SAMPLE CMC SECTION FOR HYPERPOLARIZED PYRUVATE (13C) INJECTION

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TABLE OF CONTENTS **Volume 2**

TABLE OF CONTENTS **Volume 2**

TABLE OF CONTENTS **Volume 2**

TABLE OF CONTENTS **Volume 2**

TABLE OF CONTENTS **Volume 2**

TABLE OF CONTENTS **Volume 2**

7 CHEMISTRY, MANUFACTURING AND CONTROL

Introduction

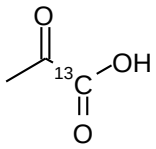
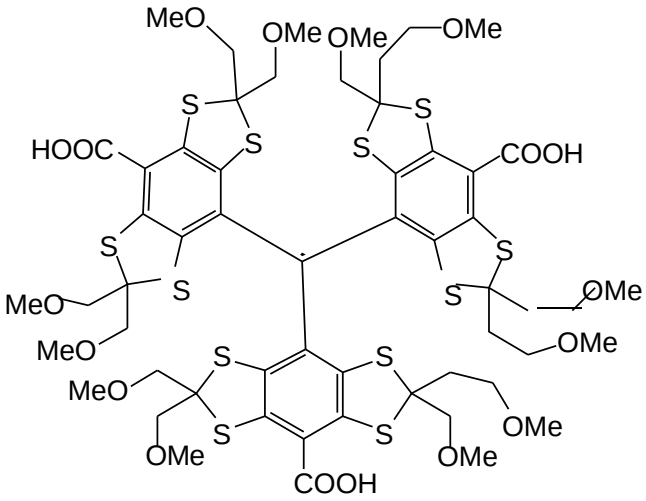
Sections 7.1, 7.2 and 7.3 of Item 7 of this IND are presented in the CTD format prescribed for Chemistry, Manufacturing and Control.

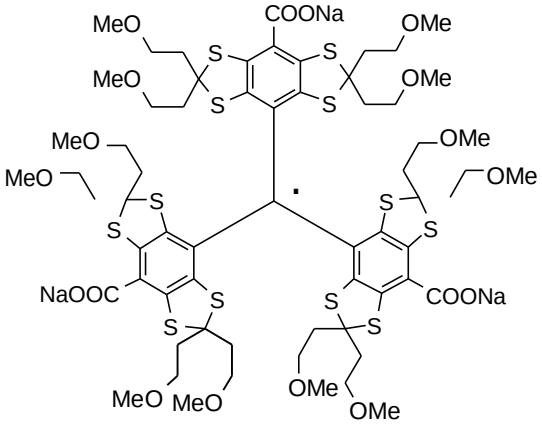
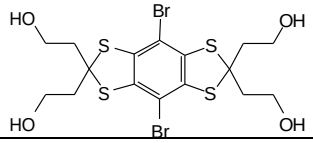
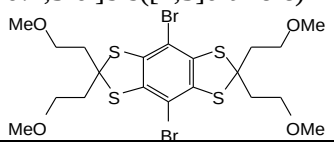
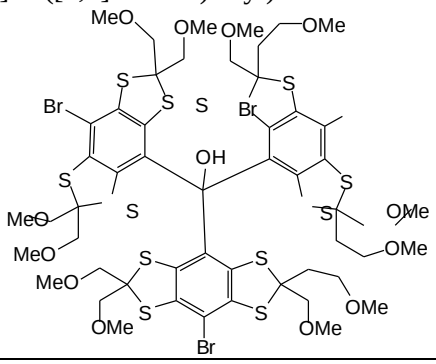
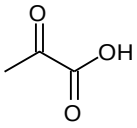
Format and Content	
Section 3.2.S	**DRUG SUBSTANCE**
3.2.S.1	**General Information**
3.2.S.1.1	Nomenclature
3.2.S.1.2	Structure
3.2.S.1.3	General Properties
3.2.S.2	**Manufacture**
3.2.S.2.1	Manufacturer(s)
3.2.S.2.2	Description of Manufacturing Process and Process Controls
3.2.S.2.3	Control of Materials
3.2.S.2.4	Controls of Critical Steps and Intermediates
3.2.S.2.5	Process Validation and/or Evaluation
3.2.S.2.6	Manufacturing Process Development
3.2.S.3	**Characterization**
3.2.S.3.1	Elucidation of Structure and Other Characteristics
3.2.S.3.2	Impurities
3.2.S.4	**Control of Drug Substance**
3.2.S.4.1	Specification
3.2.S.4.2	Analytical Procedures
3.2.S.4.3	Validation of Analytical Procedures
3.2.S.4.4	Batch Analyses
3.2.S.4.5	Justification of Specification
3.2.S.5	**Reference Standards or Materials**
3.2.S.6	**Container Closure Systems**
3.2.S.7	**Stability**
3.2.S.7.1	Stability Summary and Conclusions
3.2.S.7.2	Post-Approval Stability Protocol and Stability Commitment
3.2.S.7.3	Stability Data
3.2.P Part I	**DRUG PRODUCT – DRUG PRODUCT KIT COMPONENTS**
3.2.P.1	**Description and Composition of the Drug Product**
3.2.P.2	**Pharmaceutical Development**
3.2.P.2.1	Components of the Drug Product
3.2.P.2.2	Drug Product
3.2.P.2.3	Manufacturing Process Development
3.2.P.2.4	Container Closure System
3.2.P.2.5	Microbiological Attributes

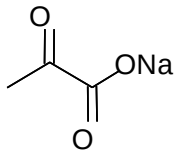
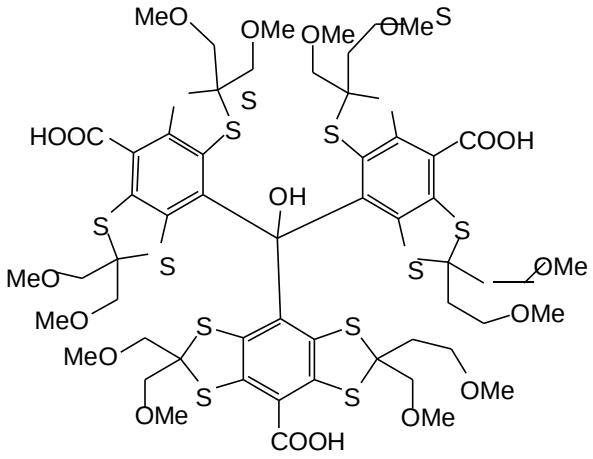
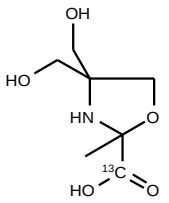
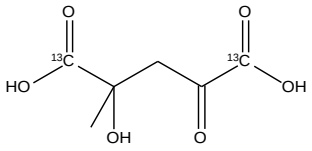
3.2.P.3	**Manufacture**
3.2.P.3.1	Manufacturer(s)
3.2.P.3.2	Batch Formula
3.2.P.3.3	Description of Manufacturing Process and Process Controls
3.2.P.3.4	Controls of Critical Steps and Intermediates
3.2.P.3.5	Process Validation and/or Evaluation
3.2.P.4	**Control of Excipients**
3.2.P.4.1	Specification(s)
3.2.P.4.2	Analytical Procedures
3.2.P.4.3	Validation of Analytical Procedures
3.2.P.4.4	Justification of Specifications
3.2.P.4.5	Excipients of Human or Animal Origin
3.2.P.4.6	Novel Excipients
3.2.P.5	**Control of Drug Product**
3.2.P.5.1	Specification(s)
3.2.P.5.2	Analytical Procedures
3.2.P.5.3	Validation of Analytical Procedures
3.2.P.5.4	Batch Analyses
3.2.P.5.5	Characterization of Impurities
3.2.P.5.6	Justification of Specification(s)
3.2.P.6	**Reference Standards or Materials**
3.2.P.7	**Container Closure System**
3.2.P.8	**Stability**
3.2.P.8.1	Stability Summary and Conclusion
3.2.P.8.2	Post-Approval Stability Protocol and Stability Commitment
3.2.P.8.3	Stability Data
3.2.P Part II	**DRUG PRODUCT – HYPERPOLARIZED PYRUVATE (^{13}C) INJECTION**
3.2.P.1	**Description and Composition of the Drug Product**
3.2.P.2	**Pharmaceutical Development**
3.2.P.2.1	Components of the Drug Product
3.2.P.2.2	Drug Product
3.2.P.2.3	Manufacturing Process Development
3.2.P.2.4	Container Closure System
3.2.P.2.5	Microbiological Attributes
3.2.P.3	**Manufacture**
3.2.P.3.1	Manufacturer(s)
3.2.P.3.2	Batch Formula
3.2.P.3.3	Description of Manufacturing Process and Process Controls
3.2.P.3.4	Controls of Critical Steps and Intermediates
3.2.P.3.5	Process Validation and/or Evaluation
3.2.P.4	**Control of Excipients**
3.2.P.4.1	Specification(s)
3.2.P.4.2	Analytical Procedures
3.2.P.4.3	Validation of Analytical Procedures
3.2.P.4.4	Justification of Specifications
3.2.P.4.5	Excipients of Human or Animal Origin
3.2.P.4.6	Novel Excipients

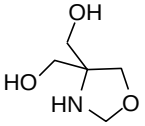
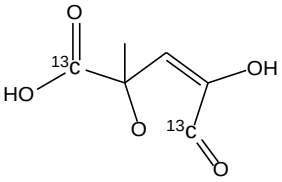
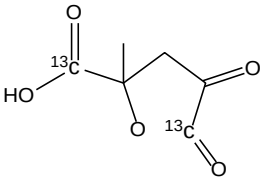
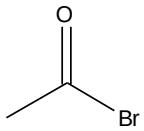
3.2.P.5	**Control of Drug Product**
3.2.P.5.1	Specification(s)
3.2.P.5.2	Analytical Procedures
3.2.P.5.3	Validation of Analytical Procedures
3.2.P.5.4	Batch Analyses
3.2.P.5.5	Characterization of Impurities
3.2.P.5.6	Justification of Specification(s)
3.2.P.6	**Reference Standards or Materials**
3.2.P.7	**Container Closure System**
3.2.P.8	**Stability**
3.2.P.8.1	Stability Summary and Conclusions
3.2.P.8.2	Post-Approval Stability Protocol and Stability Commitment
3.2.P.8.3	Stability Data
3.2.A	**APPENDICES**
3.2.A.3	**Novel Excipients**
3.2.A.3.1	General Information
3.2.A.3.2	Manufacture
3.2.A.3.3	Characterization
3.2.A.3.4	Control of Novel Excipient
3.2.A.3.5	Reference Standards or Materials
3.2.A.3.6	Container Closure System
3.2.A.3.7	Stability

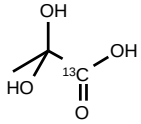
CMC LIST OF ABBREVIATIONS

Term	Meaning
AH110896	**[1-^{13}C]Pyruvic acid** 2-Oxopropanoic acid ^{13}C Pyruvic acid 
AH110896 sodium salt	**Sodium [1-^{13}C]pyruvate** Sodium salt of [1-^{13}C]pyruvic acid Pyruvic acid-1-^{13}C Sodium salt Sodium Pyruvate-1-^{13}C
AH111501	Tris{8-carboxyl-2,2,6,6-tetra[2-(1-methoxyethyl)]-benzo(1,2-d:4,5-d')bis(1,3)dithiole-4-yl}methyl acid (trityl radical) 

AH111501 sodium salt	Trisodium salt of AH111501 
AH111576	2,2',2",2"'-(4,8-Dibromobenzo[1,2-d:4,5-d']bis([1,3]dithiole)-2,2,6,6-tetrayl)tetraethanol 
AH111586	4,8-Dibromo-2,2,6,6-tetrakis(2-methoxyethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole) 
AH111709	(8-Bromo-2,2,6,6-tetrakis(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-yl)(8-bromo-2,2,6-tris(2-methoxyethyl)-6-(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-yl)(8-bromo-2-(2-methoxyethyl)-2,6,6-tris(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-yl)methanol 
AH111710	**Pyruvic acid** ^{12}C Pyruvic acid Naturally abundant pyruvic acid 

AH111710 sodium salt	**Sodium pyruvate** Naturally abundant sodium pyruvate 
AH111743	8-((8-carboxy-2,2,6,6-tetrakis(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-yl)(8-carboxy-2,2,6-tris(2-methoxyethyl)-6-(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-yl)(hydroxy)methyl)-2-(2-methoxyethyl)-2,6,6-tris(methoxymethyl)benzo[1,2-d:4,5-d']bis([1,3]dithiole)-4-carboxylic acid 
AH112615	4,4-Bis-hydroxymethyl-2-methyl-oxazolidine-2-carboxylic acid 
AH112623	Parapyruvate 2-Hydroxy-2-methyl-4-oxo-pentanedioic acid 
AH113127	(4-Hydroxymethyl-oxazolidin-4-yl)-methanol

	
AH113462/E	Enol lactone 
AH113462/K	Keto lactone 
Acetyl bromide	
ATR	Attenuated total reflection
n-BuLi	n-Butyllithium
°C	Degrees centigrade
CFU	Colony forming units
cGMP	Current good manufacturing practice
cm	Centimetre
cm^{-1}	Wave number
CO_2	Carbon dioxide
CRO	Contract research organization
CSP	Compounded sterile preparations
$Cu^{13}CN$	Cuprous [1–^{13}C]cyanide
CVS	Cardiovascular system
DMI	1,3-dimethyl-2-imidazolidinone
DMSO	Dimethyl sulfoxide
DNP	Dynamic nuclear polarization
EDTA	Ethylenediaminetetracetic acid
EU	Endotoxin units
FEP	Fluorinated ethylene propylene
FID	Flame ionization detection
FT-IR	Fourier transform infrared spectroscopy
g	Gram
µg	Microgram
GC	Gas chromatography
Gd	Gadolinium
GHz	Giga Hertz
GMP	Good manufacturing practice

HCl	Hydrochloric acid
He	Helium
H_2O	Water
HPLC	High-performance liquid chromatography
HS-GC	Headspace gas chromatography
Hydrated [1-^{13}C]pyruvic acid	
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ID	Identity
IQ	Installation qualification
IR	Infrared spectroscopy
ISO	International Organization for Standardization
K	(degrees) Kelvin
KF	Karl Fisher titration
kg	Kilogram
l	Litre
LAF	Laminar air flow
LAL	Limulus amebocyte lysate
LC-MS	Liquid chromatography mass spectrometry
LSG	Life science grade
M	Molar
mAU	Milli absorbance units
mbar	Millibar
MeOH	Methanol
mg	Milligram
MHz	Mega Hertz
Mixture of pyruvic acid and 0.2 mM AH111501 sodium salt	Drug product kit component in clinical studies GE-101-001/GE-101-003
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt	Drug product kit component in clinical study GE-101-002
ml	Millilitre
mm	Millimeter
mM	Millimolar
µm	Micrometer
µM	Micromolar
mmol	Millimol
mOsm	Milliosmol
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MS	Mass spectrometry
mW	Milliwatt
m/m	Mass per mass
mol/mol	Mol per mol
NA	Not applicable
Na	Sodium

NaCl	Sodium chloride
Na_2EDTA	Disodium ethylenediaminetetraacetate
NaOH	Sodium hydroxide
ND	Not detected
NF	National Formulary
NLT	Not less than
nm	Nanometer
NMR	Nuclear magnetic resonance
NMT	Not more than
NP	Not performed
OQ	Operational qualification
P	Phosphorus
PEEK	Polyetheretherketone
pH	Minus the decimal logarithm of the hydrogen ion activity in a solution
Ph.Eur.	European Pharmacopeia
pK_a	Acid dissociation constant
ppm	Parts per million
PQ	Performance qualification
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
QC	Quality control
RF	Radio frequency
RH	Relative humidity
RRT	Relative retention time
s	Second
Sn	Tin
T	Tesla
T_1	Time constant for hyperpolarization decay
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMEDA	N, N, N´,N´-tetramethylethylenediamine
TR	Repetition time
TRIS	Tris (hydroxymethyl) aminomethane Trometamol
TRIS/EDTA dissolution medium	Component of the drug product kit for nonclinical studies and clinical studies GE-101-001/GE-101-003: solution used to dissolve the non-hyperpolarized mixture of pyruvic acid and AH111501 sodium salt
TRIS/EDTA buffer solution	Component of the drug product kit for clinical study GE-101-002; solution used to neutralize, buffer and dilute dissolved [1-^{13}C]pyruvic acid during compounding of Hyperpolarized Pyruvate (^{13}C) Injection
TVAC	Total viable aerobic count
UCSF	University of California in San Francisco
USP	United States Pharmacopoeia
UV	Ultraviolet
UV/Vis	Ultraviolet-visible
VTI	Variable temperature insert

v/v	Volume per volume
WFI	Water for injection
wk	Week
WVL	Wavelength
w/w	Weight per weight

Bold: preferred term

7.1 Drug Substance

3.2.S.1 General Information ([1-^{13}C]pyruvic acid)

The active ingredient in Hyperpolarized Pyruvate (^{13}C) Injection is hyperpolarized [1-^{13}C]pyruvate. The drug substance is defined as [^{13}C]pyruvic acid, which is neutralized to [1-^{13}C]pyruvate during the compounding process.

In several pre-clinical and clinical studies and during evaluation of stability, pyruvic acid has been used instead of [1-^{13}C]pyruvic acid (see Sections 3.2.P.2.2.1 Formulation Development for Hyperpolarized Pyruvate (^{13}C) Injection and Section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info). In the Section 3.2.S Drug Substance, data are presented for both pyruvic acid and for [1-^{13}C]pyruvic acid. For simplicity, the terminology used in headings and captions is [1-^{13}C]pyruvic acid. Batches containing pyruvic acid are specified by footnotes.

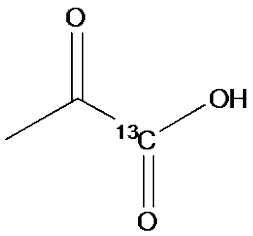
3.2.S.1.1 Nomenclature ([1-^{13}C]pyruvic acid)

The drug substance used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection is [1-^{13}C]pyruvic acid.

Company code: W6578
Chemical name: [1-^{13}C]pyruvic acid
CAS registry number: 127-17-3

3.2.S.1.2 Structure ([1-^{13}C]pyruvic acid)

Figure 1 Structure of [1-^{13}C]pyruvic acid



Molecular formula: $C_3H_4O_3$
Molecular weight: 89.06

3.2.S.1.3 General Properties ([1-^{13}C]pyruvic acid)

Appearance: Colorless to yellow, clear, viscous liquid
pKa:Ka:aranWater solubility: Complete
The structure of [1-^{13}C]pyruvic acid has been confirmed by spectroscopic analysis (see Section 3.2.S.3.1 Elucidation of Structure and other Characteristics).

3.2.S.2.1 Manufacturer ([1-^{13}C]pyruvic acid) (W6578)

The drug substance [1-^{13}C]pyruvic acid (W6578) is manufactured according to Good Manufacturing Practice at the following facility:

Aldrich Chemical Company
ISOTEC Facility
3858 Benner Road
Miamisburg, Ohio 45342
USA

3.2.S.2.2 Description of Manufacturing Process and Process Controls ([1-^{13}C]pyruvic acid)

Figure 2 Total synthesis of [1-^{13}C]pyruvic acid

3.2.S.2.2.1 Synthesis of [1-^{13}C]pyruvic acid

[redacted]

Figure 3 Synthesis of [1-^{13}C]pyruvic acid

3.2.S.2.3 Control of Materials ([1-^{13}C]pyruvic acid)

[redacted]

3.2.S.2.3.1 Raw materials used in the synthesis of [1-^{13}C]pyruvic acid

[redacted]

3.2.S.2.4 Control of Critical Steps and Intermediates ([1-^{13}C]pyruvic acid)

[redacted]

3.2.S.2.5 Process Validation and/or Evaluation ([1-^{13}C]pyruvic acid)

[redacted]

3.2.S.2.6 Manufacturing Process Development ([1-^{13}C]pyruvic acid)

[redacted].

