U.S. Department
of Transportation

**Pipeline and Hazardous
Materials Safety
Administration**

1200 New Jersey Avenue SE
Washington, DC 20590

JUN 18 2015

Mr. David Rivers
R&S Compliance Group, LLC.
2707 W. Price Ave.
Tampa, FL 33611

Reference No. 14-0248

Dear Mr. Rivers:

This is in response to your December 30, 2014 letter requesting clarification of the Hazardous Materials Regulations (HMR; 49 CFR Parts 171-180) applicable to new explosives. You state your company receives a nitroglycerin and lactose mixture classified as "UN 3319, Nitroglycerin Mixture, Desensitized, Solid, N.O.S., 4.1" transported under approval EX2000120151. You further state that this raw material is used in the production of a pharmaceutical product. Various machines that come in contact with the raw material are cleaned and the cleaning process results in effluent waste material containing a methanol and water mixture with contaminate of nitroglycerin. You present in-house test data from tests run on several lots of effluent waste showing trace concentrations of nitroglycerin between 0.00616- 0.01220 mg/ml. You ask several questions regarding the need for classification of both effluent waste material and tablet nitroglycerin. Your questions are paraphrased and answered as follows:

Q1. In order for § 173.56 to apply, does the resulting effluent material resulting from the alteration process (testing and cleaning) of the originally classified explosive mixture need to meet the definition of a Class 1 material in § 173.50(a) first? Or does § 173.56 apply first because the original ingredient that was altered was subject to § 173.56 and therefore all products or by-products of the initial classified explosive, regardless of the characteristics, or if the product meets the definition of Class 1 per § 173.50(a), require formal testing, classification and approval per § 107.705?

A1. Per § 173.56(a) a new explosive is an explosive produced by a person who has not previously produced that explosive, or has previously produced that explosive but has made a change in the formulation, design, or process so as to alter any of the properties of the explosive. Any product containing an energetic ingredient (e.g., Nitroglycerin) is therefore subject to the requirements of § 173.56, and any modification of such product previously approved is also subject to the same requirements to determine if the modifications meet the definition of a new explosive. However, if the modification results in a new material that may be classified in a hazard class or division other than Class 1 under a proper shipping name specifically listed in the Hazardous Materials Table (HMT) in § 172.101 (e.g. UN1204), the modified material would not be subject to the provisions of §173.56 unless the proper shipping name has a special provision requiring approval under § 173.56.

Q2. If the latter is true, does the final pharmaceutical product, nitroglycerin tablets, require the same approval to transport to pharmacies as the tablet is a result of an alteration of an initially classified explosive?

A2. The nitroglycerin tablets would require approval in accordance with § 173.56(b).

Q3. Would the resulting effluent material, containing residual amounts of what would be a classified explosive in other forms or concentrations, be properly described for transportation by other characteristics? In this case UN1204 Nitroglycerin solution in alcohol.

A3. In accordance with § 173.22, it is the shipper's responsibility to properly classify a hazardous material. This Office generally does not perform this function. However, if the effluent waste can be appropriately described by an existing HMT entry for a hazard class or division other than Class 1 it may be offered for transport under that entry. In your scenario, if the nitroglycerin solution in alcohol and water contains not more than 1 percent nitroglycerin in alcohol with no other energetic ingredients, the effluent would most appropriately be offered as UN1204.

I trust this information is helpful. If you have further questions, please do not hesitate to contact this office.

Sincerely,

Duane A. Pfund

Duane A. Pfund
International Standards Coordinator
Standards and Rulemaking Division

Webb
17356
New Explosives
14-0248

Goodall, Shante CTR (PHMSA)

From: Betts, Charles (PHMSA)
Sent: Wednesday, December 31, 2014 12:05 PM
To: Goodall, Shante CTR (PHMSA)
Cc: Dodd, Alice (PHMSA); Foster, Glenn (PHMSA); Fink, William (PHMSA)
Subject: Fw: Request for Interpretation
Attachments: Request for interp less than 2 nitro.pdf; STM-AR-CV-0159 Nitroglycerin Cleaning Verification Method.pdf; STM-AR-FP-0164 Nitroglycerin Sublingual Tablets 0 3 mg 0 4 mg 0 6 mg.pdf

Shante -

Please log and assign for response. Thanks

From: Fink, William (PHMSA)
Sent: Wednesday, December 31, 2014 09:08 AM Eastern Standard Time
To: Betts, Charles (PHMSA); INFOCNTR (PHMSA)
Cc: Singh, Harpreet (PHMSA)
Subject: FW: Request for Interpretation

Charles,

R&S is requesting a letter of interp.

Bill Fink

From: Alston, Barbara CTR (PHMSA)
Sent: Tuesday, December 30, 2014 4:43 PM
To: Fink, William (PHMSA); Herrera, Jacqueline (PHMSA)
Subject: FW: Request for Interpretation

Good Afternoon Team,
I spoke with Mr. Rivers on the phone and he has sent information pertaining to his questions/request, please see attachments. Many thanks, Barbara

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From: David Rivers [mailto:david@rscompliance.com]
Sent: Tuesday, December 30, 2014 4:17 PM
To: Alston, Barbara CTR (PHMSA)
Subject: Request for Interpretation

Barbara,
Thank you for speaking with me today. The attached is a scenario requesting an interpretation as to the applicability of 173.56, "New Explosive" of an effluent waste from a pharmaceutical manufacturing and cleaning process. The narrative demonstrates the specifics of our situation. If needed, I also attached testing procedures that we use that ultimately creates the "by-product" we are trying to ensure we classify for transportation appropriately given the raw material Nitroglycerin.

Your help is greatly appreciated. Happy New Year!

David O. Rivers, M.S.

R&S
Compliance Group, LLC
2707 W. Price Ave., Tampa, FL 33611 Phone: (813) 433-4979

December 30, 2014

US Department of Transportation
PHMSA – Energetic Materials
Attn: Division of Approvals and Permit, PHH30
1200 New Jersey Ave. SE, East Building 2nd Floor
Washington, DC 20590-0001

R&S Compliance Group, llc would like to request a letter of interpretation as to the intent of the definition of a "New Explosive" described in 173.56. This section requires that a classification and new EX Approval be granted for the transportation of a classified explosive if the original classified explosive is altered "…as to change the formulation, design or process so as to alter the properties of the explosive."

Specifically, we receive a Nitroglycerin and Lactose Mixture classified as;

"**Nitroglycerin Mixture, Desensitized, Solid, NOS** *with more than 2 percent but not more than 10 percent nitroglycerin, by mass",* transported under Approval **EX2000120151** and reclassified by the Associate Administrator as hazard class 4.1, UN3319.

