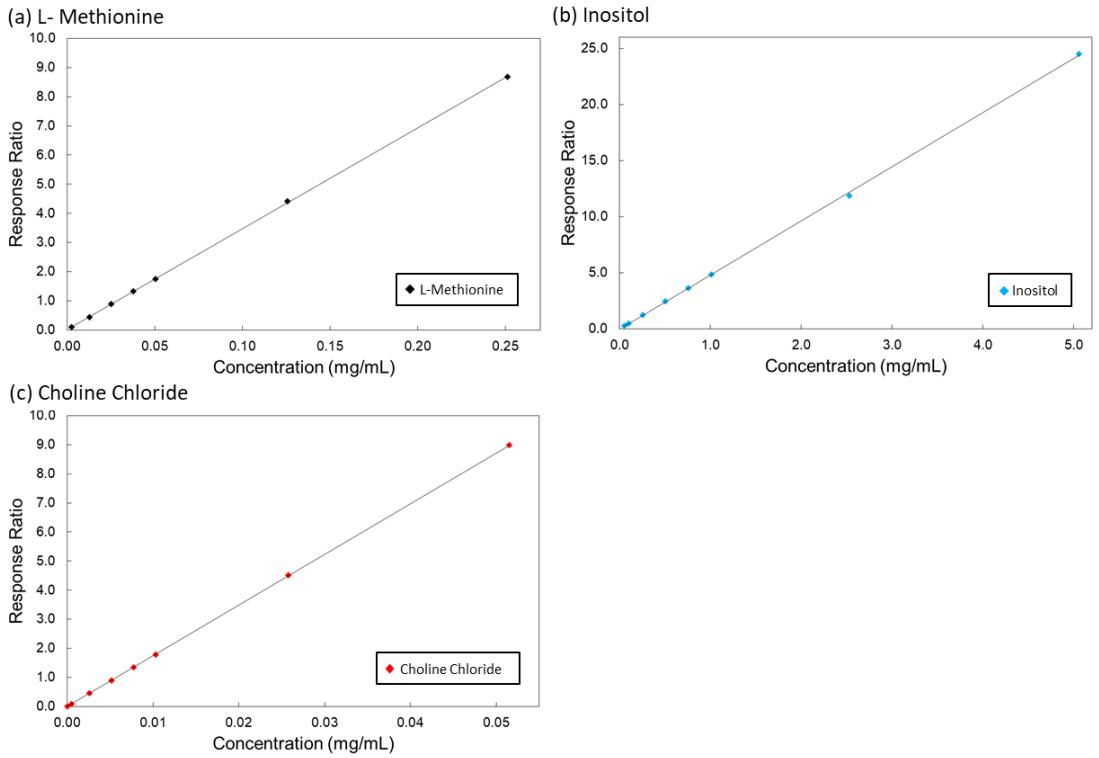
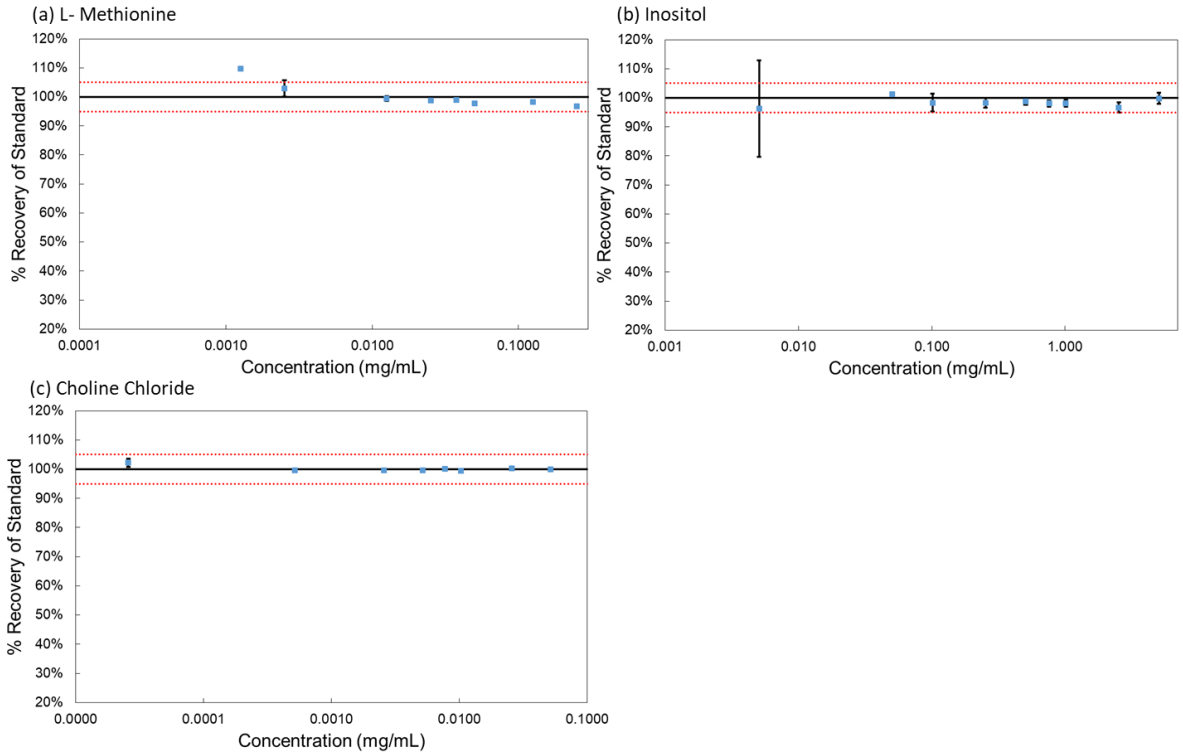