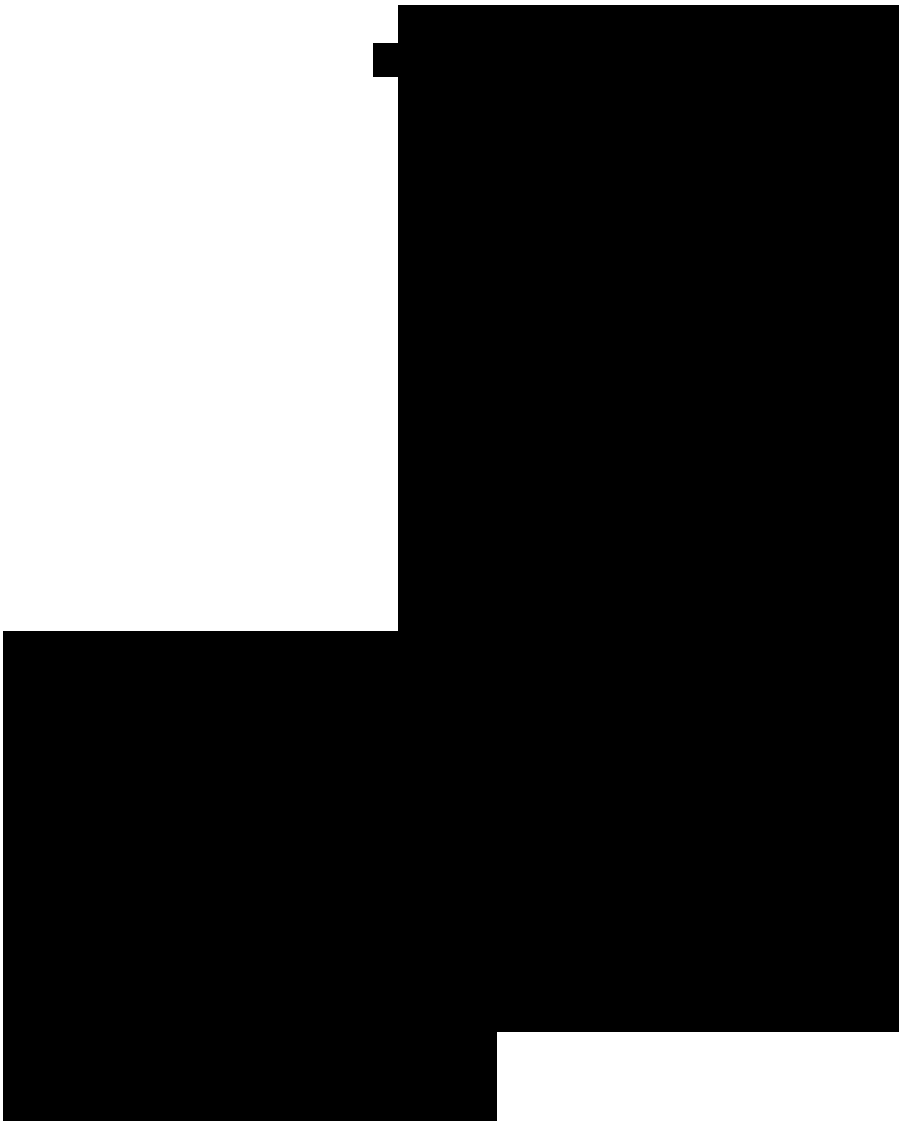
3.2.S.3.1 Elucidation of Structure and other Characteristics ([1-^{13}C]pyruvic acid)

[redacted].

3.2.S.3.1.1 NMR Spectroscopy

[redacted]

Figure 4. Spectra from ^{1}H (upper) and ^{13}C (lower) NMR analysis on [1-^{13}C]pyruvic acid



3.2.S.3.2 Impurities ([1-^{13}C]pyruvic acid)

3.2.S.3.2.1 Potential impurities from the synthesis and degradation products

[redacted]

3.2.S.3.2.2 Analytical procedures

[redacted]

3.2.S.3.2.3 Observed impurities and degradation products

(a) [redacted]

[redacted]

Figure 2. Typical chromatogram from analysis of [1-^{13}C]pyruvic acid

(b) Other impurities

3.2.S.4.1 Specification ([1-^{13}C]pyruvic acid)

Table 1 Specification for [1-^{13}C]pyruvic acid

Tests	Analytical Procedures	Acceptance Criteria
Description Appearance	Visual inspection	Clear, colorless to yellow, viscous liquid
Identification ID of [1-^{13}C]pyruvic acid by NMR	1H-NMR, 13C-NMR	Spectrum conforms with reference
Purity Impurities of [1-^{13}C]pyruvic acid by HPLC AH113462E Total unspecified impurities	HPLC with UV detection	 NMT 3.00% area NMT 2.00% area
Isotopic Enrichment	MS	NLT 99% ^{13}C atom
Water Content	USP	NMT 1.0%
Residual solvent Diethyl ether	USP	 NMT 2500 ppm
Microbiological tests Total viable aerobic count Bacterial endotoxins	 USP USP	 NMT 10 CFU/mL NMT 25 EU/mL
Assay Assay of [1-^{13}C]pyruvic acid	HPLC with UV detection	 NLT 95% (dried basis)

NMT=Not more than
NLT=Not less than

3.2.S.4.2 Analytical Procedures ([1-^{13}C]pyruvic acid)

The analytical procedures used to control [1-^{13}C]pyruvic acid are summarized in the following sections.

3.2.S.4.2.1 Description of [1-^{13}C]pyruvic acid

[1-^{13}C]Pyruvic acid is visually inspected in diffuse daylight.

3.2.S.4.2.2 Identification of [1-^{13}C]pyruvic acid by NMR

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively. Samples were dissolved in deuterium oxide. The ^{1}H chemical shift values were reported on the δ scale in parts per million (ppm) relative to the deuterium oxide peak at 4.63 ppm. The ^{1}H chemical shift values for [1-^{13}C]pyruvic acid were at 2.1 ppm and 1.3 ppm. The ^{13}C chemical shift values were reported on the δ scale in parts per million (ppm) relative to methyl group of [1-^{13}C]pyruvic acid at 28.01 ppm. The^{13}C chemical shift values for [1-^{13}C]pyruvic acid were at 28 ppm, 29 ppm 96 ppm, 166 ppm, 178 ppm, and 200 ppm. The peaks at 166 ppm and 178 ppm in the ^{13}C spectrum correspond to Carbon 1 and were roughly 50 times taller than the rest of the peaks in the ^{13}C spectrum, due to ^{13}C labeling. All spectra were recorded with sample spinning.

3.2.S.4.2.3 Impurities of [1-^{13}C]pyruvic acid by HPLC

[1-^{13}C]Pyruvic acid is dissolved in 25 ml in 75% Acetonitrile (~12/25 mg/ml) and analyzed by using ion exclusion HPLC with an isocratic mobile phase of 100 ml of stock solution of hydrochloric acid (Stock solution = 8.3 ml of concentrated HCl per liter of deionized H_2O) in 1000 ml of final solution and UV detection at 210 nm. AH113462/E is reported specifically in % area. In addition, the sum of unspecified impurities is reported as % area.

3.2.S.4.2.4 Isotopic enrichment

[1-^{13}C]pyruvic acid enrichment was quantified using mass spectrometry. Pyruvic acid was directly infused into the mass spectrometer; ^{13}C-pyruvic acid (*m/z* 89) enrichment was quantified using ESI-MS, negative mode (ions *m/z* 88 and *m/z* 87 were used for the calculation).

3.2.S.4.2.5 Water content

Analysis is performed according to USP<921>.

3.2.S.4.2.6 Residual solvents

Analysis is performed according to USP<467>.

3.2.S.4.2.7 Total viable aerobic count

Analysis is performed according to USP<61>.

3.2.S.4.2.8 Bacterial endotoxins

Analysis is performed according to USP<85>.

3.2.S.4.2.9 Assay of [1-^{13}C]pyruvic acid by HPLC

The purity of [1-^{13}C]pyruvic acid is determined by HPLC assay using an external sodium pyruvate standard. The chromatographic conditions are the same as those presented in Section 3.2.S.4.2.3.

3.2.S.4.3 Batch Analyses ([1-^{13}C]pyruvic acid)

Several batches of [1-^{13}C]pyruvic acid have been used for compounding the mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt for pre-clinical and clinical studies. Results for three batches provided in Table 1 are representative of the quality of all batches used.

Table 1 Batch analysis data for [1-^{13}C]pyruvic acid

	Acceptance criteria	EB2117	MBBB1825V	MBBB4902
Batch size		365 g	430 g	875 g
Place of manufacture		Sigma-Aldrich, Isotec, Miamisburg, OH	Sigma-Aldrich, Isotec, Miamisburg, OH	Sigma-Aldrich, Isotec, Miamisburg, OH
Date of manufacture		11 July 2013	09 January 2014	05 March 2015
Use		Technical studies	Technical studies	Technical studies
Test				
Appearance	Clear, colorless to yellow, viscous liquid	Clear, colorless to yellow, viscous liquid	Clear, colorless to yellow, viscous liquid	Clear, colorless to yellow, viscous liquid
Identification	Conforms	Conforms	Conforms	Conforms
Impurities by HPLC AH113462/E	NMT 3.0% area	ND	ND	ND
Total unspecified impurities	NMT 2.0% area	ND	0.19%	0.13%
Isotopic enrichment	NLT 99% ^{13}C	99.9%	99.9%	99.9%
Water content	NMT 1.0%	0.88%	0.29%	0.13%
Residual solvent Diethyl ether	NMT 2500 ppm	NMT 2500 ppm	8 ppm	6 ppm
Total viable aerobic count	NMT 10 CFU/g	Meets requirement	NMT 10 CFU/g	0 CFU/g
Bacterial endotoxins	NMT 25 EU/mL	Meets requirement	NMT 25 EU/ml	<2.50 EU/mL
Assay of [1-^{13}C]pyruvic acid	NLT 95%	99.80%	99.70%	96.20%

3.2.S.4.4 Justification of Specification ([1-^{13}C]pyruvic acid)

Batches will be released only if the results comply with the acceptance criteria provided in Table 1 in Section 3.2.S.4.1. Final specifications should reflect the process performance. The project is in early development and specifications are preliminary and will be evaluated throughout the development phase. Considering the early stage of the project where only limited batch data are available, the specifications are considered justified.

3.2.S.5 Container Closure System ([1-^{13}C]pyruvic acid)

The container closure system used for [1-^{13}C]pyruvic acid is a Duran Schott amber glass bottle of suitable size (50 mL), Ph.Eur and USP type I, supplied by Duran Schott, USA. The bottles are closed with a thermoplastic screw cap, lined with a Teflon (PTFE) coated sealing plate, supplied by Duran Schott, USA.

3.2.S.6.1 Post-approval Stability Protocol and Stability Commitment ([1-^{13}C]pyruvic acid)

3.2.S.6.1.1 Batch tested

Stability testing is being performed on one batch of pyruvic acid manufactured at Aldrich Chemical Company, ISOTEC Facility, Miamisburg, Ohio, USA. Batch information is given in Table 1.

Table 1 Batch subject to stability studies

Batch number	Batch use	Manufacturing date
MBBB4902V	Technical and pre-clinical studies	January 1, 2015

3.2.S.6.1.2 Storage conditions and testing frequency

Storage conditions and testing frequency are stated in Table 2.

Table 2 Stability study protocol for pyruvic acid

Batch number	Storage conditions			Sampling points (months)
MBBB4902V	°C	Position	Light	
	-20	Upright	Dark	0-3-6-12-18-24

3.2.S.6.1.3 Analytical procedures and specification

The analytical test results from selected stability indicating parameters have been evaluated according to the acceptance criteria presented in Section 3.2.S.4.1 Specifications. The analytical procedures used are described in Section 3.2.S.4.2 Analytical Procedures.

7.2 Drug Product Part 1

3.2.P DRUG PRODUCT (STERILE FLUID PATH COMPONENTS)

Hyperpolarized Pyruvate (^{13}C) Injection (drug product) is a sterile solution for intravenous injection. The compounding of Hyperpolarized Pyruvate (^{13}C) Injection is performed by an automated compounding device known as SpinLab. For each patient dose, SpinLab utilizes a single sterile fluid path which contains the following three drug product components:

- Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt
- TRIS/EDTA buffer solution
- Sterile Water for Injection (WFI)

The following 3.2.P sections describe the individual drug product components. For aspects related to the compounding of the drug product, Hyperpolarized Pyruvate (^{13}C) Injection, reference is made to 3.2.P for Hyperpolarized Pyruvate (^{13}C) Injection.

Commercially available USP quality Sterile Water for Injection (Hospira Inc., USA) is provided by the clinical site. Aspects of this drug product component will therefore not be addressed.

3.2.P.1 Description and Composition of the Drug Product (Sterile Fluid Path Components)

3.2.P.1.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The composition of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is presented in Table 1.

Table 1 Composition of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Ingredient	Quantity per container	Function	Reference to specification
[1-^{13}C]pyruvic acid	1.44 g	Drug Substance	See section 3.2.S.4.1
AH111501 sodium salt	27.7 mg	Excipient	See section 3.2.A.3.4.1

Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is compounded less than 24 hours prior to patient administration. The solution is prepared as a high-risk compounded sterile preparation in compliance with USP <797>. The solution is filtered twice through separate sterilizing (0.2 micron) filters before introduction into the cryovial. The cryovial is part of the custom made container closure system (see Section 3.2.P.7 Container Closure System). Each cryovial contains 1.47 g of solution. The density of the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is 1.26 g/ml.

3.2.P.1.2 TRIS/EDTA buffer solution

The receiving vessel of the sterile fluid path contains a TRIS/EDTA buffer solution that consists of an aqueous solution of tris (hydroxymethyl) aminomethane (TRIS), disodium ethylenediaminetetraacetate (Na_2EDTA) and sodium hydroxide (NaOH).

The composition of TRIS/EDTA buffer solution is presented in Table 2.

Table 2 Composition of TRIS/EDTA buffer solution

Ingredient	Quantity per vial	Function	Reference to specification
TRIS	1089 mg (corresp. to 333 mM)	Buffer	Ph.Eur/USP
NaOH	648 mg (corresp. to 600 mM)	Base	Ph.Eur/USP
Na_2EDTA	9.0 mg	Chelating agent	Ph.Eur/USP
Water for Injection	To 27 ml[1]	Solvent	Ph.Eur/USP

TRIS/EDTA buffer solution is compounded less than 24 hours prior to patient administration. The buffer solution is prepared as a high-risk compounded sterile preparation in compliance with USP <797>. The solution is filtered twice through separate sterilizing (0.2 micron) filters before introduction into the receiving vessel. The receiving vessel is part of the sterile fluid path, the custom-made container closure system (see Section 3.2.P.7 Container Closure System). The quantity of TRIS/EDTA buffer solution aseptically instilled into the receiving vessel is 18 mL.

3.2.P.2.1 Components of the Drug Product (Drug Product Kit Components)

3.2.P.2.1.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

(a) Drug substance

The drug substance, [1−^{13}C]pyruvic acid, is a colorless to yellow, clear, viscous liquid. [1−^{13}C]Pyruvic acid is described in Section 3.2.S Drug Substance.

Upon neutralization in the TRIS/EDTA buffer solution, the [1−^{13}C]pyruvic acid is converted to [1−^{13}C]pyruvate.

(b) Excipients

AH111501 sodium salt is a stable trityl radical, and is added to [1−^{13}C]pyruvic acid to enable hyperpolarization.

AH111501 sodium salt is a green to black, fine to granular powder. AH111501 sodium salt is further described in Section 3.2.A.3 Novel Excipients.

3.2.P.2.1.2 TRIS/EDTA buffer solution

The TRIS/EDTA buffer solution is an aqueous solution containing 333 mM TRIS, 600 mM NaOH and 333 mg/l Na_2EDTA.

TRIS is used as buffer to stabilize the pH of the Hyperpolarized Pyruvate (^{13}C) Injection at a physiologically acceptable level.

NaOH is added to neutralize the [1−^{13}C]pyruvic acid in Mixture of [1−^{13}C]pyruvic acid and 15

mM AH111501 sodium salt to [1−^{13}C]pyruvate in the Hyperpolarized Pyruvate (^{13}C) Injection.

Na_2EDTA has been included in the formulation as a chelating agent to capture traces of paramagnetic metal ions that might be present.

3.2.P.2.2 Drug Product (Drug Product Kit Components)

3.2.P.2.2.1 Formulation Development

(a) Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is dissolved in WFI and neutralized/buffered in TRIS/EDTA buffer solution to form a solution with a physiologically acceptable pH.

The concentration of AH111501 sodium salt of 15 mM has been chosen for optimization of ^{13}C nuclear polarization in Hyperpolarized Pyruvate (^{13}C) Injection. For clinical trials GE-101-001 and GE-101-003, pyruvic acid was used instead of [1-^{13}C]pyruvic acid. For these trials the Pyruvate Injection was not compounded hence; in order to mimic the maximum content of AH111501 in Hyperpolarized Pyruvate (^{13}C) Injection, the kit component used during the clinical trials GE-101-001 and GE 101-003 was Mixture of pyruvic acid and 0.2 mM AH111501 sodium salt. In addition, some pre-clinical studies were performed using pyruvic acid instead of [1-^{13}C]pyruvic acid. See Section 3.2.P.2.2.1 Formulation development for Hyperpolarized Pyruvate (^{13}C) Injection and section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info) for further details.

The amount of [1-^{13}C]pyruvic acid and AH111501 sodium salt mixture per cryovial is 1.47 g, which upon dissolution in the total volume of WFI and TRIS/EDTA buffer solution, gives 250 mM [1-^{13}C]pyruvate in the final Hyperpolarized Pyruvate (^{13}C) Injection.

(b) TRIS/EDTA buffer solution

The function of TRIS/EDTA buffer solution is to neutralize the [1-^{13}C]pyruvic acid to [1-^{13}C]pyruvate and to assure a physiologically acceptable pH of the drug product Hyperpolarized Pyruvate (^{13}C) Injection.

TRIS/EDTA buffer solution has not been used during pre-clinical studies or during clinical trials GE-101-001 and GE-101-003. For these studies, the Mixture of [1-^{13}C]pyruvic acid and AH111501 sodium salt was dissolved in a single, manual step in TRIS/EDTA dissolution medium. For compounding of Hyperpolarized Pyruvate (^{13}C) Injection, the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt will first be dissolved in WFI and then neutralized and buffered in TRIS/EDTA buffer solution. See Section 3.2.P.2.2.1 Formulation Development for Hyperpolarized Pyruvate (^{13}C) Injection for details.

The amount of [1-^{13}C]pyruvic acid to be dissolved is 1.67 g (equivalent to 18.75 mmol). This amount of acid is neutralized and buffered with 22.5 ml of TRIS/EDTA buffer solution (equivalent to 8.33 mmol of TRIS and 15.00 mmol of NaOH) to a target pH of 7.6 (at 37°C) in the Hyperpolarized Pyruvate (^{13}C) Injection.

3.2.P.2.2.2 Overages

(a) Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

There are no overages included in the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt.

(b) TRIS/EDTA buffer solution

There are no overages included in the TRIS/EDTA buffer solution.