This raw material is used in the production of a pharmaceutical product, a Nitroglycerin Tablet. Each LOT of final product, the table themselves, must be tested for quality control according to Good Manufacturing Practices (GMP) and Food and Drug Administration (FDA) requirements. The machine used to extract the active ingredient from the raw material received, Nitroglycerin, also must be cleaned. Other machines which have contact surfaces that come in contact with the pharmaceutical mixture during the blending, mixing and tablet formation process must be washed. This testing and cleaning process results in effluent waste material containing a Methanol and Water Mixture with contaminates of Nitroglycerin.

The in-house lab ran a test on this effluent waste material from several lots of effluent waste and found the following concentrations of trace Nitroglycerin per volume. (In house test procedures attached)

Approximate Conc. of Nitroglycerin per container		
Container 1	**0.01220**	mg/mL
Container 2	**0.00068**	mg/mL
Container 3	**0.00194**	mg/mL
Container 4	**0.00616**	mg/mL

*Results are "Grab" samples from 55 gallon drums of effluent and not meant to meet the testing criteria prescribed in 173.52, 173.57 and 173.58

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Our question is if the requirements to seek proper classification and EX Approval from the Associate Administrator per 173.56 apply to the effluent waste from laboratory testing and/or cleaning processes by determining if these activities meets the definition of "altering" an existing explosive when the resulting effluent material to be transported, (transportation for disposal in this case), does not meet the definition of a Class 1 explosive ,173.50(a).

#1

In order for 173.56 to apply, does the final effluent material resulting from the alteration processes (testing and cleaning) of the original classified explosive mixture (Nitroglycerin Mixture, Desensitized, Solid, NOS) need to meet the definition of a CLASS 1 material (173.50(a) FIRST? *"an explosive means ...designed to function by explosion..."*,?

If the resulting effluent material did in fact meet the explosive definition, we would agree an approval would we required as a New Explosive defined in 173.56. But what the resulting materials does not meet the definition of an explosive 173.50(a), would further examination of this effluent mixture and written determination be required from the Associate Administrator to state that the material does not need an approval?

#2

Or, does 173.56 apply simply because the original ingredient that was altered was subject to 173.56 before initially offered for transportation, therefore, all products or by-products of the initial classified explosive (in this case, Nitroglycerin Mixture, Desensitized, Solid, NOS, **EX2000120151)**, regardless of the resulting characteristics or meeting the definition of Class 1 set forth in 173.50 (a), require formal testing, classification and approval per 107.705.

If the latter, #2, is true, does the final pharmaceutical product, the common Nitroglycerin tablet for heart patients require the same approval to transport to pharmacies as the tablet is the result of an alteration of an initially classified explosive?

Or, does this resulting effluent material, even though it contains residual amounts of what otherwise would be a classified explosive in other forms or concentrations, simply need to be properly described for transportation to meet the definition of its characteristics, 173.22(a), not requiring an EX Approval, and in this case by 173.120(a)(2) as a flammable liquid such as;

UN1204, Nitroglycerian Solution in Alcohol *with not more than 1 percent nitroglycerin*

*This above authorized name does not list a special provision requiring an EX Approval from the Associate Administrator in order to describe a product as with the original Nitroglycerin product received and used, even though the description has the word "Nitroglycerin" within the name. This description would be selected by shippers where appropriate as required by "Shipper's Responsibility"

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We understand that there is not a threshold of an explosive concentration that PHMSA has established for an "altered" explosive, clarified according to interpretation #09-0058 which would need an EX Approval if, as stated in paragraph three, … "new compositions containing any amount of explosive material…". This sentence refers to an "explosive" material presumably "explosive" by definition, 173.50(a).

However, does the above described effluent waste, scenario, and furthermore the final pharmaceutical product, meet the intent or definition of a "New Explosive", subject to 173.56 simply because of "alteration" though a sampling and cleaning process and eventual formation and packaging or a pharmaceutical tablet involving previously "Classified Explosive" requiring an EX Approval for initial transport, where the "Process" is not intended to produce an a different "explosive", by definition, from the original "explosive" when the final effluent mixture does not exhibit characteristics of a CLASS 1 material, by definition?

Your attention and interest to our request is appreciated. Please feel free to contact me with any questions you may have.

Regards,

David O. Rivers, M.S.
CHMM, CSP, CET, CDGP
R&S Compliance Group, llc.
Ph: 813-433-4979
Fx: 813-436-5392
david@rscompliance.com
www.rscompliance.com

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #: STM-AR-FP-0164
	Effective Date: MAR 14 2014 Supersedes: 05/16/13
Prepared by:	Date: 03/11/14
Reviewed by: Dsha George	Date: 03/11/14
Reviewed by:	Date: 03/11/14
Approved by: Radha Goolabs	Date: 03/12/14 03/13/14

I. PURPOSE

The purpose of this procedure is to describe the test methods used for analysis of Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg and Intermediate products.

TESTS

A. Description
B. Identification
C. Assay
D. Blend Uniformity
E. Uniformity of Dosage Units
F. Degradation Products
G. Dissolution
H. Water Content
I. Disintegration

II. APPLICABILITY

This method applies to Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg and Intermediate products. This method applies to Analytical Research Department.

III. PROCEDURE

EHS Safety Statements

1. Material Safety Data Sheets for all chemicals involved should be read prior to starting any chemical handling. All recommendations of work practices and Personal Protective Equipment (PPE) should be followed.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

2. All solutions and prepared samples must be properly labeled, including hazard information, if they are to be stored longer than a single work shift.

3. All waste, including rinsings, should be labeled appropriately and handled per Watson Waste Management Policy, SOP GN-0026 and Risk Management Procedure RM-0018 (located on the Portal). If a hazardous material was used in a filter media, the filter media is considered hazardous material and must be disposed of appropriately.

4. All glassware should be inspected prior to use. Defective glassware should not be used.

5. Thick walled glassware should be used when vacuum degassing media and mobile phases.

6. Waste receptacles should be verified to have enough space to hold the volume of waste to be generated.

7. Flammable solvents should be dispensed inside of a chemical fume hood. Flammable solutions should be covered if stored outside of the hood.

8. Appropriate PPE should be worn based on the requirements of Risk Management Procedure RM-0009 (located on the Portal) and the MSDS.

9. When weighing powdered laboratory chemicals and products, excess materials should not be placed into the regular trash receptacles. Excess products should be placed inside a non-hazardous waste receptacle and excess chemical powders should be disposed of appropriately based on hazards present.

10. All PPE should be inspected prior to donning. PPE should be rechecked often for damage and replaced as needed.

11. Concentrated acids and bases should be handled with care and dispensed inside a chemical fume hood with appropriate PPE.

12. Incompatible waste streams should be segregated and collected separately for disposal.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

A. Description

Visually inspect the sample and compare with the description given in the respective Analytical research in-process and finished product specifications.
Refer to the specification item number given in Section VI

B. Identification

1. HPLC

The major peak in the assay sample chromatogram corresponds to that of the retention time for the Nitroglycerin peak in the Standard chromatogram.