Figure 6: Plot of the percent recovery of a L-methionine, inositol, and choline chloride standard analyzed on the validated SIA at a wide range of concentrations. Parallel horizontal lines correspond to 95% and 105% recovery.



System Suitability

The chromatographic separation obtained on the LC-MS system was checked for its ability to accurately quantify the samples being tested. For six replicate standard injections, the %RSD of the area was calculated along with the average peak retention (k'), peak symmetry (USP tailing), and efficiency (theoretical plates). For a typical sample prepared according to the method procedure, the average peak resolution was calculated to account for any inactive or impurities present in a sample. These system suitability results are shown in Table 12 and all are within USP/ICH recommended limits.

The %RSD of the area of the six standard injections is less than the target of 3.0% and indicates the method meets injection repeatability requirements. The target of a k' retention equal to or greater than 2.0 indicates the peaks are very well retained on the column and could be expected to provide ideal separation from possible breakdown products during the stability study. In general, as retention increases, resolution will improve. The peak symmetry (referred to as USP tailing) equal to or less than 2.0 indicates the peaks have very little tailing or fronting. Increased symmetry of the peak reduces the possibility of interference during the assay, which also provides increased confidence in accuracy and precision. The efficiency value or USP plate count is a measure of peak sharpness. Our value of theoretical plates indicates the peaks are very sharp and therefore are able to provide better separations with reduced likelihood of interferences during the validation study. Normally, a target resolution equal to or greater than 2.0 would indicate the peak was at least baseline separated from its neighboring peak. Since the LC-MS method used SIM mode, only that analyte peaks of interest were observed. Therefore resolution values are not applicable.

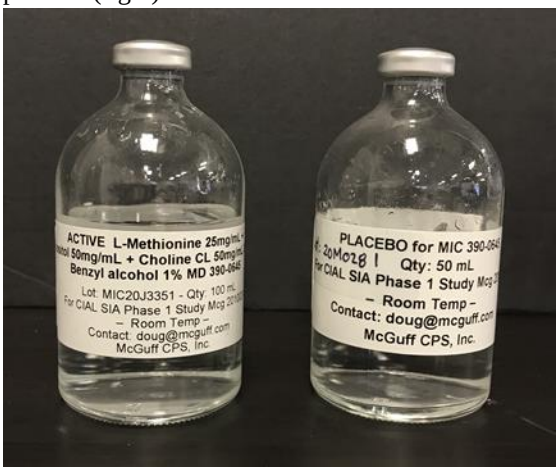
Table 12: System suitability results of the validated SIA using six standard injections.

Active Component	%RSD Area (Target ≤2.0)	Retention k' (Target ≥2.0)	USP Tailing (Target ≤2.0)	Efficiency USP Plate Count (Target ≥2,000)
L-Methionine	1.63%	14.501	1.100	29186
Inositol	1.38%	17.290	1.167	47192
Choline Chloride	1.57%	6.655	1.325	3610

Physical Characteristics Results

The container used for the compounded MIC injectable formulation for the SIA and validation was a 100 mL clear glass injection vial with rubber stopper and aluminum crimp cap. The injectable formulation was a transparent colorless liquid and did not change noticeably over the course of the validation testing. A picture of the sample is shown in Figure 7.

Figure 7: Customer sample container used for the stability indicating assay (SIA) and validation (left) and the placebo (right).



Conclusion

This concludes the development and validation of the SIA for the compounded formulation of L-methionine, inositol, and choline chloride (referred to as MIC), received from McGuff Compounding Pharmacy Services, Inc. The data within this report demonstrates the method used to quantify the potency has been validated as a stability indicating assay (SIA). The method was shown to be stability indicating through forced degradation of the sample along with other validation parameters. The validated SIA can be used to analyze a variety of samples prepared with this formulation. Beyond use dating (BUD) of this formulation can be established by conducting a potency-over-time study, utilizing this stability indicating method (VAL.900.105). All samples containing these active and inactive ingredients received from McGuff Compounding Pharmacy Services, Inc. will be analyzed using the validated SIA for any category of testing.