3.2.P.2.3 Manufacturing Process Development (Drug Product Kit Components)

3.2.P.2.3.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Terminal sterilization of the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is not possible due to degradation of [1-^{13}C]pyruvic acid. The current process is therefore performed by aseptic processing.

3.2.P.2.3.2 TRIS/EDTA buffer solution

Terminal sterilization of TRIS/EDTA buffer solution in various container closure systems has been tested, but generation of particles occurred during sterilization. This is probably caused by the high pH of the TRIS/EDTA buffer solution. The current process is therefore performed by aseptic processing.

3.2.P.2.4 Container Closure System (Sterile Fluid Path Components)

3.2.P.2.4.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The compounding process for Hyperpolarized Pyruvate (^{13}C) Injection requires a custom made container closure system, the sterile fluid path, for the Mixture of [1-^{1}3C]pyruvic acid and 15 mM AH111501 sodium salt. This container closure system is described in more detail in Section 3.2.P.7 Container Closure System.

3.2.P.2.4.2 TRIS/EDTA buffer solution

The compounding process for Hyperpolarized Pyruvate (^{13}C) Injection requires a custom made container closure system, the sterile fluid path, for the TRIS/EDTA buffer solution. This container closure system is described in more detail in Section 3.2.P.7 Container Closure System.

3.2.P.2.5 Microbiological Attributes (Sterile Fluid Path Components)

3.2.P.2.5.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Not applicable.

The mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 is compounded immediately prior to patient administration. A sample of the final Hyperpolarized Pyruvate (^{13}C) Injection is tested for

sterility and pyrogenicity subsequent to patient administration.

3.2.P.2.5.2 TRIS/EDTA buffer solution

Not applicable

The TRIS/EDTA buffer solution is compounded immediately prior to patient administration. A sample of the final Hyperpolarized Pyruvate (^{13}C) Injection is tested for sterility and pyrogenicity subsequent to patient administration.

3.2.P.3.1 Manufacturer(s) (Sterile Fluid Path Components)

3.2.P.3.1.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The compounding of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt for clinical use is conducted in accordance with compliance of USP <797> and the regulations promulgated by the California State Board of Pharmacy at the licensed pharmacy on the following academic campus:

University of California, San Francisco
Department of Clinical Pharmacy
San Francisco, California 94118

3.2.P.3.1.2 TRIS/EDTA buffer solution

The compounding of TRIS/EDTA buffer solution for clinical use is conducted in accordance with compliance of USP <797> and the regulations promulgated by the California State Board of Pharmacy at the licensed pharmacy on the following academic campus:

University of California, San Francisco
Department of Clinical Pharmacy
San Francisco, California 94118

3.2.P.3.2 Single Dose Compounding Formula (Sterile Fluid Path Components)

3.2.P.3.2.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is compounded by aseptic processing. The compounding formula for a single dose prepared immediately prior to patient administration is given in Table 1.

Table 1 Compounding formula for Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Ingredient	Quantity per container
[1-^{13}C]pyruvic acid	1.44 g
AH111501 sodium salt	27.7 mg

3.2.P.3.2.2 TRIS/EDTA buffer solution

The product comprises an aqueous solution of TRIS, NaOH, and Na2EDTA. The product is compounded by aseptic processing. The compounding formula for a single dose prepared immediately prior to patient administration is given in Table 2.

Table 2 Compounding formula for TRIS/EDTA buffer solution

Ingredient	Quantity per vial
TRIS	1089 mg (corresp. to 333 mM)
NaOH	648 mg (corresp. to 600 mM)
Na_2EDTA	9.0 mg
Water for Injection	To 27 ml[1]

[1]Quantity of sterile TRIS/EDTA buffer solution aseptically instilled into receiving vessel of sterile fluid path is 18 mL.

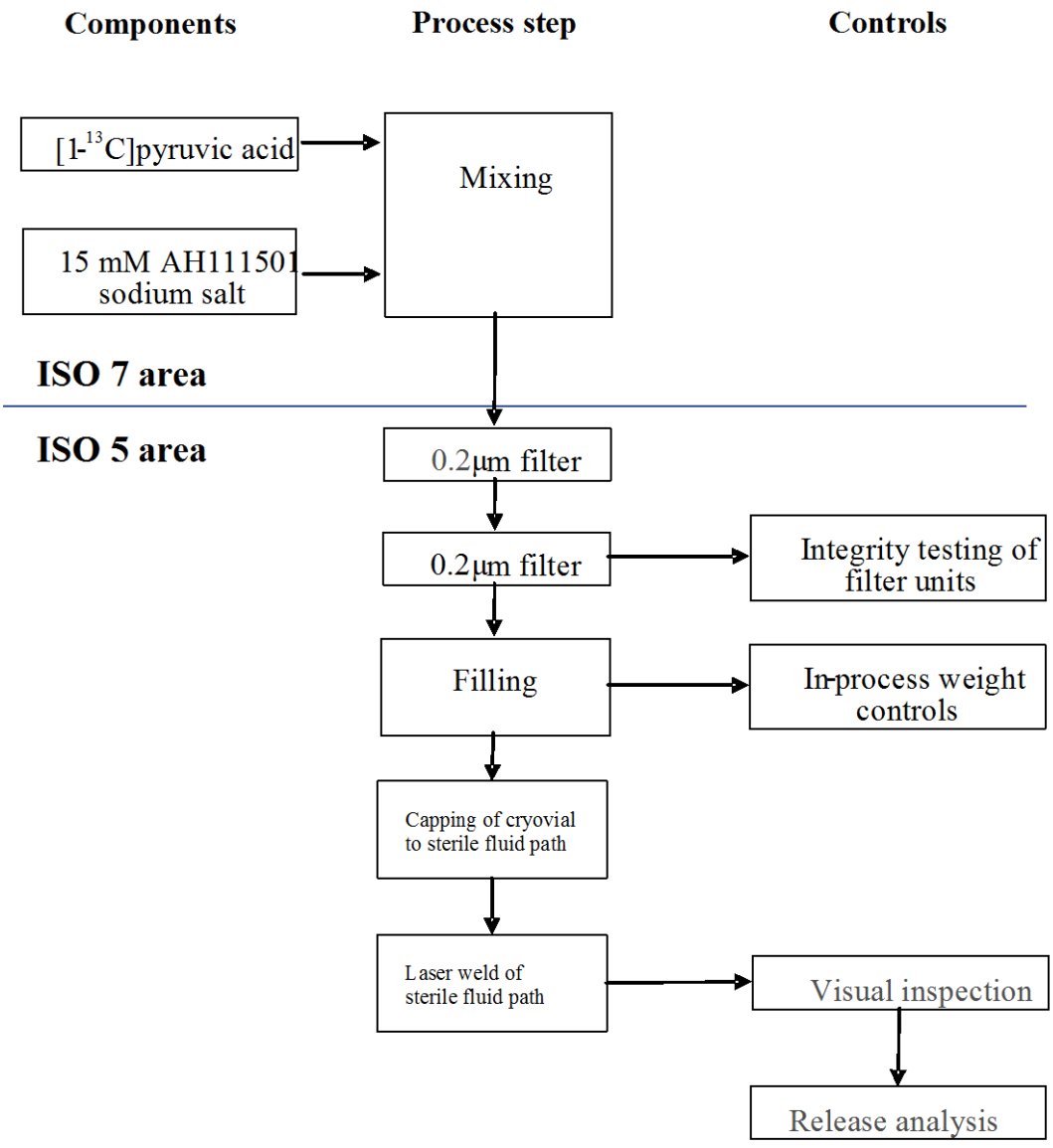
3.2.P.3.3 Description of Manufacturing Process and Process Controls (Drug Product Kit Components)

3.2.P.3.3.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The preparation of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is performed in an ISO 7 area.

[1-^{13}C]Pyruvic acid and AH111501 sodium salt are weighed out and added to the preparation vessel in successive order. The solution is allowed to stir to ensure a homogenous solution prior to filtration. As the solution is transferred from the preparation vessel in an ISO 7 area to the filling vessel in an ISO 5 area, it is filtered through two 0.2 μm sterilizing filters. Filling is performed in an ISO 5 area (LAF unit). The filling weight is calibrated to target; each cryovial shall contain 1.47 g of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt, therefore, the filling weight depends on the assay of the specific batch of [1-^{13}C]pyruvic acid used. Each container is weighed during the filling operation. The compounding process is illustrated in Figure 1.

Figure 1 **Flow chart illustrating the manufacturing process of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt**



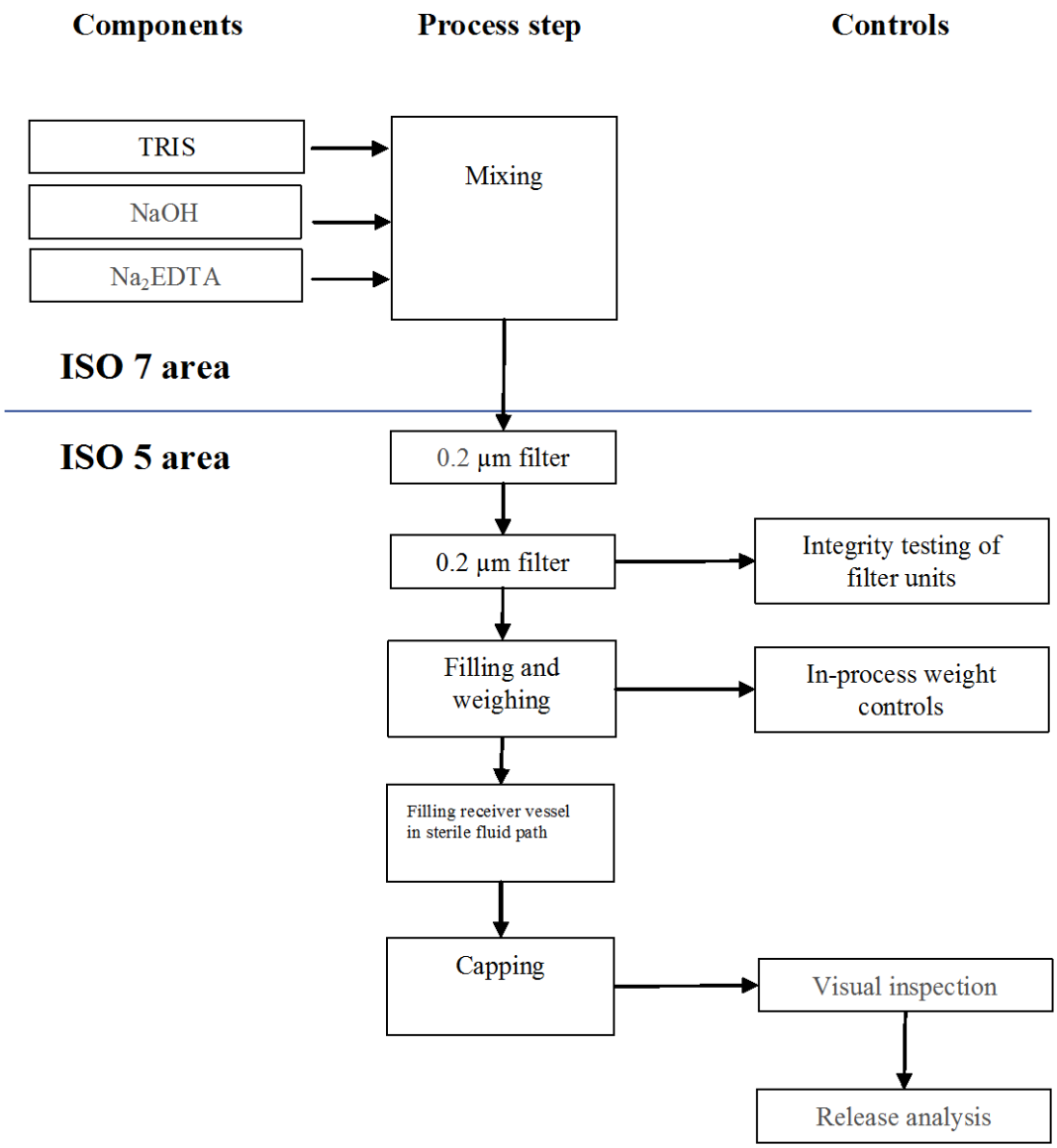
3.2.P.3.3.2 TRIS/EDTA buffer solution

The preparation of TRIS/EDTA buffer solution is performed in an ISO 7 area.

Approximately 90% of the total amount of WFI is added to the preparation vessel. TRIS, Na_2EDTA and NaOH are added successively, allowing each one to dissolve completely by sufficiently stirring between each addition. The bulk solution is adjusted to its final weight by addition of WFI and allowed to stir to ensure a homogenous solution prior to filtration. As the solution is transferred from the preparation vessel in an ISO 7 area to the filling vessel in a ISO 5 area, it is filtered through two 0.2 µm sterilizing filters. Aseptic filling of the TRIS/EDTA buffer solution into the receiving vessel of the sterile fluid path is performed in an ISO 5 area (LAF unit). Weight controls are taken regularly during filling to assure acceptable fill volume for the whole batch.

The manufacturing process is illustrated in Figure 2.

Figure 2 Flow chart illustrating the manufacturing process of TRIS/EDTA buffer solution



3.2.P.3.4 Controls of Critical Steps and Intermediates (Sterile Fluid Path Components)

3.2.P.3.4.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

A schematic representation of the process flow and the in-process controls is presented in Figure 1, Section 3.2.P.3.3.1. In addition, environmental monitoring (microbiological and non-viable particles) of the production area is performed.

3.2.P.3.4.2 TRIS/EDTA buffer solution

A schematic representation of the process flow and the in-process controls is presented in Figure 2, Section 3.2.P.3.3.2. In addition, environmental monitoring (microbiological and non-viable particles) of the production area is performed.

3.2.P.3.5 Process Validation and/or Evaluation (Sterile Fluid Path Components)

3.2.P.3.5.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

The aseptic compounding process has been validated by simulation of the aseptic process using a microbial nutrient medium. No growth has been observed in any of the media fill batches. Monitoring of the clean room and personnel is carried out and controlled on a routine basis to assure an environment suitable for aseptic processing.

3.2.P.3.5.2 TRIS/EDTA buffer solution

The aseptic compounding process has been validated by simulation of the aseptic process using a microbial nutrient medium. No growth has been observed in any of the media fill batches. Monitoring of the clean room and personnel is carried out and controlled on a routine basis to assure an environment suitable for aseptic processing.

3.2.P.4 Control of Excipients (Sterile Fluid Path Components)

The only excipient in the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is the novel excipient AH111501 sodium salt, which is described in Section 3.2.A.3 Novel Excipients. Section 3.2.P.4 Control of Excipients will therefore provide information on excipients in the TRIS/EDTA buffer solution only.

3.2.P.4.1 Specifications (Sterile Fluid Path Components)

Excipients in the TRIS/EDTA buffer solution are listed below:

Sodium hydroxide	Complies with Ph. Eur./USP(NF)
Trometamol	Complies with Ph. Eur./USP(NF)
Na_2EDTA	Complies with Ph. Eur./USP(NF)

3.2.P.4.2 Analytical Procedures (Sterile Fluid Path Components)

The analytical procedures for the excipients in TRIS/EDTA buffer solution are described in the Ph. Eur. and USP(NF).

3.2.P.4.3 Validation of Analytical Procedures (Sterile Fluid Path Components)

The methods are standard compendial methods.

3.2.P.4.4 Justification of Specifications (Sterile Fluid Path Components)

Batches of the excipients to be used in the manufacture of TRIS/EDTA buffer solution for clinical trials will be released according to the specifications provided in the appropriate pharmacopoeias.

3.2.P.4.5 Excipients of Human or Animal Origin (Sterile Fluid Path Components)

No excipient used in the manufacture of TRIS/EDTA buffer solution are of human or animal origin.

3.2.P.4.6 Novel Excipients (Drug Product Kit Components)

There are no novel excipients in the TRIS/EDTA buffer solution.

3.2.P.5 Control of Drug Product Kit Components

3.2.P.5.1 Specification(s) (Sterile Fluid Path Components)

3.2.P.5.1.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Not applicable.

The Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is prepared immediately prior to patient administration.

3.2.P.5.1.2 TRIS/EDTA buffer solution

Not applicable.

The TRIS/EDTA buffer solution is prepared immediately prior to patient administration.

3.2.P.5.2 Analytical Procedures (Sterile Fluid Path Components)

3.2.P.5.2.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Not applicable.

The Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is prepared immediately prior to patient administration.

3.2.P.5.2.2 TRIS/EDTA buffer solution

Not applicable.

The TRIS/EDTA buffer solution is prepared immediately prior to patient administration.

3.2.P.5.3 Validation of Analytical Procedures (Sterile Fluid Path Components)

3.2.P.5.3.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

Not applicable.

3.2.P.5.3.2 TRIS/EDTA buffer solution

Not applicable.

3.2.P.5.4 Batch Analysis (Sterile Fluid Path Components)

Not applicable.

Compounding of the mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt in addition to compounding of the TRIS/EDTA buffer solution are both prepared immediately prior to patient administration and released by a licensed pharmacist for installation into the sterile fluid path.

3.2.P.5.5 Characterization of Impurities (Sterile Fluid Path Components)

Not applicable.

3.2.P.5.6 Justification of Specification(s) (Sterile Fluid Path Components)

3.2.P.5.6.1 Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt

A sterile fluid path containing the mixture of [1-^{13}C]pyruvic acid, which is prepared immediately prior to patient administration, will be released by a licensed pharmacist for compounding by the

automated compounding device, SpinLab, only if the procedures for aseptic compounding the solution are satisfied. The project and utility of SpinLab for automatic compounding of the Hyperpolarized pyruvate (^{13}C) injection drug product is in early development and preliminary specifications may be developed and evaluated as this project continues in the development phase. Considering the early stage of the project and only single doses are compounded immediately prior to patient administration by licensed pharmacy personnel, the specifications are considered justified.

3.2.P.5.6.2 TRIS/EDTA buffer solution

A sterile fluid path containing the TRIS/EDTA buffer solution, which is prepared immediately prior to patient administration, will be released by a licensed pharmacist for compounding by the automated compounding device, SpinLab, only if the procedures for aseptic compounding the solution are satisfied. The project and utility of SpinLab for automatic compounding of the Hyperpolarized pyruvate (^{13}C) injection drug product is in early development and preliminary specifications may be developed and evaluated as this project continues in the development phase. Considering the early stage of the project and only single doses are compounded immediately prior to patient administration by licensed pharmacy personnel, the specifications are considered justified.

3.2.P.6 Reference Standards or Materials (Sterile Fluid Path Components)

Not applicable.

3.2.P.7 Container Closure System (Sterile Fluid Path)

The fluid path system is a single, sterile drug product container, container closure system that provides for rapid and complete dissolution of a frozen hyperpolarized drug product and transports the resulting hyperpolarized drug product solution from its initial location within a polarizer system to a final sterile Medrad syringe outside the polarizer system for clinical administration—injection into a patient.

The empty sterile fluid path (Figure 1A) is provided in a double bag plastic tray with a lid of the following approximate size:

60 cm (L) x 35.6 cm (width) x 10.2 cm (depth)/unit or
23.6 inch (L) x 14.0 inch (width) x 4.0 inch (depth)/unit
The empty sterile fluid path is designed to be a single-use drug product container, container closure system which upon arrival to a licensed pharmacy, can be aseptically manipulated so that it can be charged with the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt, TRIS/EDTA buffer, and sterile water for injection.