2. Thin-Layer Chromatographic Identification Test

2.1. Apparatus

A chromatographic chamber made of inert, transparent material with flat bottom or twin trough, a tightly fitted lid and a size suitable for the plate is used. The chamber is lined on at least one wall with filter paper. Sufficient mobile phase is added to the chamber that, after impregnation of the filter paper, a depth appropriate to the dimensions of the plate used is available. The chamber is closed and allowed to equilibrate.

2.2. Reagents

- Acetone, ACS grade or better
- Toluene, ACS grade or better
- Ethyl acetate, ACS grade or better
- Glacial acetic acid, ACS grade or better
- Methanol, HPLC grade or better
- Diphenylamine, ACS grade or better
- Nitroglycerin standard

2.3. Reagent Preparation

2.3.1. Mobile Phase

Mixture of toluene, ethyl acetate and glacial acetic acid in a ratio 16:4:1. Scale up or down as needed.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

2.3.2. Spray Solution

Prepare a mixture of diphenylamine in methanol (1 in 100). Scale up or down as needed.

2.4. Standard Preparation

Prepare a solution of 1 mg of nitroglycerin in 1 mL of acetone.

2.5. Sample Preparation

Transfer the amount of finely powdered Sublingual Tablets, equivalent to about 1 mg of nitroglycerin, to a centrifuge tube, add 1 mL of acetone, cover with parafilm shake by mechanical means for 30 minutes, and Centrifuge at 3000 rpm for 10 minutes, use supernatant for analysis.

2.6. Procedure

On a line parallel to and about 2 cm from the edge of a suitable thin-layer chromatographic plate coated with 0.25 mm layer of silica gel mixture:

1. Apply 10 µL of the test and standard solutions with an interval of at least 10 mm between the centers of spots. The application spots must be at 5 mm above the level of the mobile phase.
2. Allow the spots to dry
3. Place the plate in the chamber, ensuring the spots are above the surface of the mobile phase
4. Develop the chromatogram in a solvent system, until the solvent front has moved about three-fourth of the length of the plate.
5. Remove the plate from chamber, mark the solvent front and allow the solvent to evaporate.

2.7. Detection

Spray with a solution of diphenylamine in methanol (1 in 100) and irradiate the plate with short (254 nm) and long (365 nm) UV light for about 10 minutes. Determine the chromatographic retardation factor (R_F) for the principal spots. The R_F of the principal spot obtained from the test solution corresponds to the obtained from the standard solution.

Title: Nitroglycerin Cleaning Verification Method	Test Method: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11
Prepared by:	Date: 09/29/11
Reviewed by:	Date: 09/29/11
Approved by:	Date: 09/29/11

I. PURPOSE

The purpose of this procedure is to describe the residue test method for monitoring Nitroglycerin in manufacturing equipment.

II. APPLICABILITY

This STM applies to cleaning swabs for Nitroglycerin. This method applies to both Analytical Research and Quality Control Departments.

III. PROCEDURE

A. Instrumentation

High Performance Liquid Chromatography system consisting of a pump, an injector, a UV detector and a data processing system capable of integrating the areas under the curve of the peaks of interest.

Column:	Alltech, Alltima C18, 5 µm, 4.6 mm x 150 mm PN# 88052
Mobile Phase:	USP Water: Methanol (60:40)
Injection Volume:	50 µL
Run time:	Not less than 20 min
Flow rate:	1.2 mL/min
Wavelength:	220 (8) nm
Temperature:	25°C
Rt window:	10 min to 14 min

B. Reagents

1. Water, USP
2. Methanol, HPLC grade or equivalent
3. Nitroglycerin standard

C. Reagent Preparation

1 Mobile Phase: USP Water: Methanol (60:40)

Mix 600 mL of USP water with 400 mL of Methanol and mix well. This preparation may be scaled up or down as needed

Title: Nitroglycerin Cleaning Verification Method	STM #: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11

2 Diluent: USP Water: Methanol = 50:50

Mix 500 mL of USP water with 500 mL of Methanol and mix well. This preparation may be scaled up or down as needed

D. Standard Preparation:

1. Stock Solution:

Accurately weigh and transfer approximately 63.3 mg of Diluted Nitroglycerin Standard (equivalent to 0.6 mg of Nitroglycerin) into a 50 mL volumetric flask, dissolve and dilute to volume with Methanol. The concentration of Nitroglycerin stock Solution is approximately 0.012 mg/mL.

2. Working Standard Solution

Pipette 5.0 mL of the stock solution into a 50 mL volumetric flask, dilute to volume with diluent, and mix well. Scale up or down as needed.

The stock and working standard solutions are stable for 5 days at room temperatures. Concentration of Nitroglycerin working standard is about 0.0012 mg/mL.

E. Swab Sample Preparation:

Swab samples are collected and submitted in 50-mL plastic centrifuge tubes. Pipette 5.0 mL of the diluent into each of the sample tubes and mix the contents for at least 2 minutes using a vortex mixer. Transfer about 1.5 mL of the resulting solution into an HPLC vial. Swab samples and solutions are stable for 3 days.

F. Blank Swab Preparation:

A Blank Swab is prepared and submitted in a 50 mL plastic centrifuge tubes. Treat the same way as a Sample Swab.

G. HPLC Procedure:

Set up the HPLC system according to the conditions listed under "Instrumentation" (Section III.A), and allow the system to equilibrate. Make at least one injection of the diluent. Perform System Suitability Test. Calculate the reproducibility (%RSD) for six Nitroglycerin Working Standard injections and perform the system suitability calculations. Calculate the reproducibility (%RSD is $\leq$ 5.0 %), the number of theoretical plates (N$\geq$ 3000), and the tailing factor (T$\leq$ 2.0). If the system is suitable, proceed with the samples analysis. Bracket the samples with the Working Standard Solution.

Title: Nitroglycerin Cleaning Verification Method	STM #: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11

H. Calculations

Note: Label mobile phase, detergent and swab peaks in the sample chromatograms according to the table below (Refer to Validation Report ARVR-11-0068)

Peak	Peak RT (min)	RRT
Mobile Phase Peak 1	0.766	0.06
Detergent Peak	0.872	0.07
Mobile Phase Peak 2	1.274	0.11
Swab Peak 1	2.21	0.18
Mobile Phase Peak 3	3.368	0.28
Swab Peak 2	4.485	0.37
Swab Peak 3	6.232	0.52
Swab Peak 4	8.674	0.72
Nitroglycerin	12.084	1.00
Swab Peak 5	13.39	1.11

Calculate the quantity (Q) of Nitroglycerin or unknown µg /swab using the following equation:

$$\mu g \text{ Nitroglycerin /Swab} = \frac{Au}{As} \times \frac{Ws}{0.661} \times PF \times 0.1$$

Where

PF	=	Potency of Nitroglycerin Reference Standard. Use as % (e.g. 0.948% is entered as 0.948)
As	=	Average area of the Nitroglycerin peaks in chromatograms for the bracketing Working Standard Solutions.
Au	=	Peak response of Nitroglycerin in the sample chromatogram.
Ws	=	Weight of Diluted Nitroglycerin Reference Standard in mg.
0.661	=	Correction factor based on the ferrous plate recovery study, expressed in decimals.
0.1	=	Dilution factor including standard, Sample dilutions and conversion of Diluted Nitroglycerin into Nitroglycerin

Title: Nitroglycerin Cleaning Verification Method	STM #: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11

IV. REFERENCES

Current SOP # AR-0051, How to write a Standard Test Method.
Current USP
Cleaning Validation Request letter for Nitroglycerin (From Technical Service Department), received on 06/09/20011.