The key components of the empty sterile fluid path system that are in contact with the drug product are composed of USP Plastic Class V as follows:

- A Radel R Plastic sample vial which will serve to contain a mixture of the drug substance [1-^{13}C] Pyruvic Acid and the Electron Paramagnetic Agent (EPA) excipient, tris(8-carboxyl-2,2,6,6-tetra(2-(l-methoxy-2,2-d2-ethyl))-benzo[l ,2-d:4,5-d']bis(dithiole-4-yl)methyl sodium

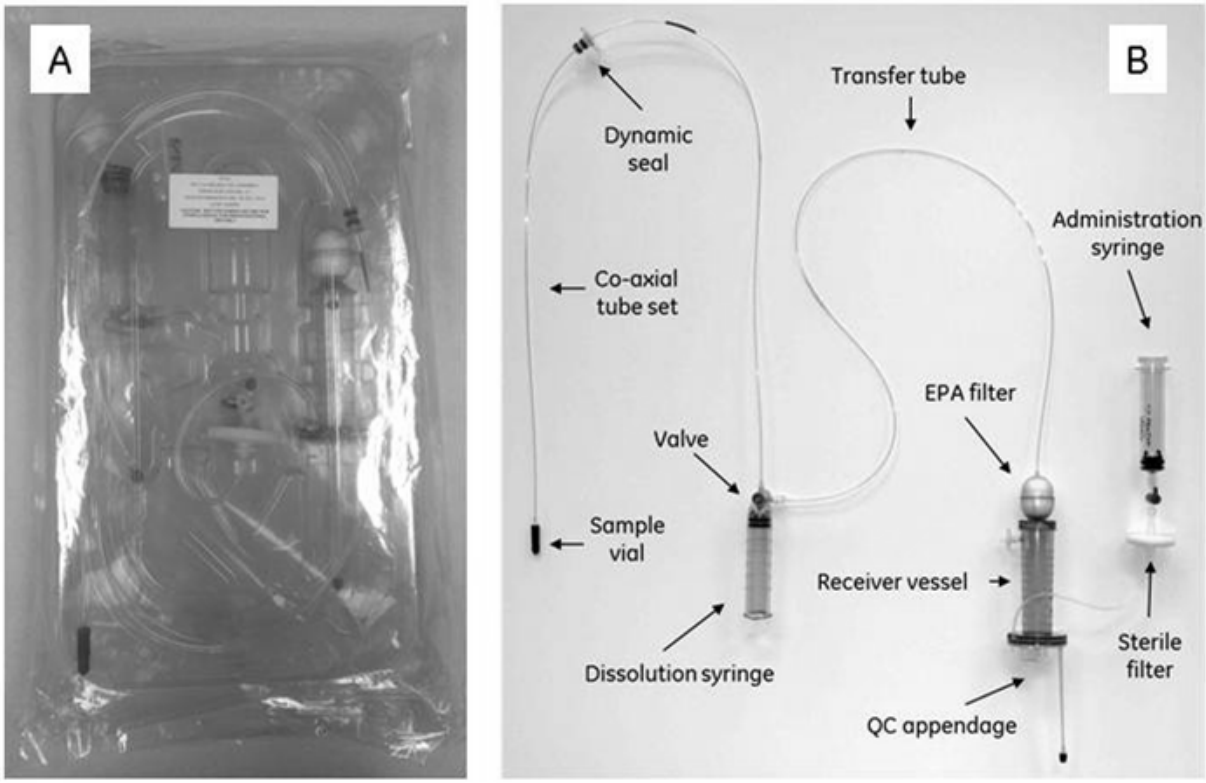
salt, AH111501 Sodium Salt.
- A Radel R Plastic syringe which will serve to contain sterile water for injection.
- A Radel R Plastic receiver vessel which will serve to contain an aqueous solution of (hydroxymethyl)aminomethane (TRIS), disodium ethylenediaminetretraacetate (Na_2EDTA) and sodium hydroxide (NaOH).
- A Radel R plastic casing containing the EPA ultrahigh molecular weight polyethylene filters.
- A Tygon plastic tubing connecting the Receiver vessel to the Sterile filter.
- The rest of the assembly is composed of Radel R Plastic co-axial and transfer tubes and Udel plastic valves.

The Dynamic seal is designed and integrated into the empty sterile fluid path however it is not in contact with the drug product.

The QC appendage is designed and integrated into the empty sterile fluid path, however it is not in contact with the drug product as an aliquot of the drug product is transferred to the QC appendage.

Commercially available SSQK 65/115VS Syringe Kits (Bayer Inc., USA) containing a sterile 65 mL Qwik-Fit Syringe which is aseptically added to the sterile empty fluid path for collection of the final drug product, Hyperpolarized Pyruvate (^{13}C) Injection, will not be addressed here and is depicted as Administration syringe.

Figure 1A Depiction of empty sterile fluid path in packaging.
Figure 1B Basic anatomy of an empty sterile fluid path.



7.3 Drug Product Part 2

3.2.P DRUG PRODUCT (HYPERPOLARIZED PYRUVATE [^{13}C] INJECTION)

Hyperpolarized Pyruvate (^{13}C) Injection (drug product) is a sterile solution for intravenous injection. Compounding the Hyperpolarized Pyruvate (^{13}C) Injection requires the following drug product components:

- Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt
- TRIS/EDTA buffer solution
- Sterile Water for Injection (WFI)

Hyperpolarized Pyruvate (^{13}C) Injection is compounded at the clinical site utilizing an automated compounding device, known as SpinLab, according to USP <797> Pharmaceutical Compounding – Sterile Preparations, just prior to administration. For each patient does, SpinLab utilizes a single sterile fluid path that is composed of a cryovial which contains the mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt. The cryovial is lowered into the polarizer and polarized for up to 120 minutes at a temperature of approximately 0.8 K. After polarization, the mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is flushed out of the cryovial with heated and pressurized sterile WFI within the sterile fluid path then passed through a mechanical filter for removal of AH111501, then emptied into a receiver vessel containing sterile WFI and TRIS/EDTA buffer solution. A sample of solution from the receiver vessel is automatically extracted for testing by an automated quality control instrument (QC System). While the QC System processes the solution sample, the remaining solution in the receiver vessel is passed through a sterilizing filter (0.2 μm) and then enters the final drug product container for patient administration, a 65 mL MedRad syringe. Based on the results from the QC System, the final release authorization for administration to humans will be performed by a licensed pharmacist.

The following 3.2.P sections describe the Hyperpolarized Pyruvate (^{13}C) Injection. For aspects related to the drug product components required for compounding the Hyperpolarized Pyruvate (^{13}C) Injection, reference is made to section 3.2.P for Drug Product Kit Components.

3.2.P.1 Description and Composition of the Drug Product (Hyperpolarized Pyruvate [^{13}C] Injection)

Hyperpolarized Pyruvate (^{13}C) Injection is compounded from three drug product components: (1) Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt, (2) TRIS/EDTA buffer solution and (3) sterile WFI.

The composition of Hyperpolarized Pyruvate (^{13}C) Injection is presented in Table 1.

Table 1 Composition of Hyperpolarized Pyruvate (^{13}C) Injection

Ingredient	Quantity per ml	Function	Reference to specification
[1-^{13}C]pyruvate	22.0 mg (corresponding to 250 mM)	Drug Substance	See section 3.2.S.4.1[1]
TRIS	12.1 mg (corresponding to 100 mM)	Excipient	See section 3.2.P.4.1 for Drug Product Kit Components
Sodium	4.1 mg (corresponding to 180 mM)	Excipient	See section 3.2.P.4.1 for Drug Product Kit Components[2]
Na_2EDTA	0.1 mg	Excipient	See section 3.2.P.4.1 for Drug Product Kit Components
AH111501 (as tri-anion)	NMT 4.6 µg (corresponding to NMT 3.0 µM)	Excipient	See section 3.2.A.3.4.1
Sterile Water for Injection	To 1 ml	Diluent	Compliant to USP

[1] Note that the referred specification is for [1-^{13}C]pyruvic acid which is neutralized to [1-^{13}C]pyruvate during the compounding process.

[2] The sodium in Hyperpolarized Pyruvate (^{13}C) Injection stems from the sodium hydroxide added for neutralization.

Hyperpolarized Pyruvate (^{13}C) Injection is supplied via a sterile disposable Medrad Qwik-Fit Syringe® for contrast media with a fill volume of 65 mL.

3.2P.2.1 Components of the Drug Product (Hyperpolarized Pyruvate (^{13}C) Injection)

3.2.P.2.1.1 Drug substance

The drug substance, [1−^{13}C]pyruvic acid, is a colorless to yellow, clear, viscous liquid. [1−^{13}C]Pyruvic acid is described in Section 3.2.S Drug Substance.

After neutralization in the TRIS/EDTA buffer solution, the [1−^{13}C]pyruvic acid is present as [1−^{13}C]pyruvate.

3.2.P.2.1.2 Excipients

AH111501 sodium salt is a stable trityl radical, and is added to [1−^{13}C]pyruvic acid to enable hyperpolarization. After hyperpolarization and compounding, the solution is passed through a filter to remove the AH111501 from the drug product.

AH111501 sodium salt is a green to black, fine to granular powder. AH111501 sodium salt is further described in Section 3.2.A.3 Novel Excipients.

The TRIS/EDTA buffer solution is an aqueous solution containing 333 mM TRIS, 600 mM NaOH and 333 mg/l Na_2EDTA.

TRIS is added as a buffer to stabilize the pH of the Hyperpolarized Pyruvate (^{13}C) Injection at a physiologically acceptable level.

NaOH is added to neutralize the [1−^{13}C]pyruvic acid in the Mixture of [1−^{13}C]pyruvic acid and 15 mM AH111501 sodium salt to [1−^{13}C]pyruvate in the Hyperpolarized Pyruvate (^{13}C) Injection.

Na_2EDTA has been included in the formulation as a chelating agent to capture traces of

paramagnetic metal ions that might be present.

3.2.P.2.2 Drug Product (Hyperpolarized Pyruvate [^{13}C] Injection)

3.2.P.2.2.1 Formulation Development

The drug product kit components used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection in the polarizer differ slightly from the components used for pre-clinical studies and clinical studies GE-101-001 and GE-101-003 (see Section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info). These differences are explained in the following sections and are summarized in Table 1.

(a) Pyruvic acid and [1-^{13}C]pyruvic acid

Drug product used for clinical studies GE-101-001 and GE-101-003 was not hyperpolarized. As the need for ^{13}C enriched material was not present, the drug substance used was pyruvic acid, whereas the drug substance used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection is [1-^{13}C]pyruvic acid. Some pre-clinical safety studies were also conducted using pyruvic acid (see Section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info).

(b) Content of AH111501 sodium salt

AH111501 is removed during compounding of Hyperpolarized Pyruvate (^{13}C) Injection, and the content of this excipient in the final drug product is NMT 3.0 µM. To mimic this situation for clinical studies GE-101-001 and GE-101-003, 0.2 mM AH111501 sodium salt was added to the pyruvic acid in order to obtain 3.0 µM AH111501 in the Pyruvate Injection. For most of the pre-clinical studies (see Section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info) and for compounding of Hyperpolarized Pyruvate (^{13}C) Injection, 15 mM AH111501 sodium salt is added to the [1-13C]pyruvic acid.

(c) Content of [1-^{13}C]pyruvate in drug product

The drug product was initially formulated to contain 500 mM [1-^{13}C]pyruvate. For this formulation, a Mixture of [1-^{13}C]pyruvic acid and AH111501 sodium salt, containing 2.23 g [1-^{13}C]pyruvic acid, was dissolved in 50 ml TRIS/EDTA dissolution medium, containing 360 mM NaOH, 200 mM TRIS and 100 mg/l Na_2EDTA. Because pre-clinical studies using this formulation revealed cardiovascular effects (see Sections 8.2.4.3 Effects on the Cardiovascular Systems (CVS) in the Pentobarbital/Fentanyl Anesthetized Dog, subsections a and b, for Item 8 Pharmacology and Toxicology Info) the product was later reformulated to contain 250 mM [1-^{13}C]pyruvate. For this formulation, Mixture of [1-^{13}C]pyruvic acid and AH111501 sodium salt, containing 2.23 g [1-^{13}C]pyruvic acid, was dissolved in 100 ml TRIS/EDTA dissolution medium, containing 180 mM NaOH, 100 mM TRIS and 100 mg/l Na_2EDTA. Some pre- clinical studies were performed with the formulation targeted 500 mM [1-^{13}C]pyruvate. For most pre-clinical (see Section 8.1 Introduction for Item 8 Pharmacology and Toxicology Info) and all clinical studies, the Pyruvate (^{13}C) Injection is targeted to contain 250 mM [1-^{13}C]pyruvate.

(d) TRIS/EDTA dissolution medium and TRIS/EDTA buffer solution

For clinical studies GE-101-001 and GE-101-003 and pre-clinical studies, the Mixture of pyruvic acid and AH111501 sodium salt was dissolved in TRIS/EDTA dissolution medium in a single step by manual dissolution (see section 3.2.P.2.3 Manufacturing Process Development). The

compounding of Hyperpolarized Pyruvate (^{13}C) Injection in the polarizer applies a two step dissolution, where the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is first dissolved in sterile WFI and subsequently neutralized, buffered and diluted in TRIS/EDTA buffer solution. The TRIS/EDTA buffer solution contains 600 mM NaOH, 333 mM TRIS and 333 mg/l Na_2EDTA.

The drug product kit components used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection in the polarizer are formulated such that the drug product is equivalent to the drug product used for clinical studies GE-101-001 and GE-101-003. The different combinations of drug product kit components investigated are summarized in Table 1 (overleaf), along with the resulting composition of the (Hyperpolarized) Pyruvate (^{13}C) Injection.

Table 1 Different drug product kit components investigated during pre-clinical and clinical trials and the resulting composition of (Hyperpolarized Pyruvate (^{13}C) Injection

Formulation	A	B	C	D
Use	Pre-clinical studies	Pre-clinical studies	Clinical study GE-101-001 GE-101-003	Clinical study GE-101-002
[1-^{13}C]pyruvic acid per container (g)[1]	2.23	2.23	2.20	1.67
Concentration of AH111501 sodium salt in Mixture of [1-^{13}C]pyruvic acid and AH111501 sodium salt (mM)	15	15	~ 0.2[2]	15
Composition of TRIS/EDTA dissolution medium[3]	360/200/100	180/100/100	180/100/100	NA
Volume of TRIS/EDTA dissolution medium (ml)	50	100	100	NA
Volume of sterile WFI for dissolution (ml)	NA	NA	NA	45[4]
Composition of TRIS/EDTA buffer solution[3]	NA	NA	NA	600/333/333
Volume of TRIS/EDTA buffer solution	NA	NA	NA	22.5
Compounding procedure	Manual dissolution	Manual dissolution	Manual dissolution	Semi-automated compounding sequence
Composition of (Hyperpolarized) Pyruvate (^{13}C) Injection:				
[1-^{13}C]pyruvate (mM)	500	250	250	250
AH111501 sodium salt (µM)	~ 516[5]	~ 262[5]	3.0	NMT 3.0[6]
TRIS (mM)	200	100	100	100
Na_2EDTA (mg/l)	100	100	100	100

NA = Not Applicable

[1] Formulations A and B containing pyruvic acid have also been used. Formulation C contains pyruvic acid. In these cases the target amount of pyruvic acid per container was 2.20 g.

[2] Approximately. Actual amount added was targeted to a concentration of 3.0 µM in the Pyruvate Injection.

[3] Represents the concentration of NaOH (mM)/TRIS (mM)/Na_2EDTA (mg/l), respectively.

[4] Whereof 30 ml in the heating vessel and 15 ml in the receiver vessel. Additionally the drug product is diluted by 7.5 ml of sterile WFI when passing the C18 column for removal of AH111501 giving a total sterile WFI volume of 52.5 ml

[5] Approximately. Actual amount added was targeted to a concentration of 15 mM in the Mixture of [1-^{13}C]pyruvic acid and AH111501 sodium salt

[6] Release limit for content of AH111501 in Hyperpolarized Pyruvate (^{13}C) Injection

3.2.P.2.2.2 Overages

There are no overages included in Hyperpolarized Pyruvate (13C) Injection.

3.2.P.2.3 Manufacturing Process Development (Hyperpolarized Pyruvate [^{13}C] Injection)

The procedure for compounding of Hyperpolarized Pyruvate (^{13}C) Injection was not used for pre-clinical studies or clinical studies GE-101-001 and GE-101-003. The Pyruvate (^{13}C) Injection for these studies was prepared by manual mixing of the drug product kit components as described in the following section.

Prior to mixing the drug product kit components, the components were allowed to reach ambient room temperature. 100 mL of TRIS/EDTA dissolution medium was then added to the vial containing the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt. Immediately, the vial was vigorously shaken for at least 30 seconds to assure homogeneity. The vial was then heated in a 80°C water bath for 10 minutes and cooled in cold tap water for 5 minutes. The vial is then stored in a 37°C water bath for a maximum of 4 hours before use.

3.2.P.2.4 Container Closure System (Hyperpolarized Pyruvate [^{13}C] Injection)

The container closure system for Hyperpolarized Pyruvate (^{13}C) Injection is custom made. The container closure system is described in more detail in Section 3.2.P.7 Container Closure System.

3.2.P.2.5 Microbiological Attributes (Hyperpolarized Pyruvate [^{13}C] Injection)

The residence time of Hyperpolarized Pyruvate (^{13}C) Injection in the drug product container is less than 60s. Based on this short user window and reproductive times of potential microbiological contaminants, microbiological growth is not considered to be significant. No container closure integrity testing has therefore been performed.

3.2.P.3.1 Manufacturer (Hyperpolarized Pyruvate (^{13}C) Injection)

Hyperpolarized Pyruvate (^{13}C) Injection is compounded at the clinical site according to USP <797> Pharmaceutical Compounding – Sterile Preparations. All drug product kit components used for compounding are manufactured according to Good Manufacturing Practice (see Section 3.2.P.3 Manufacture of Drug Product Kit Components).

The compounding of Hyperpolarized Pyruvate (^{13}C) Injection is conducted in accordance with <797> Pharmaceutical Compounding – Sterile Preparations at the following facility:

University of California San Francisco 1700 4th Street San Francisco, CA 94518-2512 USA

3.2.P.3.2 Batch Formula (Hyperpolarized Pyruvate (^{13}C) Injection)

The drug product kit components used in compounding of Hyperpolarized Pyruvate (^{13}C) Injection are provided in Table 1.

Table 1 Manufacturing formula for Hyperpolarized Pyruvate (^{13}C) Injection

Sterile fluid path component	Quantity per compounding
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt	1.47 g
Sterile WFI	56.5 mL
TRIS/EDTA buffer solution	18 mL

3.2.P.3.3 Description of Manufacturing Process and Process Controls (Hyperpolarized Pyruvate (^{13}C) Injection)

Hyperpolarized Pyruvate (^{13}C) Injection is compounded at the clinical site prior to administration. For compounding, the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is hyperpolarized by Dynamic Nuclear Polarization (DNP) for approximately 60 minutes at 1.2 K. After hyperpolarization, the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is dissolved in sterile WFI and the AH111501 is then removed from the hyperpolarized solution by mechanical filtration. The hyperpolarized solution is then neutralized, buffered and diluted in TRIS/EDTA buffer solution and subsequently passed through a sterilizing filter (0.2 μm) into the final drug product container, an empty sterile disposable Medrad syringe.

The SpinLab system used for hyperpolarization and compounding is located in an area adjacent to the MR scanner room. All compounding process steps are in accordance with USP <797> Pharmaceutical Compounding – Sterile Preparations.

Immediately after compounding, the Hyperpolarized Pyruvate (^{13}C) Injection is sampled and tested by an automatic quality control instrument (QC System). The final release authorization for administration to humans will be performed by a licensed pharmacist. After release, the Hyperpolarized Pyruvate (^{13}C) Injection will be delivered to the adjoining MR scanner room for patient administration.

3.2.P.3.3.1 Process description

(a) Compounding and filling of empty sterile fluid path

All process steps for compounding and filling of the empty sterile fluid path (SFP) used in SpinLab to prepare the hyperpolarized (^{13}C) pyruvate injection are performed within a cleanroom ISO 5 area.

A clean, sterile, empty SFP is aseptically removed from its packaging and placed into the ISO 5 area. The sterile water for injection (38 g) is aseptically introduced into the dissolution syringe of the SFP and another 18.5 g of sterile water for injection is aseptically introduced into the receiving vessel. The sterile TRIS/EDTA buffer (18 g) is aseptically introduced into the receiving vessel.

A mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 is prepared and then sterilized using a sterilizing filter (0.2 μm) and 1.47 g of the sterile solution is placed into the sterile cryovial. The cyrovial containing the sterile mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 is then attached to the empty sterile fluid path and sealed utilizing a laser welder. The cryovial is then

placed directly into a bath of liquid nitrogen for 2 minutes to flash freeze the [1-^{13}C]pyruvic acid and 15 mM AH111501 mixture. Following the flash freeze process, the SFP is then aseptically connected to a line containing sterile helium. Sterile helium at a pressure of 40 psi is introduced into the SFP for a total of 2 minutes in order to test the integrity of the laser seal of the cryovial to the SFP. If there is no drop in sterile helium pressure and the system remains at 40 psi, the seal of the cryovial to the SFP is deemed acceptable.

Following pressure testing of the SFP, the entire SFP system is then flushed with sterile helium at a rate of 4 L/min for two minutes to remove any excess water vapor that may impact polarization of the sample contained in the cryovial.

After flushing the SFP with sterile helium, the receiver vessel is aseptically connected to the dissolution syringe vial luer lock mechanisms and the final drug product container, a 65 mL Medrad syringe is attached to the SFP.

The filled SFP is then immediately transported to SpinLab for automated compounding and preparation of the hyperpolarized [^{13}C]pyruvate injection.

(b) SpinLab automated compounding process

The cryostat vial attached to the sterile fluid path (SFP) containing the mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is introduced into the cryostat through an airlock insertion system that isolates the internal cryogenic environment from the lab atmosphere. The insertion system controls the position of the sample vial inside the cryostat via motorized rollers that grip and drive the outer lumen of the SFP. SpinLab is designed to simultaneously handle up to four separate tubing/vial sets.

After positioning the sample(s) in the cryostat at the polarization position, the sample is irradiated with microwave energy tuned to the frequency of the free electrons in the EPA (plus or minus the NMR frequency of the nucleus of interest).

An RF coil in the polarization chamber is used to measure the level of hyperpolarization of the sample via a Solid state NMR (SSNMR) spectrometer. The polarization is measured independently for each sample by taking a measurement of all the samples together, and then subtracting one sample from other measurements to compute a difference. The difference is the polarization of the sample in question.

Once the sample is sufficiently polarized, a dissolution process is initiated to transfer the sample from the sample vial and into a waiting receiver. The dissolution solution is contained in a syringe-shaped feature of the disposable SFP where it is pressurized and heated in preparation for dissolution. Typical dissolution solution parameters are 50 psi at 130°C for heating the dissolution fluid, followed by 250 psi pressure at 130°C for dissoluting the hyperpolarized sample. A flow valve molded into the fluid path controls the flow of the dissolution fluid to the vial through the inner lumen. When a dissolution is commanded, the valve is opened, allowing the hot dissolution fluid to flow from the syringe to the vial through the inner lumen and back through the outer lumen. Sufficient time and volume of dissolution fluid is used, to ensure that the sample is completely liquefied, dissolved, and transferred to the receiving vessel. Dissolution typically takes 5-10 seconds.

Prior to collection in the receiving vessel, the Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is flushed through a mechanical filter for removal of AH111501. Once in

the receiving vessel, the hyperpolarized [1-^{13}C] pyruvic acid is neutralized, buffered and diluted with a sterile TRIS/EDTA buffer solution and sterile WFI. A sample of the Hyperpolarized Pyruvate (^{13}C) Injection is then withdrawn from the receiving vessel and processed by the QC System for determination of release parameters. The remaining Hyperpolarized Pyruvate (^{13}C) Injection is withdrawn from the receiving vessel, passes through a sterilizing filter (0.2μm) and fills the empty sterile Medrad syringe, ready for patient administration.

After the QC analysis is completed, the results are evaluated by the licensed pharmacist and the Hyperpolarized Pyruvate (^{13}C) Injection is released for administration to the patient or rejected. Integrity testing of the sterilizing filter (0.2 μm) is performed post administration.

(c) SpinLab polarizer

SPINlab is the name used to denote the assemblage of equipment designed by GE that uses Dynamic Nuclear Polarization (DNP) technology to hyperpolarize ^{13}C pyruvate. The SPINlab is comprised of a superconducting 5T magnet located within a helium-filled cryostat (contained within polarizer cabinet). The magnet and cryostat are suspended within an external vacuum vessle. The magnet and its radiation shield are cooled via a two-stage Gifford-McMahon cryocooler. Positioned within the magnet isocenter is the helium vessle where polarization is performed. This vessel consists of two separate volumes of liquid helium. The first, inner volume is accessed through the polarizer airlocks and is used to directly cool the sealed sample vials for temperature of 0.8 K. This vessel is thermally linked to a second, outer liquid helium volume that is generated through the condensation of helium gas expelled from the sorption pump located within the cryostat volume. The sorption pump contains a volume of activated charcoal that is charged with helium gas. The sorption pump can be isolated from the cryocooler by a gas gap therma switch, enabling the sorption pump to be heated without significantly impacting the temperature of the magnet. Elevation of the temperature of the charcoal within the sorption pump with integrated resistive heaters expels helium gas from the charcoal. This gas is transported to the outer helium vessel within the magnet center, where the helium is condensed to liquid helium by a cryocooler. Thermal connection of the sorption pump to the cryocooler through the thermal switch results in cooling of the activated charcoal and the generation of reduced pressure within the helium vessel within the magnet's center. This reduced pressure cools the condensed liquid helium to temperatures < 1 K. Because the sorption pump and the outer helium vessel are linked the helium gas is preserved within the system and the condensation cycle can be repeated without the need for additional cryogens. The microwave source has a maximum output power of up to 100 mW at 140 GHz, and a tuning range of 500 MHz. The frequency is controlled via an analogue tuning input. The components of SPINlab are shown in Figure 1.

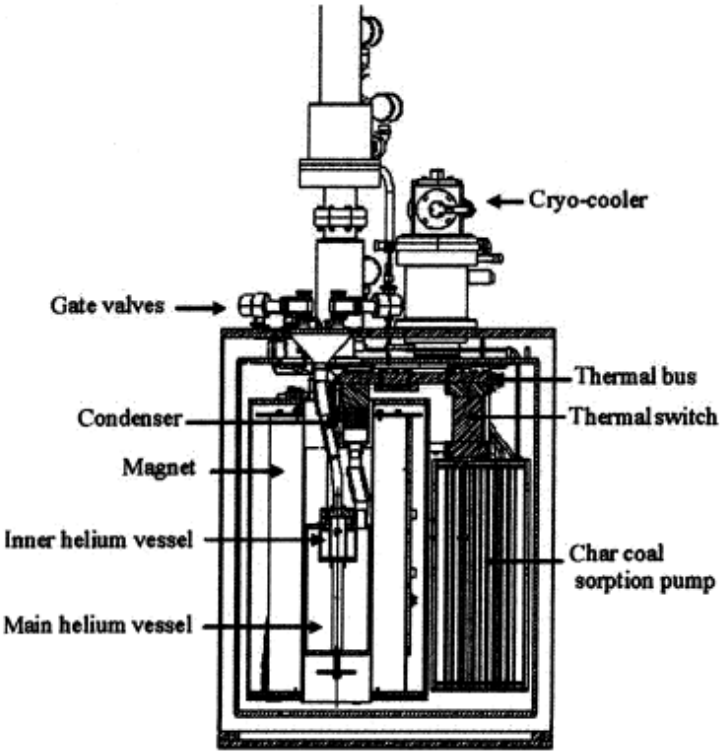


Figure 1

(e) Microbiological attributes

The classification, operation and monitoring of the pharmacy clean room used for compounding and filling the empty sterile fluid paths used in SpinLab, as well as the qualification, training and ongoing monitoring of the pharmacy personnel, comply with USP <797> Pharmaceutical Compounding - Sterile Preparations (CSP) requirements for High Risk CSP products. As per the USP <797> requirements for this risk level, sterility and bacterial endotoxin testing of each preparation is required. The microbial quality is also assured through aseptic validation, as well as post administration sterile filter integrity testing, process/environmental controls and environmental/personnel monitoring. Furthermore, the very short life span of the Hyperpolarized Pyruvate (^{13}C) Injection precludes any sterility or bacterial endotoxin testing prior to administration, thus all sterility and bacterial endotoxin testing for doses is conducted after administration.

All drug product kit components are sterilized within the cleanroom ISO 5 area by passing them through a sterilizing filter (0.2 μm) prior to introduction into the empty sterile fluid path (see Section 3.2.P.2.3 Manufacturing Process Development for Drug Product Kit Components).

The empty sterile fluid path is cleaned and sterilized prior to use.

The helium gas used during the process is Medical Grade and is sterile filtered before being in contact with the drug product or drug product kit components.

As the final step in the automated compounding procedure, the drug product is passed through a sterilizing filter (0.2 μm) within SpinLab immediately before entering the sterile Medrad syringe, the final drug product container.

3.2.P.3.4 Controls of Critical Steps and Intermediates (Hyperpolarized Pyruvate (^{13}C) Injection)

A schematic representation of the process flow and the in-process controls is presented in Figure 1 in Section 3.2.P.3.3.1 (a).

The polarizer software application monitors and controls critical system and process functions and settings such as data communication and temperature settings. Malfunctions or settings detected to be outside pre-set ranges are communicated to the operators via software-generated alarms that prevent further processing. Control of the mechanical functionality of process hardware, such as valves and fittings, and of the He driving pressure, is performed manually by the operator.

The final release analyses performed by the QC System ensure that the compounding process has executed as intended and that the Hyperpolarized Pyruvate (^{13}C) Injection is within specifications (see Table 1 in Section 3.2.P.5.1).

The post-administration integrity test of the sterilizing filter assesses whether the filter was functional during use.

3.2.P.3.5 Process Validation and/or Evaluation (Hyperpolarized Pyruvate (^{13}C) Injection)

3.2.P.3.5.1 IQ/OQ/PQ Program

The clean room, polarizer, process equipment and QC system have gone through an extensive IQ/OQ/PQ program prior to use during clinical trials. The clean room and equipment were found to be suitable for their intended use.

3.2.P.3.5.2 Microbiological aspects

The compounding process has been validated by simulation of the process using a microbial nutrient medium. No growth has been observed in any of the media fill batches.

The microbiological quality has also been demonstrated by sterility and microbial endotoxin testing of repeated runs (n=6).

3.2.P.3.5.3 Compounding process consistency

The consistency of the compounding process has been evaluated by repeated (n=10) compounding of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt and TRIS/EDTA buffer solution. All batches were within specifications (see Section 3.2.P.5.4 Batch Analyses for details).

As the QC System only determines a limited set of quality parameters, all batches were also analyzed for related substances of [1-^{13}C]pyruvate and TRIS, assay of [1-^{13}C]pyruvate, osmolality and particulate contamination. Because of limited analytical capability at the site of compounding (UCSF), samples were shipped to GE Healthcare and analyses were performed 8 to 34 days after compounding. The formation of AH112615 after compounding (see Section 3.2.P.5.5 Characterization of Impurities) causes a decrease in the assay of [1-^{13}C]pyruvate. Because of this

effect, and as the HPLC method does not detect AH112615, for this study determination of [1-^{13}C]pyruvate content was performed by quantitative 1H NMR analysis using acetate as an internal standard for calibration. It has been shown that the content of AH112615 immediately after compounding is negligible (see Section 3.2.P.5.5 Characterization of Impurities). To determine the content of [1-^{13}C]pyruvate *at time of compounding at UCSF*, the AH112625 peak was therefore integrated as [1-13C]pyruvate. The formation of AH112615 also causes a decrease in osmolality with time after compounding. For this study the osmolality *at time of compounding at UCSF* was therefore calculated from the measured osmolality and the content of AH112615 (determined by 1H NMR) at time of analysis.

Results from these analyses are stated in Table 1. As can be seen from these results, the assay of [1-^{13}C]pyruvate in (Hyperpolarized Pyruvate (^{13}C) Injection) varied in the range of 222-252 mM with an average of 241 ± 12 mM. Although the observed assay displays a larger variance than would be expected from the drug product kit components used the results are considered to demonstrate an acceptable process consistency. It should be noted that even though the QC system does not determine the assay of [1-^{13}C]pyruvate, the determination of pH constitute a relevant indirect control of this parameter. The level of control obtained through the determination of pH has been investigated in a study where a sample of Mixture of [1- ^{13}C]pyruvic acid and 15 mM AH111501 sodium salt was titrated with TRIS/EDTA buffer solution diluted in sterile WFI. Results from this study are shown in Figure 1. As can be seen from Figure 1 the pH of the solution is a well defined function of the [1- ^{13}C]pyruvate concentration. As expected, the pKa of TRIS is observed at approximately 8.1 and the depletion of buffer capacity towards the acidic range is observed at approximately 280 mM. Estimated from the observed relationship, the specification to pH (6.7 to 8.0) is equivalent to approximately 210 to 270 mM Pyruvate.

With regards to the efficacy of the drug product, the ^{13}C NMR determined by the QC system is proportional to the concentration of [1-^{13}C]pyruvate (see Section 3.2.P.5.2.1 Analytical Procedures). As the ^{13}C nuclear polarization reported by the QC system assumes a fixed concentration of [1-^{13}C]pyruvate, it varies linearly with the actual concentration of [1-^{13}C]pyruvate. Hence, this parameter represents a more relevant assurance of product efficacy than the assay of [1-^{13}C]pyruvate alone.

Osmolality varied in the range of 484-513 mOsm/kg with an average of 501 ± 12 mOsm/kg. Particulate contamination was well within the pharmacopoeia limits for all batches. The purity profile observed during this study was as expected from the purity profile of the drug product kit components. No new impurities were observed.
With regards to the purity profile of Hyperpolarized Pyruvate (^{13}C) Injection *at time of compounding at UCSF*, reference is made to 3.2.P.5.5 Characterization of Impurities.

3.2.P.4 Control of Excipients (Hyperpolarized Pyruvate (^{13}C) Injection)

There are no excipients added during compounding of Hyperpolarized Pyruvate (^{13}C) Injection. All excipients in the drug product are attributed to the drug product kit components; Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium and TRIS/EDTA buffer solution. Excipients in the drug product kit components are discussed in Section 3.2.P.4 Control of Excipients for Drug Product Kit Components and Section 3.2.A.3 Novel Excipients.

3.2.P.4.1 Specification (Hyperpolarized Pyruvate (^{13}C) Injection)

Not applicable.

3.2.P.4.2 Analytical Procedures (Hyperpolarized Pyruvate (^{13}C) Injection)

Not applicable.

3.2.P.4.3 Validation of Analytical Procedures (Hyperpolarized Pyruvate [^{13}C] Injection)

Not applicable.

3.2.P.4.4 Justification of Specifications (Hyperpolarized Pyruvate (^{13}C) Injection)

Not applicable.

3.2.P.4.5 Excipients of Human or Animal Origin (Hyperpolarized Pyruvate [^{13}C] Injection)

Not applicable.

3.2.P.4.6 Novel Excipients (Hyperpolarized Pyruvate [^{13}C] Injection)

Not applicable.

3.2.P.5 Control of Drug Product (Hyperpolarized Pyruvate (^{13}C) Injection)

The drug product kit components used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection in the polarizer differ, to some extent, from the components used for pre-clinical studies and clinical studies GE-101-001 and GE-101-003. The drug product kit components used for the compounding of Hyperpolarized Pyruvate (^{13}C) Injection in the DNP polarizer are formulated such that the final drug product is equivalent to the product used for clinical studies GE-101-001 and GE-101-003. See Section 3.2.P.2.2.1 Formulation Development for details.

3.2.P.5.1 Specification(s) (Hyperpolarized Pyruvate (^{13}C) Injection)

The specifications for Hyperpolarized Pyruvate (^{13}C) Injection, due to the rapid decay of the ^{13}C nuclear polarization, are limited to the parameters determined by the QC System (see Table 1). Control of additional parameters is assured through testing performed on the combination of the drug product kit components as described in Section 3.2.P.5.1 specification(s) for Drug Product Kit Components.

Table 1 Specification for Hyperpolarized Pyruvate (^{13}C) Injection

Test	Analytical Procedures	Acceptance Criteria
^{13}C nuclear polarization	Liquid-state NMR	NLT 15.0%[1]
Pyruvate concentration	UV absorbance	220-280 mM
Residual AH 111501	Visible absorbance	NMT 3.0 μM
pH	Ratiometric indicator	6.7-8.0
Drug product temperature	IR pyrometry	25.0-27.0°C[2]
Drug product volume	Capacitive level sensor	> 38 mL

[1] Specification to ^{13}C nuclear polarization is at time of start dissolution.
[2] Specification to temperature is at time of analysis.

3.2.P.5.2 Analytical Procedures (Hyperpolarized Pyruvate (^{13}C) Injection)

The 13C nuclear polarization of Hyperpolarized Pyruvate (^{13}C) Injection undergoes an exponential decay with a time constant of approximately 69 seconds. In order to preserve an acceptable imaging efficacy, the time between start dissolution and start of administration to the patient has to be NMT 50s (see Section 3.2.P.8.1 Stability Summary and Conclusions). Due to this limited user window, analyses to control Hyperpolarized Pyruvate (^{13}C) Injection are performed using an automated analytical system (QC System) that determines a limited set of parameters within a time span of approximately 10s. This QC System is specifically developed for the analysis of Hyperpolarized Pyruvate (^{13}C) Injection immediately prior to administration to the patients.

The QC accessory participates in the dissolution process by managing the state of the sterile fluid path. Specifically, the QC accessory controls the upper slide valve, which is used to isolate the receiver from the EPA filter, and the lower slide valve which controls fluid flow to the cuvettes and the Administration syringe. After a dissolution is complete, the QC accessory closes the upper slide valve, measures the temperature of the receiver, and opens the lower slide valve to allow the mixed solution to be drawn out. Once the cuvettes and NMR bulb are filled, the QC measures the pyruvate concentration, EPA concentration, and pH. The percent polarization is also measured. Once the Administration syringe is filled, the QC checks that the volume is above the level of a threshold sensor. All measurement results are reported to the Hyperpolarizer, where they are interpreted and displayed on the screen for an operator to decide how to proceed. After completed analysis, the software performs a comparison of the results to a pre-set list of specifications (see Table 1 in Section 3.2.P.5.1 Specifications) and reports the compliance or non-compliance of the Hyperpolarized Pyruvate (^{13}C) Injection to the specifications.

Control of additional parameters is assured through testing performed on the combination of the drug product kit components; Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt dissolved in WFI and TRIS/EDTA buffer solution (Pyruvate (^{13}C) Injection) (see Section 3.2.P.5.1 Specifications for Drug Product Kit Components).
The analytical procedures used to control Hyperpolarized Pyruvate (^{13}C) Injection are summarized in Sections 3.2.P.5.2.1 through 3.2.P.5.2.5.