V. HISTORY

New, Effective date: 07/22/11

Revision 1, effective date: SEP 29 2011
Linearity range in the summary chart of page 6 updated to: 0.0001213 mg/mL to 0.002425 mg/mL

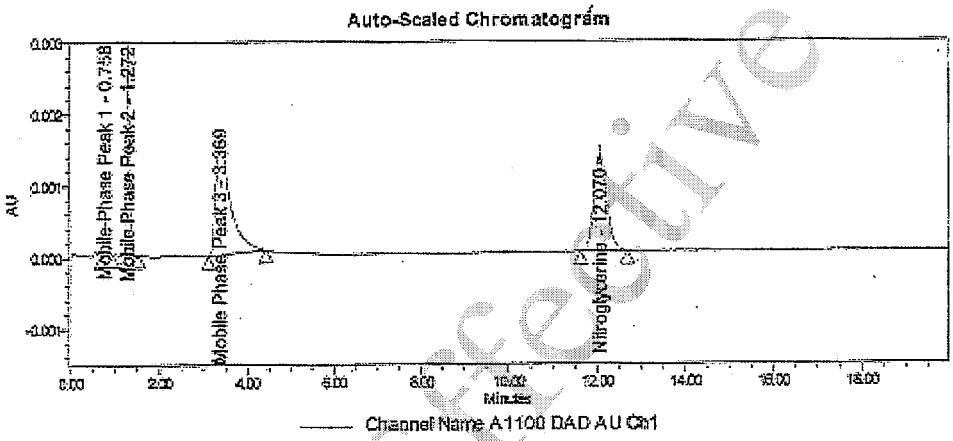
VI. SPECIFICATION

Cleaning verification criteria: NMT 5.77 µg of Nitroglycerin/ Swab

Title: Nitroglycerin Cleaning Verification Method	STM #: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11

Figure 1. Chromatogram of Nitroglycerin Working Standard Solution

SAMPLE INFORMATION			
Sample Name:	STD 1-7	Acquired By:	dtatkow
Sample Type:	Standard	Date Acquired:	7/11/2011 7:39:09 PM EDT
Vial:	43	Acq. Method Set:	CV LC 17
Injection #:	1	Date Processed:	7/18/2011 9:13:19 PM EDT
Injection Volume:	50.00 ul	Processing Method:	CV Specificity
Run Time:	20.0 Minutes	Channel Name:	A1100 DAD AU Ch1
Sample Set Name:	071111 5day std stab LC17	Proc. Chnl. Descr.:	DAD AU Ch 1 Sample 220, Bw 8



SampleName: STD 1-7

	SampleName	Name	Area	Retention Time (min)	s/n	USP Plate Count	USP Tailing
1	STD 1-7	Mobile Phase Peak1	2559	0.759	46.8	489	1.6
2	STD 1-7	Detergent Peak		0.879			
3	STD 1-7	Mobile Phase Peak2	15744	1.272	301.2	1578	2.0
4	STD 1-7	Swab Peak1		2.210			
5	STD 1-7	Mobile Phase Peak3	35455	3.368	157.0	695	2.4
6	STD 1-7	Swab Peak2		4.550			
7	STD 1-7	Swab Peak3		6.418			
8	STD 1-7	Swab Peak4		8.870			

Title: Nitroglycerin Cleaning Verification Method	STM #: STM-AR-CV-0159
	Effective Date: SEP 29 2011
	Supersedes: 07/22/11

Summary Chart

Type of Swab:	Texwipe TX714 A
Maximum Allowable Limit:	NMT 5.77 µg / Swab
Area to be Swabbed:	100 cm^2
Target Ingredient:	Nitroglycerin
Limit of Detection:	0.00006063 mg/mL
Limit of Quantitation:	0.0001213 mg/mL
Linearity:	0.0001213 mg/mL to 0.002425 mg/mL
Specificity:	No Interference
Plate Recovery:	From Ferrous Plates: 66.1 %
Swabbing Solution:	Methanol
Swab Stability:	Stable for 3 days
Swab Stability in Solution:	Stable for 3 days
Swab Sampling Technique:	As per SOP # AR-0063 Section: 8.6.1

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

C. Assay

1. Instrument

An HPLC system consisting of an autosampler, pump, a column, an adjustable wavelength UV Detector and an integrator capable of integrating the peaks of interest.

HPLC Parameters:

Column:	Phenomenex, Luna C18 (2), 5 um, 4.6 mm x 250 mm PN# 00G-4252-E0
Mobile Phase:	Methanol/Water=50/50
Injection Volume:	20 µL
Run time:	not less than 19 minutes
Flow rate:	1.0 mL/min
Wavelength: | Column	220 (8) nm, ref: off |
Temperature:	25 °C
Rt window:	13.5 minutes to 17.5 minutes

2. Reagents

- Diluted Nitroglycerin Reference Standard
- Methanol, HPLC grade or equivalent
- USP Water

3. Reagent Preparation

3.1. Mobile Phase Preparation: Methanol/Water=50/50

Mix 1000 mL of Water with 1000 mL of Methanol and sonicate to degas for 5 minutes. This preparation may be scaled up or down as needed.
Mobile Phase was demonstrated to be stable for at least 6 weeks at room temperature conditions

3.2. Diluent

Use assay Mobile Phase: Methanol/Water=50/50

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

4. Standard Preparation

4.1. Working standard solution

Accurately weigh about 158 mg of Diluted Nitroglycerin standard (equivalent to 1.5 mg of Nitroglycerin RS) into 20 mL V.F. Add 10 mL of diluent and dissolve. Dilute to volume with diluent and mix well. This preparation may be scaled up or down as needed.

4.2. Stability of Standard Solution

The standard solution has been proved to be stable for 15 days at room temperature conditions.

4.3. Standard Concentration calculation

The working solution contains approximately 0.075 mg/mL of Nitroglycerin and is calculated using the following equation:

$$Cstd = \frac{Wstd \times P}{20\,mL \times 100}$$

Where,

C_{std} = Concentration of Nitroglycerin standard in mg/mL.
W_{std} = Diluted Nitroglycerin Standard weight, mg
P = Potency of the Diluted Nitroglycerin Reference Standard in %.
100% = Conversion from Diluted Nitroglycerin to Nitroglycerin

5. Sample Preparation

5.1. Stock Sample Solution

Weigh the amount of tablets listed in the table below. Transfer into the proper volumetric flask. Add a stir bar and 80 % of the flask volume of diluent. Stir the sample for 1 hour (tablets should completely disintegrate). Remove the stir bar and rinse it inside the flask with diluent, complete to volume with diluent and mix well. Transfer about 10 mL into a centrifuge tube, cover with parafilm and centrifuge the sample at 3000 rpm for 10 minutes. (Concentration is about 0.3 mg/mL).