3.2.P.5.2.1 ^{13}C nuclear polarization

The percent polarization is determined using a liquid state NMR measurement. During calibration of the system, a measurement of a thermal sample of methanol is taken. The 90 degree flip angle of the LSNMR is also determined during this process. After a dissolution is completed and the

NMR bulb is filled, a measurement of the hyperpolarization is collected and its result compared to the thermal sample from the calibration. The adjusted ratio of these measurements is used to calculate the percent polarization. Because of the large difference in the signal level between the thermal signal and the hyperpolarized signal, the measurements are done using very different flip angles.

3.2.P.5.2.2 Residual AH111501

Before the sterile fluid path is loaded into the QC, the dark measurement is recorded. When the sterile Fluid path is loaded into the QC, and the cuvettes are still empty, the QC measures the light intensity of light that passes through the cuvette, and saves this as the blank. After the cuvettes are filled, the QC measures the intensity of the light that passes through the cuvettes and the solution. For this measurement the cuvette is roughly 2cm. the QC uses an LED at 470nm wavelength and a broad range photodiode detector. It then calculates the absorbance using the following calculation:

$$\text{EPA Absorbance} = \left(\frac{I_{filled} - I_{dark}}{I_{Empty} - I_{dark}}\right)$$

This value is then multiplied by the slope and an offset added according to the calibration results previously performed. The result is the EPA concentration.

3.2.P.5.2.3 pH

Before the sterile fluid path is loaded into the QC, the dark measurement is recorded. When the sterile Fluid path is loaded into the QC, and the cuvettes are still empty, the QC measures the light intensity of light that passes through the cuvette, and saves this as the blank. After the cuvettes are filled, the QC measures the intensity of the light that passes through the cuvettes and the solution. This measurement uses a 1cm pathlength and has two different wavelengths of interested whose ratio is used to define the pH. It then calculates the result using the following calculations:

$$\text{405 Absorbance} = \left(\frac{I_{filled} - I_{dark}}{I_{Empty} - I_{dark}}\right)$$

$$\text{450 Absorbance} = \left(\frac{I_{filled} - I_{dark}}{I_{Empty} - I_{dark}}\right)$$

$$pH\ Ratio = \frac{450\ Absorbance}{405\ Absorbance}$$

This value is then added into a 4th order polynomial to determine the pH of the solution. The terms of this polynomial were determined according to the calibration results performed previously.

3.2.P.5.2.4 Temperature

The temperature measurement is performed using a pyrometer that is facing the wall of the receiver of the fluid path. The region inspected by this sensor is thinned, to speed thermal transfer from the fluid on the inside to the outside surface for the pyrometer to read.

3.2.P.5.2.5 Volume

The volume measurement in the QC is a threshold measurement performed at the Administration syringe after the fluid movement is complete. This measurement is a capacitive measurement that was tuned by the manufacturer during system setup. The sensor was also tuned to have its threshold centered at a nominal volume of 38 mL.

3.2.P.5.3 Validation of Analytical Procedures (Hyperpolarized Pyruvate (^{13}C) Injection)

The analytical procedures are appropriately validated for the current development phase and are suitable for intended use. The validation performed at this stage is summarized in Table 1.

Table 1 Validation of analytical procedures performed at this stage

Test	Validation Parameters
^{13}C Nuclear polarization	Accuracy
Pyruvate concentration	Accuracy, repeatability, intermediate precision, linearity, range, detection and quantification limits, interference
Residual AH 111501	Accuracy, repeatability, intermediate precision, linearity, range, detection and quantification limits
pH	Accuracy, repeatability, range, interference
Temperature	Accuracy, range
Volume	Accuracy

3.2.P.5.4 Batch Analyses (Hyperpolarized Pyruvate (^{13}C) Injection)

Hyperpolarized Pyruvate (^{13}C) Injection has not been used for pre-clinical studies or clinical studies GE-101-001 and GE-101-003. For the clinical studies, non-polarized Pyruvate Injection has been used. Different formulations of Pyruvate (^{13}C) Injection have been used during non-clinical studies and clinical studies GE-101-001 and GE-101-003. The drug product kit components used for compounding of Hyperpolarized Pyruvate (^{13}C) Injection have been formulated such that the drug product is equivalent to the drug product used for clinical studies GE-101-001 and GE-101-003, as discussed in Section 3.2.P.2.2.1 Formulation Development. Results for batches of Hyperpolarized Pyruvate (^{13}C) Injection are presented in Table 1 and Table 2.

Table 1 Batch data for Hyperpolarized Pyruvate (^{13}C) Injection

	Specification	0222201502	0222201503	0223201501	0224201501	0224201502
Place of Compounding		UCSF	UCSF	UCSF	UCSF	UCSF
Date of SpinLab Compounding		23 February 2015	24 February 2015	24 February 2015	26 February 2015	26 February 2015
SpinLab Channel		1	1	1	1	1
Empty Sterile Fluid Path Batch		36662535	36662535	36662535	36662535	36662535
[1-^{13}C]pyruvic acid batch		EB2117	EB2117	EB2117	EB2117	EB2117
AH111501 batch		192226	192226	192226	192226	192226
TRIS/EDTA dissolution buffer batch		011014	011014	011014	011014	011014
Sterile Water for Injection batch		J4N545	J4N545	J4N545	J4N545	J4N545
Empty MedRad Syringe Batch		166852	166852	166852	166852	166852
Use		Technical	Technical	Technical	Technical	Technical
Test						
^{13}C nuclear polarization	NLT 15.0 %	19.1	22.5	21.2	16.4	17.2
Pyruvate Concentration	220-280 mM	267	269	270	261	267
Residual AH111501	NMT 3.0 µM	1.2	1.9	1.4	1.7	1.2
pH	6.7-8.0	7.6	7.5	7.6	7.6	7.5
Temperature	25.0-37.0 °C	33.2	34.1	34.4	33.6	33.9
Volume	> 38 mL	> 38 mL	> 38 mL	> 38 mL	> 38 mL	> 38 mL

Each batch represents one compounded dose from drug components as designated
UCSF = University of California, San Francisco

Table 2 Batch data for Hyperpolarized Pyruvate (^{13}C) Injection

	Specification	0301201502	0301201503	0301201504	0301201505	0301201506
Place of Compounding		UCSF	UCSF	UCSF	UCSF	UCSF
Date of SpinLab Compounding		2 March 2015	3 March 2015	3 March 2015	4 March 2015	4 March 2015
SpinLab Channel		2	2	2	2	2
Empty Sterile Fluid Path Batch		36662535	36662535	36662535	36662535	36662535
[1-^{13}C]pyruvic acid batch		EB2117	EB2117	EB2117	EB2117	EB2117
AH111501 batch		192226	192226	192226	192226	192226
TRIS/EDTA dissolution buffer batch		011014	011014	011014	011014	011014
Sterile Water for Injection batch		J4N545	J4N545	J4N545	J4N545	J4N545
Empty MedRad Syringe Batch		166852	166852	166852	166852	166852
Use		Technical	Technical	Technical	Technical	Technical
Test						
^{13}C nuclear polarization	NLT 15.0 %	18.3	16.8	16.8	16.3	16.7
Pyruvate Concentration	220-280 mM	259	254	248	270	262
Residual AH111501	NMT 3.0 µM	1.2	1.3	1.0	1.2	1.1
pH	6.7-8.0	7.7	7.8	7.9	7.6	7.7
Temperature	25.0-37.0 °C	33.5	34.9	32.4	33	31.6
Volume	> 38 mL	> 38 mL	> 38 mL	> 38 mL	> 38 mL	> 38 mL

Each batch represents one compounded dose from drug components as designated
UCSF = University of California, San Francisco

3.2.P.5.5 Characterization of Impurities (Hyperpolarized Pyruvate (^{13}C) Injection)

A determination of the impurities in Hyperpolarized Pyruvate (^{13}C) Injection is not part of the analyses performed by the QC System. Hence, documentation and control of the impurities in the drug product rests on analyses performed during the release testing of Pyruvate (^{13}C) Injection (see Section 3.2.P.5.5 Characterization of Impurities for Drug Product Kit Components) and the results from process verification studies.

3.2.P.5.5.1 Differences in dissolution procedures

The manual procedure for the compounding of Pyruvate (^{13}C) Injection during preparation of samples for analysis is identical to the procedure used during pre-clinical safety studies and clinical studies GE-101-001 and GE-101-003. The dissolution process during compounding of Hyperpolarized Pyruvate (^{13}C) Injection is different from the manual procedure, particularly with regards to parameters such as time, temperature, flow rates and pressure. These differences influence the purity profile such that the impurities in manually dissolved Pyruvate (^{13}C) Injection, to some extent, are different from those in Hyperpolarized Pyruvate (^{13}C) Injection. These effects and the purity profile of Hyperpolarized Pyruvate (^{13}C) Injection are discussed in Sections 3.2.P.5.5.2, 3.2.P.5.5.3 and 3.2.P.5.5.4.

3.2.P.5.5.2 Transformation between AH113462 and AH112623

During and after manual compounding of Pyruvate (^{13}C) Injection, the major impurity in the drug substance, AH113462/E, transforms through AH113462/K to AH112623 (see Section 3.2.P.5.5.1 (a) Transformation of the [1-^{13}C]pyruvic acid purity profile for Drug Product Kit Components). As the dissolution step during the semi-automated compounding of Hyperpolarized Pyruvate (^{13}C) Injection takes place in less than 10 seconds and the product is administered within 50s from start of dissolution, the transformation from AH113462/E to AH112623 will not be complete. The drug product used in the pre-clinical safety studies and for clinical studies GE-101-001 and GE-101-

003, contains insignificant amounts of AH113462/E and AH113462/K at the time of administration. Hyperpolarized Pyruvate (^{13}C) Injection, however, may contain these impurities at time of administration; therefore, AH112623, AH113462/E and AH113462/K have been qualified in specific pre-clinical studies (see Sections 8.4.2.5 AH112623 (Parapyruvate) and 8.4.4.5 AH113462 (Lactone) for Item 8 Pharmacology and Toxicology Info).

3.2.P.5.5.3 Absence of AH112615 in Hyperpolarized Pyruvate (^{13}C) Injection

During and after manual compounding of Pyruvate (^{13}C) Injection a reaction product, AH112615, may form between [1-^{13}C]pyruvic acid and TRIS (see Section 3.2.P.5.5.1 (b) Formation of AH112615 in Pyruvate (^{13}C) Injection for Drug Product Kit Components). As the dissolution process applied during the semi-automated compounding of Hyperpolarized Pyruvate (^{13}C) Injection takes place within 10 seconds and the product is administered immediately, the formation of AH112615 will be negligible prior to administration. Opposed to the pre-clinical test item or the drug product used for clinical studies GE-101-001 and GE- 101-003, where AH112615 was the major impurity, Hyperpolarized Pyruvate (^{13}C) Injection contain insignificant amounts of this impurity. However, because of its presence in the drug product administered during clinical studies GE-101-001 and GE-101 003, AH112615 has been qualified in specific pre-clinical studies (see Sections 8.4.2 (d) AH112615 (Reaction product between pyruvic acid and TRIS) and 8.4.4 (c) AH112615 (Reaction product between pyruvic acid and TRIS) for Item 8 Pharmacology and Toxicology Info.)

3.2.P.5.5.4 Impurities in Hyperpolarized Pyruvate (^{13}C) Injection

To document the impurities in Hyperpolarized Pyruvate (^{13}C) Injection, a process verification study was performed where two batches of Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt were compounded with two batches of TRIS/EDTA buffer solution. The compounding was performed in triplicate for each combination, resulting in twelve samples of Hyperpolarized Pyruvate (^{13}C) Injection. After dissolution, the system is dynamic with regards to the impurity profile. For this reason, HPLC analysis will not be able to produce a true picture of the impurity profile at time of administration. Therefore, 1H NMR analysis was performed in addition to HPLC analysis. Since relaxation-driven polarization transfer from 13C to 1H prevents an early analysis, the 1H NMR was performed approximately 10 minutes after the start of compounding. To determine the impurity profile at a time relevant for the administration of drug product to the patient, impurities related to [1-^{13}C]pyruvic acid were also determined by 13C NMR analysis. This analysis was performed approximately 24 seconds after start of compounding. For comparison, impurities related to [1-^{13}C]pyruvic acid were determined by HPLC and 1H NMR analyses for all four combinations of manually dissolved Pyruvate (^{13}C) Injection.

Results from this study are summarized in Table 1 and Table 2.

As can be observed from these results, apart from the differences discussed in Sections 3.2.P.5.5.2 and 3.2.P.5.5.3, the impurities in Hyperpolarized Pyruvate (^{13}C) Injection are essentially equivalent to the impurities in Pyruvate (^{13}C) Injection.

- HPLC – The content of AH112623 in compounded Hyperpolarized Pyruvate (^{13}C) Injection (3-6 days after compounding) ranges from 1.27 to 2.97% area as compared to manually dissolved Pyruvate (^{13}C) Injection (immediately after dissolution), which ranges from 0.87 to 1.56% area. No new impurities are observed by HPLC in Hyperpolarized

Pyruvate (^{13}C) Injection.

- 1H NMR – The content of AH112623 in compounded Hyperpolarized Pyruvate (^{13}C) Injection (10 minutes after compounding) ranges from 0.45 to 0.70% area as compared to manually dissolved Pyruvate (^{13}C) Injection (immediately after dissolution), which ranges from 0.47 to 0.91%. The content of AH113462/E in compounded Hyperpolarized Pyruvate (13C) Injection ranges from 0.16-0.50% area compared to not detected for manually dissolved Pyruvate (^{13}C) Injection. The content of AH113462/K in compounded Hyperpolarized Pyruvate (^{13}C) Injection ranges from not detected to 0.05% area compared to not detected for manually dissolved Pyruvate (^{13}C) Injection. The content of AH112615 in compounded Hyperpolarized Pyruvate (^{13}C) Injection ranges from 0.33 to 0.48% area compared to 2.46 to 3.09% area for manually dissolved Pyruvate (^{13}C) Injection. No new impurities are observed by 1H NMR in ^{13}C) Injection. The content of impurities related to TRIS in Hyperpolarized Pyruvate (^{13}C) Injection was insignificantly different from the content observed in manually dissolved Pyruvate (^{13}C) Injection. The content of residual ethanol is low and reproducible between 1.04 and 2.58 % area. With a [1-^{13}C]pyruvate content of 250 mM.

- ^{13}C NMR - The content of AH112623 in compounded Hyperpolarized Pyruvate (^{13}C) Injection (24 seconds after compounding) ranges from 0.37 to 0.61% area as compared to manually dissolved Pyruvate (^{13}C) Injection (immediately after dissolution), which ranges from 0.47 to 0.91% (by 1H NMR). The content of AH113462/E in compounded Hyperpolarized Pyruvate (^{13}C) Injection ranges from 0.19-0.42% area compared to not detected for manually dissolved Pyruvate (^{13}C) Injection (by 1H NMR). The content of AH113462/K in compounded Hyperpolarized Pyruvate (^{13}C) Injection ranges from 0.07 to 0.15% area compared to not detected for manually dissolved Pyruvate (^{13}C) Injection (by 1H NMR). The content of AH112615 in compounded Hyperpolarized Pyruvate (^{13}C) Injection ranges from not detected to 0.07% area compared to 2.46 to 3.09% area for manually dissolved Pyruvate (^{13}C) Injection (by 1H NMR). No new impurities are observed by ^{13}C NMR in Hyperpolarized Pyruvate (^{13}C) Injection.

Based on these results, it is concluded that, with exception of AH113462/E and AH113462/K, the impurities in Hyperpolarized Pyruvate (^{13}C) Injection are identical to the impurities in Pyruvate (^{13}C) Injection and that control of the latter represents a relevant assurance of impurities in Hyperpolarized Pyruvate (^{13}C) Injection.

Table 1 Impurities in Hyperpolarized Pyruvate (^{13}C) Injection and Pyruvate (^{13}C) Injection by HPLC

Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF099/198-510. TRIS/EDTA buffer solution batch FFF139/107-706.		
	Pyruvate (^{13}C) Injection	Hyperpolarized Pyruvate (^{13}C) Injection
Time of analysis and dissolution procedure	Immediately after manual dissolution	3-6 days after compounding[1]
AH112623	1.37	2.30, 2.36, 2.10
AH113462/K	0.13	0.08, 0.07, ND
AH113462/E	0.16	0.06, ND, 0.06
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF099/198-510. TRIS/EDTA buffer solution batch FFF139/108-706.		
	Pyruvate (^{13}C) Injection	Hyperpolarized Pyruvate (^{13}C) Injection
Time of analysis and dissolution procedure	Immediately after manual dissolution	3-6 days after compounding[1]
AH112623	1.56	2.49, 2.38, 2.97
AH113462/K	0.14	ND, ND, 0.08
AH113462/E	0.14	ND, ND, 0.07
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF139/106-706. TRIS/EDTA buffer solution batch FFF139/107-706.		
	Pyruvate (^{13}C) Injection	Hyperpolarized Pyruvate (^{13}C) Injection
Time of analysis and dissolution procedure	Immediately after manual dissolution	3-6 days after compounding[1]
AH112623	0.87	1.99, 1.77, 1.60
AH113462/K	ND	0.06, ND, ND
AH113462/E	ND	ND, 0.07, ND
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF139/107-706. TRIS/EDTA buffer solution batch FFF139/108-706.		
	Pyruvate (^{13}C) Injection	Hyperpolarized Pyruvate (^{13}C) Injection
Time of analysis and dissolution procedure	Immediately after manual dissolution	3-6 days after compounding[1]
AH112623	1.15	1.56, 1.30, 1.27
AH113462/K	0.06	ND, ND, ND
AH113462/E	ND	0.07, 0.06, ND

All results are stated in % area.

ND = not detected (≤ 0.05)

[1] Due to instrumental problems the HPLC analysis could not be performed immediately after compounding.

Table 2 Impurities in Hyperpolarized Pyruvate (^{13}C) Injection and Pyruvate (^{13}C) Injection by ^{1}H and ^{13}C NMR

Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF099/198-510. TRIS/EDTA buffer solution batch FFF139/107-706.			
	Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{13}C NMR
Time of analysis and dissolution procedure	Immediately after manual dissolution	10 minutes after compounding	24 s after compounding
AH112623	0.80	0.61, 0.63, 0.58	0.49, 0.47, 0.61
AH112615	2.46	0.36, 0.33, 0.42	0.05, 0.05, ND
AH113462/K	ND	ND, ND, 0.05	0.13, 0.14, 0.11
AH113462/E	ND	0.42, 0.49, 0.43	0.42, 0.40, 0.36
Ethanol	ND	1.06, 1.47, 2.58	NP
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF099/198-510. TRIS/EDTA buffer solution batch FFF139/108-706.			
	Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{13}C NMR
Time of analysis and dissolution procedure	Immediately after manual dissolution	10 minutes after compounding	24 s after compounding
AH112623	0.91	0.66, 0.65, 0.70	0.54, 0.50, 0.49
AH112615	2.85	0.48, 0.36, 0.45	0.06, ND, ND
AH113462/K	ND	ND, ND, ND	0.15, 0.11, 0.10
AH113462/E	ND	0.46, 0.46, 0.50	0.39, 0.35, 0.35
Ethanol	ND	1.79, 1.07, 2.14	NP
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF139/106-706. TRIS/EDTA buffer solution batch FFF139/107-706.			
	Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{13}C NMR
Time of analysis and dissolution procedure	Immediately after manual dissolution	10 minutes after compounding	24 s after compounding
AH112623	0.47	0.51, 0.50, 0.55	0.39, 0.42, 0.45
AH112615	3.00	0.45, 0.45, 0.33	0.07, ND, 0.05
AH113462/K	ND	ND, ND, ND	0.11, 0.14, 0.08
AH113462/E	ND	0.16, 0.19, 0.20	0.20, 0.22, 0.19
Ethanol	ND	1.04, 1.49, 0.92	NP
Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF139/106-706. TRIS/EDTA buffer solution batch FFF139/108-706.			
	Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{1}H NMR	Hyperpolarized Pyruvate (^{13}C) Injection by ^{13}C NMR[1]
Time of analysis and dissolution procedure	Immediately after manual dissolution	10 minutes after compounding	24 s after compounding
AH112623	0.66	0.53, 0.46, 0.45	0.45, 0.37
AH112615	3.09	0.33, 0.42, 0.36	0.07, 0.07
AH113462/K	ND	ND, ND, ND	0.12, 0.07
AH113462/E	ND	0.19, 0.18, 0.18	0.21, 0.31
Ethanol	ND	1.16, 1.70, 1.10	NP

Results for related impurities are stated in % area. Results for ethanol are stated in % mol/mol [1-^{13}C]pyruvate.
ND = not detected (≤ 0.05% area).
NP = not performed
[1] Due to instrumental error only two of the three samples were analyzed.

3.2.P.5.6 Justification of Specification(s) (Hyperpolarized Pyruvate (^{13}C) Injection)

Batches will be released only if the results comply with the acceptance criteria presented in Section 3.2.P.5.1 Specifications. Final specifications should reflect the process performance. The project is in early development and specifications are preliminary and will be evaluated throughout the development phase. Considering the early stage of the project where only limited batch data are available, the specifications are considered justified.

3.2.P.6 Reference Standards or Materials (Hyperpolarized Pyruvate (^{13}C) Injection)

A batch of AH111501 sodium salt has been qualified as a working reference standard (WS1010.002). The batch has been characterized by visual appearance, identification by MS, water content by Karl Fisher titration, inorganic residuals by ICP-AES, residual solvents by HS-GC and purity by HPLC and HPLC-MS.

3.2.P.7 Container Closure System (Hyperpolarized Pyruvate (^{13}C) Injection)

The container closure system for Hyperpolarized Pyruvate (^{13}C) Injection is a sterile, disposable 65 mL Qwik-Fit Syringe® that originates from the MEDRAD Syringe-Kit SSQK65 for Spectris Solaris. The 65 mL Qwik-Fit Syringe® is aseptically attached to the sterile fluid path via the sterilizing filter during the aseptic filling and assembly of the sterile fluid path.

3.2.P.8.1 Stability Summary and Conclusion (Hyperpolarized Pyruvate (^{13}C) Injection)

The stability-indicating parameter for Hyperpolarized Pyruvate (^{13}C) Injection is the level of ^{13}C nuclear polarization, which decays rapidly after compounding. The stability testing performed has therefore been limited to determination of the ^{13}C nuclear polarization and relaxation time (T1).

3.2.P.8.1.1 Batches tested

Stability testing has been performed on six samples of Hyperpolarized Pyruvate (^{13}C) Injection compounded from Mixture of [1-^{13}C]pyruvic acid and 15 mM AH111501 sodium salt batch FFF106/140-806 and TRIS/EDTA buffer solution batch FFF106/142-806.

3.2.P.8.1.2 Storage conditions and testing frequency

Testing was performed inside an MRI scanner located next to the clean room where the compounding of the sample took place. For testing frequency, see Section 3.2.P.8.1.3 Analytical Procedures and Specification.

3.2.P.8.1.3 Analytical procedures and specification

The level of ^{13}C nuclear polarization was determined using a 3T MRI scanner. The hyperpolarized ^{13}C NMR signal was obtained using a 5 degree RF pulse. During the relaxation of the non-equilibrium polarization, a total of 64 spectra with 5 degree pulse and TR=3s were collected; the first of which was used for calculating ^{13}C polarization. The relaxation time (^{13}C T1) was calculated by fitting these data to a mono-exponential decay curve. After relaxation to thermal equilibrium, a thermal ^{13}C NMR spectrum was collected (90 degree pulse, 64 averages, TR=10s, after addition of 10 µl Gd/ml solution) in order to calculate the ^{13}C polarization.

No shelf-life specifications have been established for Hyperpolarized Pyruvate (^{13}C) Injection. Assurance of quality at time of administration rests on analyses performed before release and the time limit for administration after the dissolution step is completed, which is stated in the imaging

protocol.

3.2.P.8.1.4 Summary of results

The stability results are presented in Section 3.2.P.8.3 Stability Data.

3.2.P.8.1.5 Conclusion

With a relaxation time of 69s, the polarization decreases by 7% (relative) each 5 seconds. To optimize imaging signal, administration should hence take place as quickly as practically possible. To assure the level of polarization during clinical use, and hence a certain level of imaging signal, the drug product will be administered within 50s from time of start dissolution. With a release specification of NLT 15.0% and a relaxation time of 69s, this user window limit will assure a polarization of NLT 7.3% at time of administration.

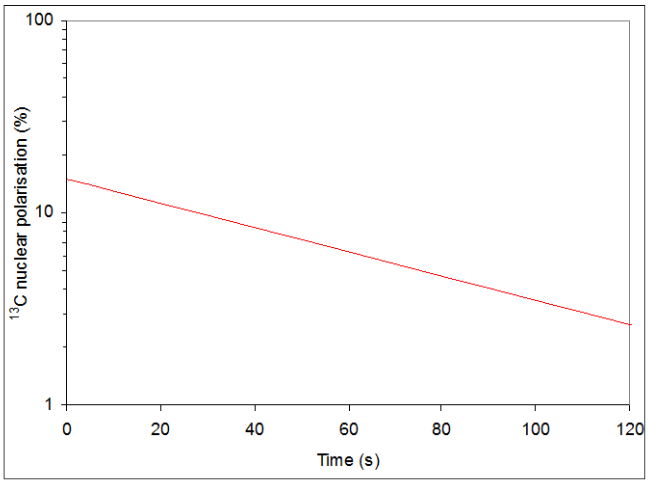
3.2.P.8.2 Post-approval Stability Protocol and Stability Commitment (Hyperpolarized Pyruvate (^{13}C) Injection)

Not applicable.

3.2.P.8.3 Stability Data (Hyperpolarized Pyruvate (^{13}C) Injection)

The average relaxation time determined for the six samples investigated was 68.8 ± 1.3s, with a range of 67.1 to 71.0s. A line derived from the stability results on Hyperpolarized Pyruvate (^{13}C) Injection is shown in Figure 1. In Figure 1, the line represents a sample released at specification limit (NLT 15.0% at start of dissolution), decaying with the average measured relaxation time (69s).

Figure 1 The ^{13}C nuclear polarization of Hyperpolarized Pyruvate (^{13}C) Injection versus time after start dissolution



7.4 Appendices

3.2.A.3.1 General information

AH111501 sodium salt is a stable trityl radical, and is added to [1-^{13}C]pyruvic acid to enable hyperpolarization.

3.2.A.3.1.1 Nomenclature

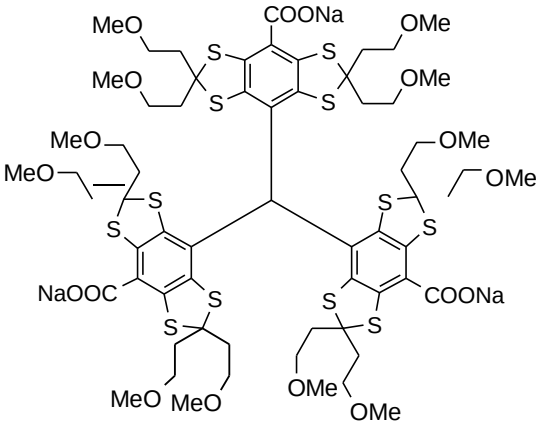
Company code: AH111501 sodium salt

Chemical name: Methyl, tris[8-carboxy-2,2,6,6-tetrakis(2-methoxyethyl)benzo[1,2-d:4,5-d']bis[1,3]dithiol-4-yl]-, trisodium salt

CAS registry number: 874536-54-6

3.2.A.3.1.2 Structure

Figure 1 Structure of AH111501 sodium salt



Molecular formula: $C_{64}H_{84}O_{18}S_{12}Na_3$

Molecular weight: 1595.11

The structure of AH111501 sodium salt has been confirmed by spectroscopic analysis (see Section 3.2.A.3.3).

3.2.A.3.1.3 General Properties

Appearance: Green to black, fine to granular powder

3.2.A.3.2 Manufacture (AH111501 Sodium salt)

3.2.A.3.2.1 Manufacturer

Manufacturer of AH111501 sodium salt:

Syncom BV
Kadijk 3
9747 AT Groningen
The Netherlands

3.2.A.3.2.2 Description of Manufacturing Process and Process Controls

[redacted]

Figure 1 Total synthesis of AH111501 Sodium Salt

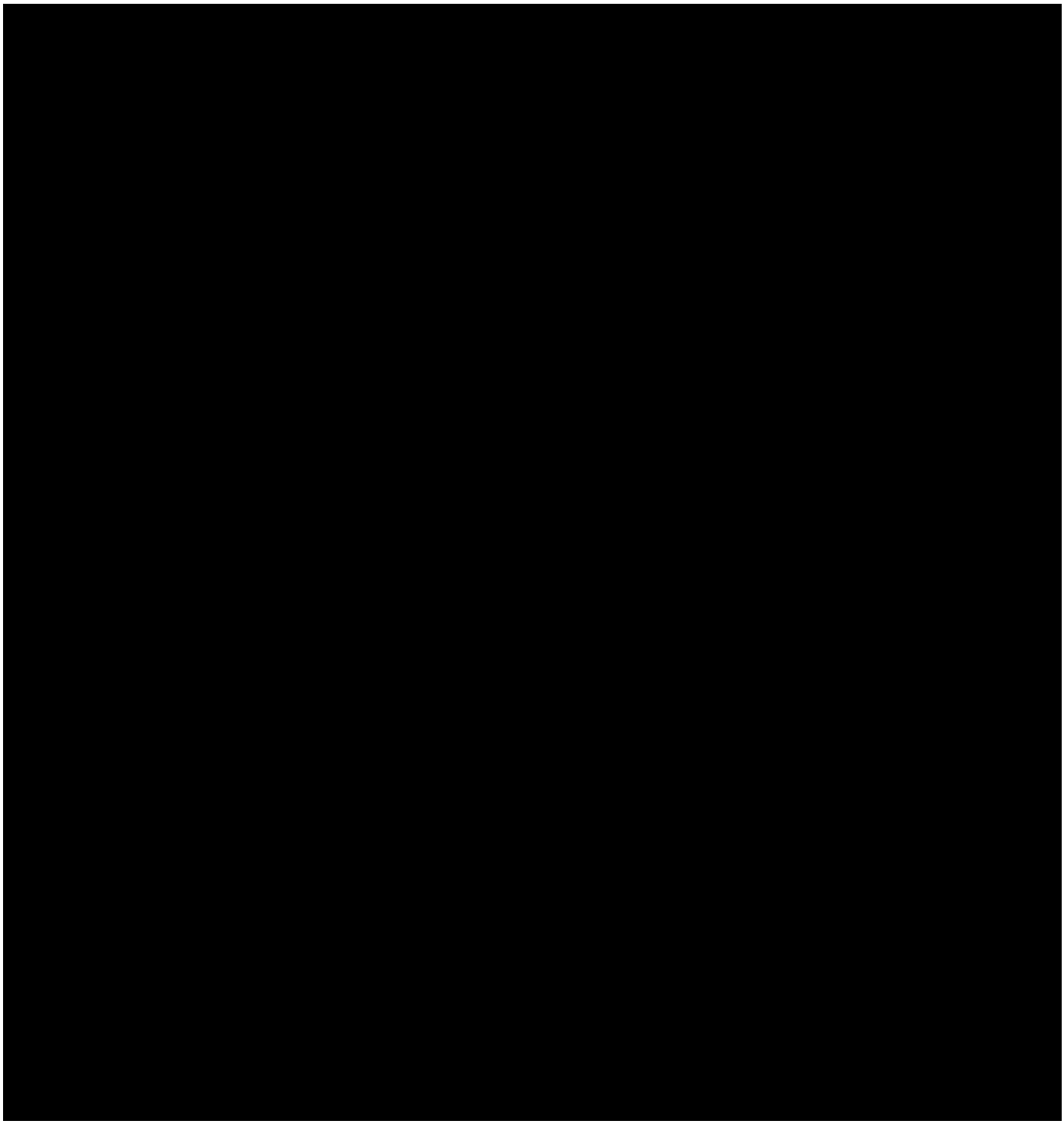
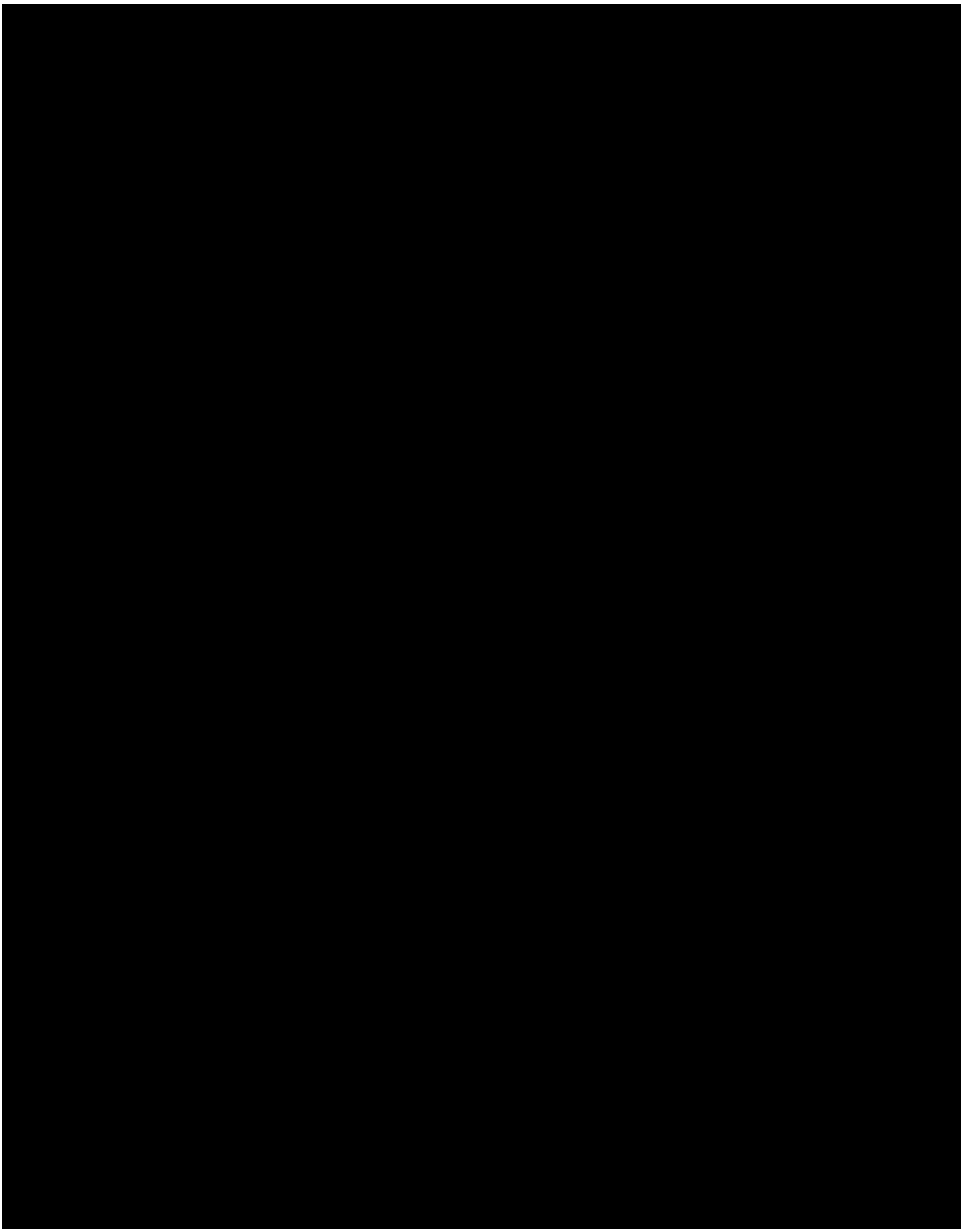
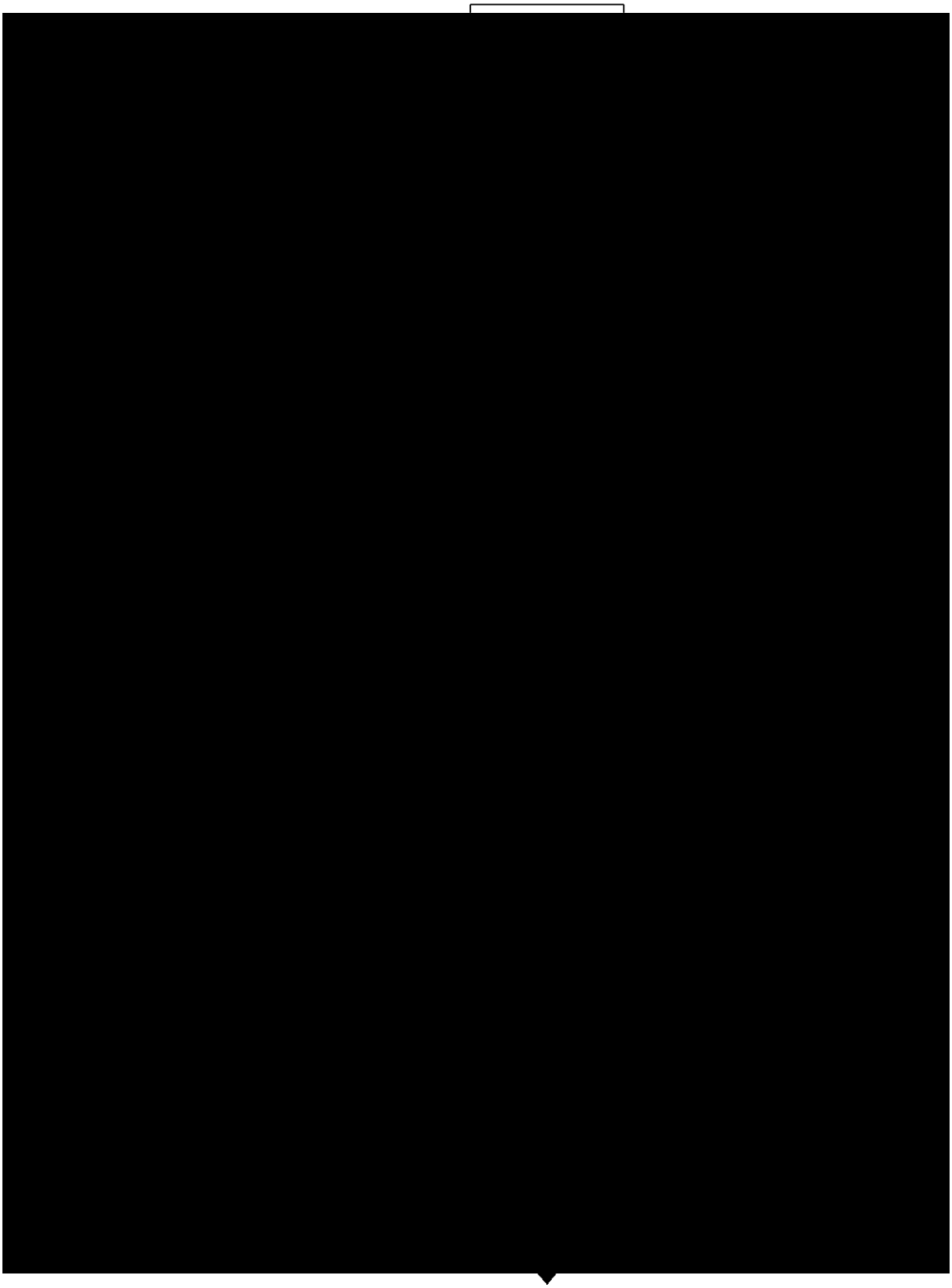