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Strength	# of Tablets	Volumetric Flask in mL
0.3 mg	20	20
0.4 mg	20	25
0.6 mg	25	50

5.2. Working Sample Solution

Dilute 6.0 mL of the centrifuged supernatant stock solution into a 25 mL volumetric flask and dilute to volume with diluent, mix well. Transfer an aliquot into HPLC vial to analyze. (Concentration is in range 0.072 to 0.077 mg/mL).

5.3. Stability of Sample Solution

The Stock and working assay sample solutions were proved to be stable for at least 3 days at room temperature conditions

6. Procedure

6.1. Refer to SOP AR-0049 "High Performance Liquid Chromatographic Analysis"

6.2. System Suitability Criteria

Make five replicate injections of the Working Assay Standard-1 solution. Calculate the reproducibility (% RSD) of the five consecutive injections, theoretical plates (N) and the tailing factor (T) for the Nitroglycerin peak. The system is suitable if: RSD $\leq$ 2.0%, N $\geq$ 3000 and T $\leq$ 2.5.

Inject the Working Assay Standard Solution-2 twice. Compare the potency (%) of Standard-2 relative to Standard-1:

6.3. Standard Comparison Calculation

$$Std\,Comp.(\%) = \frac{Astd2 \times Wstd1}{Astd1 \times Wstd2} \times 100$$

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Where,

A_{std1} = Peak area of Standard-1 (Average of five injections)
A_{std2} = Peak Area of Standard-2 (Average of two injections)
W_{std1} = Standard-1 weight, mg
W_{std2} = Standard-2 weight, mg

Standard-1 is suitable for use if Standard comparison (%) value is within the range 100.0 ± 2.0%.

6.4. Sample Analysis

Inject working sample solutions bracketed by working standard –1.

6.5. Factors to be used in Empower

Factor	Description	Custom Field in Empower Set up
Standard Concentration	Standard Concentration in mg/mL	Component Editor Table/ STANDARDS ONLY
20 (for 0.3mg and 0.4mg) or 25 (for 0.6mg)	Number of Tablets for analysis	Sample Weight
83.3333 (for 0.3mg), 104.1667 (for 0.4mg) or 208.3333 (for 0.6 mg)	Dilution volume in mL	Dilution
0.3, 0.4 or 0.6	Nitroglycerin Strength in mg	Component Editor Table/ STANDARDS & UNKNOWNS (under LC)

7. Calculations

7.1. Peak identification

Follow table below for possible peaks observed in the assay sample chromatograms based on the specificity study during the method validation (Reference: 2492p9)

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Component name	Peak	Retention Time (minutes)	RRT
Placebo	No peak observed	NA	NA
2-Mononitroglycerin	Peak observed	2.964	0.20
1-Mononitroglycerin	Peak observed	3.120	0.21
1,3-Dinitrolgycerin	Peak observed	5.240	0.36
1,2-Dinitroglycerin	Peak observed	5.838	0.40
Mobile Phase (Diluent)	Peak observed	6.154	0.42
Nitroglycerin	Peak observed	14.673	NA

7.2. Assay Calculation

From the HPLC analysis, obtain the peak area of the sample (A_{spl}) and Standard-1 (A_{std}). The percentage of Nitroglycerin per tablet is calculated from the equation:

$$\% \text{ LC} = \frac{Aspl \times Cstd \times VF \times 25mL}{Astd \times LC \times \#tablets \times 6.0mL} \times 100\%$$

Where,

Cstd	=	Nitroglycerin Standard-1 concentration, mg /mL
Aspl	=	Peak area of the sample.
Astd	=	Average Area of Bracketing standard-1.
LC	=	Label claim in mg
# tablets	=	number of tablets used in the analysis
VFstock	=	Volume of the Stock Solution in mL

D. Blend Uniformity

1. Instrument, Reagent, Reagent Preparation, Standard Preparation and Procedure

Refer to Assay section III.C.1, III.C.2, III.C.3, III.C.4 & III.C.6.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

2. Sample Preparation

Samples of one to six dosage units are provided from various blenders and drum locations. Perform the following for each sample:

Tap the vial several times on the bench top. Weigh accurately the vial containing the sample **(with the cap included in the initial weight)** and record the weight (G).

Quantitatively transfer the content of the vial to a volumetric flask (follow table below according strength and dosage units provided as a reference only).
NOTE: Ensure to wash the vial and the inside portion of the cap with diluent to recover any possible active trapped in the cap.
Add a stir bar into the volumetric flask and diluent up to 70 % of the flask volume and stir for 1 hour. Remove the stir bar and rinse it inside the flask with diluent, complete to volume with diluent and mix well. Transfer an aliquot into a centrifuge tube, cover with parafilm and centrifuge the sample at 3000 rpm for 10 minutes. Transfer an aliquot of supernatant into HPLC vial to analyze

The Nitroglycerin concentration in the prepared Blend Uniformity Samples should be within the range of 0.045 mg/mL to 0.1125 mg/mL

Dosage Units	Strengths in mg	VFstock in mL
1 dosage unit	*0.3*	5
	0.4	5
	0.6	10
2 dosage units	*0.3*	10
	0.4	10
	0.6	20
3 dosage units	*0.3*	10
	0.4	20
	0.6	25

Rinse the empty sample vials and caps with acetone and:
-Dry the rinsed vials (**without the cap**) in the oven at 105°C for about 30 minutes,
-Dry the rinsed caps at room temperature for at least 2 hours or until needed ensuring that they are completely dry

Weigh the empty vial **(with the cap)** and record its weight (T). Finally calculate the weight of the sample (N) = (G – T).