Figure 2 Flow chart for the production of AH111501 Sodium Salt



Reagents (continued…) Processing (continued…) Analysis (continued…)



[redacted]

3.2.A.3.2.3 Control of Materials

[redacted]

3.2.A.3.2.3.1 Raw materials used in the synthesis of AH111501 Sodium Salt

[redacted] te

[redacted]

3.2.A.3.2.4 Controls of Critical Steps and Intermediates

[redacted]

3.2.A.3.2.5 Process Validation and/or Evaluation

[redacted]

3.2.A.3.2.6 Manufacturing Process Development

[redacted]

3.2.A.3.3 Characterization (AH111501 Sodium Salt)

3.2.A.3.3.1 Elucidation of Structure and other Characteristics

A) Description of AH111501 Sodium Salt

[redacted]

B) [redacted]

[redacted]

C) [redacted]

[redacted]

D) [redacted]

[redacted]

E) [redacted]

[redacted]

F) [redacted]

[redacted]

G) [redacted]

[redacted]

3.2.A.3.3.2 Impurities

a Potential Impurities from the Synthesis and Degradation Products

[redacted]

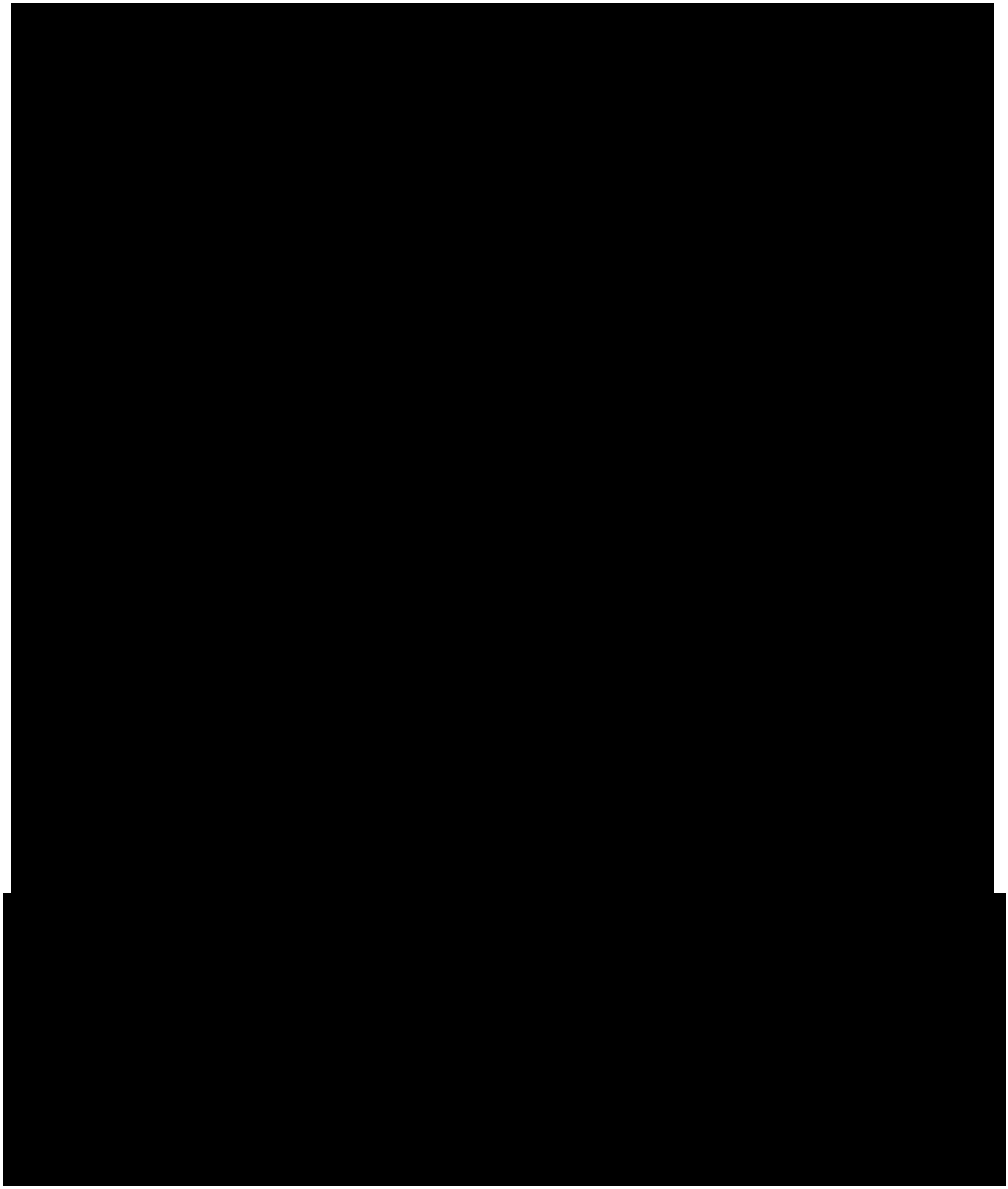
b Analytical Procedures

[redacted]

c Observed Impurities and Degradation Products

[redacted]

Figure 3 Typical chromatogram from HPLC analysis of batch AH111501 sodium salt



ii Other Impurities

[redacted]

3.2.A.3.4 Control of Novel Excipient (AH111501 Sodium Salt)

3.2.A.3.4.1 Specification

Table 1 Specification for AH111501 Sodium Salt

Tests	Analytical procedures	Acceptance criteria
Description Appearance	Visual inspection	Green to black, fine to granular powder
Identification ID of AH111501[1] by MS	Mass spectrometry	1527.3 ± 1.0 Dalton
Purity Total impurities of AH111501 sodium salt by HPLC	HPLC with UV detection	NMT 7.00% area
Water content	KF	NMT 8.0% m/m
Residual solvents Acetonitrile Ethyl acetate Toluene	GC	 NMT 100 µg/g NMT 1000 µg/g NMT 100 µg/g
Inorganic residues Sodium Phosphorous Tin	ICP-AES	 3.0 to 8.0% m/m NMT 30 µg/g NMT 30 µg/g
Microbiological tests Total Viable Aerobic Count Bacterial endotoxins	 Ph. Eur./USP Ph. Eur./USP	 NMT 5 CFU/10 mg NMT 1.5 EU/mg
Assay Content of AH111501 tri anion[2]	HPLC with UV detection	75.0 to 100.0% m/m

[1] Identification of AH111501 is determined in solution and the mass is calculated as the neutral free acid
[2] Assay is determined for dissolved AH111501 sodium salt and is hence reported for the dissociated free acid

3.2.A.3.4.2 Analytical Procedures

The analytical procedures used to control AH111501 sodium salt are summarized below.

A) Description of AH111501 Sodium Salt

AH111501 sodium salt is visually inspected in diffuse daylight.

B) Identification and impurities of AH111501 by HPLC-MS

AH111501 sodium salt is dissolved in water (0.3 mg/mL) and analyzed by reversed phase HPLC using a Zorbax Eclipse XDB C18 column (4.6 * 150mm; 5 µm) and applying a gradient of 0.1% (v/v) trifluoroacetic acid in water and acetonitrile containing 0.1% (v/v)

trifluoroacetic acid (gradient: 55/45 (0 min) → (17 min) → 38/62 (0 min) → (10 min)). By means of UV-detection at 270 nm purity has been established. Total impurities are reported as % area.

C) Water content by Karl Fisher (KF) titration

The water content in AH111501 sodium salt is determined by volumetric Karl Fisher titration, using a Hydranal two-component system (Metrohm 718 STAT Titrino).
Due to the fluffy and static properties of the material, reproducibility between the weighing of the samples is relatively poor.

D) Residual Solvents by Headspace Gas Chromatography (HS-GC)

Residual solvents in AH111501 sodium salt are determined by automated HS-GC with mass detector (MSD). Residual amounts of tetrahydrofuran (THF), toluene, n-heptane, ethylacetate (EtOAc), diethylether (Et_2O) acetonitrile (ACN) and N,N-dimethylacetamide (DMA) are determined. For the high boiling DMA we used DMI (1,3-dimethyl-2-imidazolidinone) as headspace solvent for the standards and the samples. For all other solvents water was used. This also means that the headspace method for DMA differs somewhat from the method for the other solvents. The GCMS system consisted of an Agilent 6890 GC, 5973 MSD and 7694 Headspace sampler. The column used was an Alltech AT 624 (30 m x 320 µm x 1.8 µm; #13756).

E) Inorganic Residues by ICP-AES

The concentration of sodium in AH111501 sodium salt is determined by ICP-AES

F) Total Viable Aerobic Count

Analysis is performed according to Ph. Eur. and USP.

G) Bacterial Endotoxins

Analysis is performed according to Ph. Eur. and USP

3.2.A.3.4.3 Validation of Analytical Procedures

The analytical procedures are appropriately validated for the current development phase and are suitable for their intended use. The validation performed at this stage is summarized in Table 2.

Table 2 Validation of Analytical procedures Performed at this Stage

Test	Validation parameters
Identification of AH111501 by MS	Accuracy, specificity, repeatability
Impurities by HPLC	Accuracy, repeatability, intermediate precision, specificity, linearity, range, detection and quantification limits
Water content by KF	Accuracy, repeatability, intermediate precision, linearity, range
Residual solvents by HS-GC	Accuracy, repeatability, intermediate precision, specificity, linearity, range, detection and quantification limits
Inorganic residues by ICP	Accuracy, repeatability, intermediate precision, specificity, linearity, range, detection and quantification limits
Total Viable Aerobic Count	The validation showed that AH111501 sodium salt did not have any inhibitory effect on growth of the test organisms
Bacterial endotoxins	The validation showed that the chosen test dilution of AH111501 sodium salt did not inhibit or enhance the positive product control
Assay of AH111501 tri-anion by HPLC	Accuracy, repeatability, intermediate precision, linearity, range

3.2.A.3.4.4 Batch Analyses

A batch of AH111501 sodium salt have been used for the manufacture of Mixture of pyruvic acid and AH111501 sodium salt for pre-clinical and technical studies. This batch has been repurified for subsequent pre-clinical and technical studies. The results for these batches presented in Table 3 are representative of the quality of the original and repurified batches used.

Table 3 Batch analysis data for AH111501 sodium salt

	Acceptance Criteria	**Lot#160923-1 (25-10-2011)**	**Lot#160923-1 Re-analysis (3-4-2013)**	**Lot#160923-1 Re-analysis (27-5-2014)**
Batch size		45 g		
Place of manufacture		Syncom b.v. Groningen, The Netherlands.		
Date of manufacture		24 august 2011		
Use		Pre-clinical studies, technical studies	Pre-clinical studies, technical studies	Pre-clinical studies, technical studies
Test				
Appearance	Green to black, fine to granular powder	Dark green, fine to coarse powder	Dark green, fine to coarse powder	Dark green, fine to coarse powder
Identification	1527.3 +/- 1.0 Dalton	1549.7, (+ sodium)	1549.7, (+ sodium)	Not determined
Total impurities of AH111501 sodium salt by HPLC	NMT 7.00% area	4.8%	5.4%	5.6 %
Water content	NMT 8% m/m	5.5 %	5%	Not determined
Assay of AH111501 trianion	75-100% m/m	94.0% m/m	Not determined	Not determined
Residual solvents: Acetonitrile Ethyl Acetate Toluene Tetrahydrofuran Ethylether Heptane Dimethylacetamide	 NMT 100 µg/g NMT 1000 µg/g NMT 100 µg/g NMT 100 µg/g NMT 1000 µg/g NMT 1000 µg/g NMT 100 µg/g	 NMT 25 µg/g 31 µg/g NMT 25 µg/g NMT 25 µg/g 2 µg/g 36 µg/g NMT 25 µg/g	Not determined	Not determined
Inorganic residues: Sodium Phosphorus Tin	 3.0 to 8.0% m/m NMT 30 µg/g NMT 30 µg/g	 3.69% NMT 3 µg/g NMT 3 µg/g		
Total Viable Aerobic Count	NMT 5 CFU/10 mg	NMT 1 CFU/10 mg	< 0.3 CFU/10 mg	
Bacterial endotoxins	NMT 1.5 EU/mg	NMT 0.25 EU/mg	<0.1 EU/mg	
pH	6.5<pH<7.5			

3.2.A.3.4.5 Justification of Specification

Batches will be released only if the results comply with the acceptance criteria provided in Section 3.2.A.3.4.1. Final specifications should reflect the process performance. The project is in early development and specifications are preliminary and will be evaluated throughout the development phase. Considering the early stage of the project where only limited batch data are available, the specifications are considered justified.

3.2.A.3.5 Reference Standards or Materials (AH111501 Sodium Salt)

A batch of AH111501 sodium salt has been qualified as a working reference standard (WS1010.002). The batch has been characterized by visual appearance, identification by MS, water content by Karl Fisher titration, inorganic residues by ICP-AES, residual solvents by HS- GC and purity by HPLC.

3.2.A.3.6 Container Closure System (AH111501 Sodium Salt)

The container closure system used for the AH111501 sodium salt is a Schott Fiolax amber screw neck glass bottle of suitable size (5 ml), Ph. Eur. and USP type I, supplied by Schott Glaswerke, Germany. The bottles are closed with a white PP screw cap, lined with a Teflon (PTFE) coated sealing plate, supplied by Schott Glaswerke, Germany.

3.2.A.3.7 Stability (AH111501 Sodium Salt)

3.2.A.3.7.1 Stability Summary and Conclusions

(a) Batch Tested

Stability testing has been performed on one batch of AH111501 sodium salt manufactured at GE Healthcare AS, Oslo, Norway. Batch information is given in Table 1.

Table 1 Batch Subjected to Stability Studies

Batch number	Batch use	Manufacturing date
FKJ.0157/131-01	Clinical study GE-101-001, Pre-clinical studies, technical studies	24 June 2005

(b) Storage Conditions and Testing Frequency

Storage conditions and testing frequency are stated in Table 2.

Table 2 Stability study protocol for AH111501 Sodium Salt

Batch number	Storage condition				Sampling points (months)
	Temp. (°C)	%RH	Position	Light	
FKJ.0157/131-01	5	Ambient	Inverted	Dark	0-6-8.5-12.5-18-24
	25	60	Inverted	Dark	0-6-9.5

Ambient = the humidity is monitored, but not regulated

(c) Analytical Procedures and Specification

The analytical test results from selected stability indicating parameters have been evaluated according to the acceptance criteria presented in Section 3.2.A.3.4.1. The analytical procedures used are described in Section 3.2.A.3.4.2.

The HPLC method applied initially was found to display an insufficient resolution, and a new method has been developed. The new method has been applied from, and including, the 8.5 months sampling point.

(d) Summary of Results

The stability results to date are presented in Table 3 and Table 4 in Section 3.2.A.3.7.3. All results remained within the acceptance criteria for samples stored at 5°C for 24 months, and stored at 25°C/60% RH for 9.5 months.

(e) Conclusion

Based on the stability data presented, AH111501 sodium salt is supported by a re-test period of 24 months when stored at 5°C (2-8°C), and protected from light in the container closure system described in Section 3.2.A.3.6.

3.2.A.3.7.2 Post-approval Stability Protocol and Stability Commitment

Not applicable.

3.2.A.3.7.3 Stability Data

Stability results on AH111501 sodium salt are given in Table 3 and Table 4.

Table 3 Stability results for AH111501 Sodium Salt batch FKJ.0157/131-01 at 5°C and ambient humidity

Test	Specification SP EN 1010.1021	Initial (0)	5°C/ambient humidity (months) 6	8.5	12.5	18	24
Appearance	Green to black, fine to granular powder	Dark green, fine to coarse powder	Fine, green powder	Fine, green powder	Fine, green powder	Fine, green powder	Fine, green powder
Total impurities of AH111501 sodium salt by HPLC	NMT 7.00 (% area)	1[1]	1	6.28, 6.20, 6.25	6.08 , 6.09, 6.12	5.91, 5.95, 5.90	5.96, 5.96, 6.00
Assay of AH111501 tri ation	75.0 to 100.0 (% m/m)	1	1	84.8, 84.7, 85.2	83.9, 84.0, 83.6	86.0, 86.9, 86.1	85.9, 85.7, 86.1
Water content	8.0 (% m/m)	7.0, 7.3, 8.1[2]	2.5, 2.6, 2.4	1.6, 1.5, 1.4	3.6, 3.6, 3.7	2.7, 2.8, 2.4	3.8, 3.3, 3.2
Total Viable Aerobic Count	NMT 5 CFU/10 mg	0	—	—	1	—	1
Bacterial endotoxins	NMT 1.5 EU/mg	NMT 0.5	—	—	NMT 0.5	—	NMT 0.5

— = Not performed NMT = Not More Than

[1] The HPLC method applied during the initial release and the 6 months testing was found to display an insufficient resolution and a new method has been developed.

[2] The content of water determined during the initial analysis was significantly higher than the results obtained during the stability testing. The reason for this is unexplained but may be due to an analytical error.

Table 4 Stability results for AH111501 Sodium Salt batch FKL0157/131-01 at 25°C/60% RH

Test	Specification	Initial	25°C/60% RH (months)	
	SP EN 1010.1/021	00	6	9.5
Appearance	Green to black, fine to granular powder	Dark green, fine to coarse powder	Fine, green powder	Fine, green powder
Total impurities of AH111501 sodium salt by HPLC	NMT 7.00 (% area)	1	1	6.08, 6.09, 6.06
Assay of AH111501 tri anion	75.0 to 100.0 (% m/m)	1	1	85.7, 85.5, 85.2
Water content	8.0 (% m/m)	7.0, 7.3, 8.1[2]	2.4 , 2.2, 2.8	2.6, 2.5, 2.5
Total Viable Aerobic Count	NMT 5 CFU/10 mg	0	—	—
Bacterial endotoxins	NMT 1.5 EU/mg	NMT 0.5	—	—

— = Not performed NMT = Not More Than

[1] The HPLC method applied during the initial release and the 6 months testing was found to display an insufficient resolution and a new method has been developed.

[2] The content of water determined during the initial analysis was significantly higher than the results obtained during the stability testing. The reason for this is unexplained but may be due to an analytical error.

7.5 Labeling

CAUTION: New Drug- Limited by Federal law to investigational use

Hyperpolarized Pyruvate (^{13}C) Injection Single Use Only

Study:_____ Batch #: MMDDYYYY-NN

Beyond-Use-Date: *Immediate Use Upon Preparation*

Pharmacist Initials: _____ Date: MMDDYYYY

University of California, San Francisco, 1700 4th Street

San Francisco, California, 94518-2512, USA

7.6 Environmental Analysis

The sponsor believes that the Investigational Product qualifies for categorical exclusion from an environmental assessment, and that, to our knowledge, no extraordinary circumstances exist. Under the provisions of 21 CFR 25.15 (d) and 21 CFR 25.31 (e), a categorical exclusion is requested.