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

3. **Procedure**

3.1. Factors to be used in Empower

Factor	Description	Custom Field in Empower Set up
Standard Concentration	Standard Concentration in mg/mL	Component Editor Table/ STANDARDS ONLY
Weight	Sample Weight in mg	Sample Weight
Volumetric Flask Volume	Dilution Factor in mL	Dilution
Theoretical potency	Theoretical potency in decimals (Not as in percentage)	Component Editor Table/ STANDARDS & UNKNOWNS (under LC)

4. **Calculations**

The percentage of Nitroglycerin is calculated using the equation below:

$$\%LC = \frac{Aspl}{Astd} \times \frac{Cstd \times VF \times 100\%}{Ws \times \%Th} \times 100\%$$

Where
Aspl = Peak area response of the sample.
Astd = Average area response of the bracketing standard.
Cstd = Concentration of Nitroglycerin standard in mg/mL
Ws = Sample weight in mg
%Th = Theoretical percentage of the active in the blend.
VFs = Volume of the Volumetric Flask in mL

E. Uniformity of Dosage Units (Content Uniformity)

1. **Instrument, Reagent, Reagent Preparation, Standard Preparation and Procedure**

Refer to Assay section III.C.1, III.C.2, III.C.3, III.C.4 & III.C.6.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

2. Sample Preparation

Weigh ten tablets individually. Transfer into separate volumetric flasks (VF) (follow table below). Add a stir bar and 70 % of the flask volume of diluent and stir for 1 hour. Remove the stir bar and rinse it inside the flask with diluent, complete to volume with diluent and mix well. Transfer an aliquot into a centrifuge tube, cover with parafilm and centrifuge the sample at 3000 rpm for 10 minutes. Transfer an aliquot of supernatant into HPLC vial to analyze

Strength	VF in mL
0.3 mg	5
0.4 mg	5
0.6 mg	10

Concentration of Nitroglycerin is from 0.06 mg/mL to 0.08 mg/mL

3. Procedure

3.1. Factors to be used in Empower

Factor	Description	Custom Field in Empower Set up
Standard Concentration	Standard Concentration in mg/mL	Component Editor Table/ STANDARDS ONLY
1	Number of Tablets for analysis	Sample Weight
5 (for 0.3mg and 0.4mg) or 10 (for 0.6 mg)	Dilution volume in mL	Dilution
0.3, 0.4 or 0.6	Nitroglycerin Strength in mg	Component Editor Table/ STANDARDS & UNKNOWNS (under LC)

4. Calculations:

From the HPLC analysis, obtain the peak area of the sample (A_{spl}) and Standard-1 (A_{std}). The percentage of Nitroglycerin per tablet is calculated from the equation:

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

$$\%LC = \frac{Aspl \times Cstd \times VF}{Astd \times LC} \times 100\%$$

Where,

Cstd	=	Standard-1 concentration, mg /mL
Aspl	=	Peak Area of the Sample.
Astd	=	Average Area of Bracketing standard-1.
LC	=	Label Claim in mg
VFstock	=	Volume of the Volumetric Flask in mL

5. Criteria: (AS PER USP Monograph)

The content of each of the 10 Sublingual Tablets is within the range of 75.0 % and 135.0 % of the labeled claim. If the content of not more than 1 Sublingual Tablet is outside the range of 75.0 % and 135.0 % and if the content of none of the Sublingual Tablets is outside the range of 60.0 % and 150.0 %, test 20 additional units. The requirements are met if the content of each of the additional 20 units falls within the range of 75.0 % and 135.0 % of the labeled claim.

F. Degradation Products

1. Instrumental

An HPLC system consisting of an autosampler, pump, a column, an adjustable wavelength UV Detector and an integrator capable of integrating the peaks of interest.

HPLC Parameters:

Column:	Phenomenex, Luna C18 (2), 5 um, 4.6 mm x 250 mm PN# 00G-4252-E0
Mobile Phase:	A: 0.05M Phosphate Buffer pH 3.0/Methanol=95/5 B: 0.05M Phosphate Buffer pH 3.0/Methanol=50/50
Injection Volume:	20 µL
Run time:	65 min (Refer to the gradient table below)
Flow rate:	1.0 mL/min
Wavelength:	215 (8) nm, ref: off
Column Temperature:	25 °C
Rt window:	45 minutes to 52 minutes

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Gradient Table

Time (min)	Flow (ml/min)	Mobile Phase A	Mobile Phase B
0	1.0	100	0
7	1.0	100	0
40	1.0	0	100
55	1.0	0	100
56	1.0	100	0
65	1.0	100	0

2. Reagents

- Diluted Nitroglycerin Reference Standard
- Methanol, HPLC grade or equivalent
- USP Water
- 85% o-Phosphoric Acid, ACS grade or equivalent
- Potassium Phosphate Monobasic (KH_2PO_4), ACS grade or better

3. Reagents Preparation

3.1. Preparation of 0.05 M Potassium Phosphate Monobasic Buffer pH 3.00 ± 0.05

Accurately weigh about 13.6 g of Monobasic Potassium Phosphate and dissolve in 2000 ml of water, mix well and adjust pH to 3.00 ± 0.05 with o-Phosphoric Acid (85%). This preparation may be scale up or down as needed.

3.2. Mobile Phase A: 0.05M Phosphate Buffer pH 3.0/ Methanol =95/5

Mix 950 mL of 0.05M Phosphate Buffer pH 3.0 with 50 mL of Methanol and sonicate to degas for 5 minutes. This preparation may be scaled up or down as needed.
Mobile Phase was demonstrated to be stable for at least 6 weeks at room temperature conditions

3.3. Mobile Phase B: 0.05M Phosphate Buffer pH 3.0/ Methanol =50/50

Mix 500 mL of 0.05M Phosphate Buffer pH 3.0 with 500 mL of Methanol and sonicate to degas for 5 minutes. This preparation may be scaled up or down as needed.
Mobile Phase was demonstrated to be stable for at least 6 weeks at room temperature conditions

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

3.4. Diluent: Methanol/Water=50/50

Mix 500 mL of Methanol with 500 mL of water and mix well. This preparation may be scaled up or down as needed.

4. Impurity Standard Preparation

4.1. Working Impurity Solution

Dilute 2.0 mL of the standard solution from Section III.C.4.1 (Conc. about 0.075 mg/mL) into a 50 mL volumetric flask, dilute to volume with diluent. Mix well. Label as the "Impurity Standard".

4.2. Stability of the Standard Solutions

The stock and the working impurity solutions were proved to be stable for 17 days at room temperature conditions

4.3. Standard Concentration calculation

Concentration of Nitroglycerin Impurity Standard is approximately 0.003 mg/mL. Calculate the Impurity Standard concentration in mg/mL as follow:

$$Cstd = \frac{Wstd \times P \times 2.0mL}{20mL \times 100 \times 50mL}$$

Where,
C_{std} = Concentration of Nitroglycerin standard in mg/mL.
W_{std} = Diluted Nitroglycerin Standard weight, mg
P = Potency of the Diluted Nitroglycerin Reference Standard in %.
100% = Conversion from Diluted Nitroglycerin to Nitroglycerin

5. Sample Preparation

5.1. Working Impurity Sample

Use the Stock Sample Solution centrifuged supernatant prepared in Section III.C.5.1. Transfer an aliquot into HPLC vial for analysis. (Conc. about 0.3 mg/mL).

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

5.2. Stability of the Impurity Sample Solution

The impurity sample solution was proved to be stable for 3 days at room temperature conditions

6. Procedure

6.1. Refer to SOP AR-0049 "High Performance Liquid Chromatographic Analysis"

6.2. System Suitability Criteria

Make at least two injections of the diluent solution and six replicate injections of the Impurity Standard Solution. Calculate the reproducibility (% RSD) of the six replicate injections, theoretical plates (N) and the tailing factor (T) for the Nitroglycerin peak. The system is suitable if: RSD $\leq$ 5.0%, N $\geq$ 6000 and T $\leq$ 2.

6.3. Sample Analysis

Inject the sample solutions bracketed by impurity standard.

6.4. Factors to be used in Empower

Factor	Description	Custom Field in Empower Set up
Standard Concentration	Standard Concentration in mg/mL	Component Editor Table/ STANDARDS ONLY
0.3, 0.4 or 0.6	Nitroglycerin Label Claim in mg	Label Claim in the Sample set custom field
20 (for 0.3mg and 0.4mg) or 25 (for 0.6mg)	Number of Tablets for analysis	Sample Weight
20 (for 0.3mg), 25 (for 0.4mg) or 50 (for 0.6 mg)	Dilution factor in mL	Dilution
1/RRF	The inverse of the Relative Response Factor of each impurity	Under RELATIVE RESONSE tab of the Component Table in the Processing Method

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

7. Calculations

7.1 Identification and Limit of Quantitation (LOQ) of Impurities

For identification of known degradation impurities refer to the table below. Table summarizes the Relative Response Factor (RRF), Relative retention Time (RRT) and LOQ values of degradation compounds. Any degradation impurity that is below the quantitation limit should be reported as BQL.

RRF, RRT and LOQ of Nitroglycerin & Degradation Impurities

Related Compound	RRF	Approximate RRT To Nitroglycerin Peak	LOQ
2-Mononitroglycerin	0.84	0.12	0.05 %
1-Mononitroglycerin	0.86	0.14	0.05 %
1,3-Dinitroglycerin	1.21	0.49	0.05 %
1,2-Dinitroglycerin	1.04	0.57	0.05 %
Nitroglycerin	1.00	1.00	0.05 %
1-Chloro-2,3-Dinitro-2,3-propanediol	1.00	1.02	0.05 %

The ***Limit of Quantitation*** of the method was proved to be about 0.000151 mg/mL for Nitroglycerin and **unknowns** (equivalent to **0.05 %** of the Impurity sample concentration). Therefore unknown impurities below 0.05 % will be reported as below the quantitation limit (BQL).

7.2. Identification of Placebo peaks

No placebo peak is observed in sample chromatograms

7.3. Degradation Products Calculation

From the HPLC analysis, obtain the peak area of individual Impurity/Degradants. The amount of each Impurity/Degradant is calculated from the equation:

$$\% \text{ Impurity} = \frac{Aspl \times Cstd \times VF}{Astd \times LC \times \#tablets \times RRF} \times 100\%$$

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Where,

Cstd	=	Impurity Standard concentration, mg /mL
Aspl	=	Peak area of impurity in the sample.
Astd	=	Average Area of Bracketing standard.
LC	=	Label claim in mg
# tablets	=	number of tablets used in the analysis
VFstock	=	Volume of the Stock Volumetric Flask in mL
RRF	=	Relative Response Factor

$\sum_{i=1}^{n}$ of Individual Unknown impurities + Known Impurities = % Total impurities

G. Dissolution

1. Instrument

1.1. Dissolution Conditions

USP <711> Apparatus 2 (Paddles) operated under the following conditions:

Medium:	0.05 M Potassium Phosphate Buffer pH 6.5
Volume:	500 mL
Temperature:	37 °C ± 0.5 °C
Speed:	50 rpm
Filter Tips:	10 µm

1.2. Sample Collection Parameters

Dissolution VK7000 tester coupled with Vankel sampling station(s) (VK8000) with the following parameters:

Sample volume:	5 mL
Prime time:	30 sec
Purge time:	60 sec
Media replacement:	Disable
Time intervals:	1, 3, 5, 8, and 10 minutes

1.3 HPLC Parameters:

An HPLC system consisting of an autosampler, pump, a column, an UV Detector and an integrator capable of integrating the peaks of interest

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Column:	Alltech, Alltima C8, 5 µm, 4.6 mm x 150 mm, PN 88072
Mobile Phase:	Water/Methanol=50/50
Injection Volume:	80 µL
Run time:	Not less than 8 min
Flow rate:	1.0 mL/min
Wavelength:	220(8) nm
Temperature:	35 C
Rt window:	5 min to 8 min

1.4. Degassing Conditions

Degas the necessary volume of medium by sonicating for 20 minutes in ~25" of Hg vacuum (reading on gauge) in a suitable container

2. Reagent

- Diluted Nitroglycerin Reference Standard
- Methanol, HPLC grade or equivalent
- Potassium Phosphate Monobasic (KH_2PO_4), ACS grade or equivalent
- USP Water
- Sodium Hydroxide Pellets, ACS or equivalent
- Hydrochloric Acid (HCl), concentrated, ACS grade

3. Reagent Preparation

3.1 Media Preparation: 0.05M Potassium Phosphate Buffer pH 6.5

Dissolve 41 g of monobasic potassium Phosphate and 3.4 g of sodium hydroxide pellets (NaOH) by stirring in a 1000 ml of Water in a suitable container. Completely transfer this solution into a 6 L container, dilute to volume (6 L) and mix well. Adjust the pH with 1M sodium hydroxide or sodium hydroxide pellets so that the final pH of the buffer is in the range 6.50 ± 0.05. This preparation may be scaled up or down as needed.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

3.2 Mobile Phase Preparation: Water/Methanol=50/50

Mix 1000 mL of Water and 1000 mL of Methanol, mix well and sonicate for 5 minutes. This preparation may be scaled up or down as needed.
Mobile Phase was demonstrated to be stable for at least 6 weeks at room temperature conditions

4. Standard Preparation

4.1 Stock standard solution

Weigh about 63 mg of Diluted Nitroglycerin Reference Standard (equivalent 0.6 mg of Nitroglycerin) and transfer in a 25 ml volumetric flask. Dissolve in Methanol with sonication. Dilute to volume with Methanol. This preparation may be scaled up or down as needed. (Conc.: ~ 0.024 mg/mL).

4.2 Working standard solution

Pipette 5.0 mL of stock standard solution into a 100 mL volumetric flask and dilute to volume in dissolution medium, mix well. This preparation may be scaled up or down as needed. (Conc.: ~ 0.0012 mg/mL).

4.3. Stability of the Standard Solutions

The stock and working standard solutions were proved to be stable for 14 days at room temperate conditions

4.4. Standard Concentration calculation

The concentration of the "Working Standard Solution" in mg /mL is calculated using the following equation:

$$Cstd = \frac{Wstd \times 5.0mL \times P}{25mL \times 100mL \times 100}$$

Where,
C_{std} = nitroglycerin Working standard concentration (mg /mL)
W_{std} = Weight of Diluted Nitroglycerin Reference Standard (mg).
P = Potency of the Diluted Nitroglycerin Reference Standard in %.
100%= Conversion from Diluted Nitroglycerin to Nitroglycerin

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

5. Sample Preparation

Dissolution samples will be collected after being filtered through 10 µm full flow filter (on line). The collected dissolution samples are centrifuged at 2500 RPM for 5 minutes. Transfer samples into HPLC vials to be analyzed.

5.1. Sample Solution Stability

The dissolution sample solutions were proved to be stable for 3 days at room temperature conditions

6. Procedure

6.1 Preparation of Dissolution VK7000 tester coupled with Vankel sampling station(s) (VK8000)

6.1.1 Dissolution System Cleaning: Follow the procedure in SOP # AR-0040
Note: Before starting the Dissolution test, clean thoroughly the dissolution apparatus as follow:
- ***Apply a cleaning cycle with Water/ Methanol = 1/ 1***
- ***Apply a cleaning cycle with warm water***

6.1.2 Valve Calibration: Follow the procedure in SOP # AR-0040
6.1.3 Dissolution Apparatus set-up: Follow the procedure in SOP # AR-0040
6.1.4 Dissolution Program set-up: Follow the procedure in SOP # AR-0040
6.1.5 Dissolution start-up: Follow the procedure in SOP # AR-0040. Make sure that enough clean test tubes are placed in the fraction collector tray to collect the dissolution samples.

6.2. Dissolution Process

- Transfer 500 mL of dissolution media into the dissolution vessels and allow the temperature inside the vessels to equilibrate to 37 ± 0.5° C.
- Drop one tablet in each vessel
- Run the dissolution apparatus and
- Collect a sample at the specified time points
- Analyze the samples by HPLC

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

6.3. Refer to SOP AR-0049 "High Performance Liquid Chromatographic Analysis"

6.4. System Suitability Criteria

Inject at least once the dissolution medium. Make five replicate injections of the standard-1 solution. Calculate the reproducibility (RSD) of the five consecutive injections, theoretical plates (N) and the tailing factor (T) for the Nitroglycerin peak. The system is suitable if: RSD $\leq$ 2.0%, N $\geq$ 2500, and T $\leq$ 2.

Inject the Standard Solution-2 twice. Compare the potency (%) of Standard - 2 relative to Standard - 1

6.5. Standard Comparison Calculation

$$Std\ Comp.(\%) = \frac{Astd2 \times Wstd1}{Astd1 \times Wstd2} \times 100$$

Where,

A_{std1} = Peak area of Standard-1 (Average of five injections)
A_{std2} = Peak Area of Standard-2 (Average of two injections)
W_{std1} = Standard-1 weight, mg
W_{std2} = Standard-2 weight, mg

Standard-1 is suitable for use if Standard comparison (%) value is within the range 100.0 ± 3.0%.

6.6. Sample Analysis

Inject dissolution samples bracketed by working standard – 1. The cumulative % RSD should be NMT 3.0 %.

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

6.7. Factors to be used in Empower

Factor	Description	Custom Field in Empower Set up
Standard Concentration	Standard Concentration in mg/mL	Component Editor Table/ STANDARDS ONLY
500	Dissolution volume in mL	Initial Volume of Media in each vessel in the Sample set method
5	Sample volume in mL	Volume removed on each transfer in the Sample set method
0.3, 0.4 or 0.6	Nitroglycerin Strength in mg	Dissolution Claimed amount in the Sample set method

7. Calculations:

Note: Mobile Phase peak can be observed in the dissolution sample chromatograms at RRT ~ 0.47.

From the HPLC analysis, obtain the peak area of the sample (A_{spl}) and Standard-1 (A_{std}). The percentage of Nitroglycerin per tablet is calculated from the equations:

Concentration of Nitroglycerin at each time point:

$$Cn = \frac{Aspl_{(n)} \times Cstd}{Astd}$$

% Nitroglycerin Dissolved:

$$\% \text{ Dissolved} = \frac{\{C(n) \times [500mL - 5\,(n-1)]\} + [\sum_{i=1}^{n-1} C(i) \times 5]}{LC} \times 100\%$$

Where:
Astd = Average Area of Bracketing standard-1 between samples analyzed
Aspl = Area of Nitroglycerin peak in the chromatogram

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

C(n) = Concentration of dissolution samples taken at the *n*th time point, in mg/mL

n = The *n*th time point ($n \geq 1$)

LC = Label Claim in mg

$\sum_{i=1}^{n=1} C(I)$ = Summation of dissolution sample's concentration(s) from the first to the (*n-i*)th time point.(Only applicable for $n \geq 2$)

H. Water Content

1. Instrument

751 GPD Metrohm Titrino or equivalent

2. Reagents

- Methanol, HPLC grade or better
- Water, USP
- Hydranal Composite 5 or other suitable Karl Fisher Titrant

3. Procedure

3.1. Standardization

Standardize the Karl Fisher instrument according to SOP # AR-008520 Fill the titration vessel with methanol until it is ¼ full. Neutralize the water in the vessel by pressing the start button on the Titrino.

3.2. Sample Preparation

Grind at least 20 tablets using a mortar and pestle. Weigh approximately 0.5 g of the sample and transfer to the titration vessel and return the weighing boat to the balance. Obtain the net sample weight transferred to the titration vessel and proceed with the titration.

4. Calculation

Calculate the % of Water from the following equation:

$$\% \text{ Water} = \frac{EP \times Titer \times 0.1}{W \text{ spl}}$$

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Where:
EP = The titration endpoint in mL
Titer = Constant equivalent to the amount of water needed to neutralize one mL of titrant, mg/mL
Wspl = The net weight of the sample, grams

I. Disintegration

1. Instrument

1.1. Disintegration Conditions

Apparatus:	Basket-rack assembly of six open-ended transparent tubes with a woven stainless steel wire cloth, which has a plain square weaves of 1.8 to 2.2 mm aperture and wire diameter of 0.57 to 0.66 mm
Medium:	Water
Volume:	700 mL
Temperature:	37 ± 2° C

2. Procedure

- Transfer 700 mL of water into the disintegration vessel and allow the temperature inside the vessels to equilibrate to 37 ± 2° C.
- Drop one tablet in each the six tubes of the basket
- Run the disintegration apparatus for 2 minutes
- Annotate the time needed for the tablets to completely disintegrate
- At the end of the 2 minutes, lift the basket from the fluid, and observe that the tablets have disintegrated completely

3. Acceptance criteria

Follow USP General Chapter <701> Disintegration: 2 minutes, determined as set forth for sublingual tablets

IV. REFERENCES

- Nitroglycerin Technical Package, Copperhead Chemical Company.
- Watson Florida Laboratories SOP # AR-0067, "Dissolution Media Preparation Procedure".

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

- SOP # AR-0040, "Care and Use of VanKel Dissolution System VK7000 Tester Coupled with VK8000 Sampling Station."
- Current USP <921> Water Determination (Karl Fisher)

V. HISTORY

New
Effective date: 09/29/11

Revision 01, Effective date: 10/06/11

Proposed Change	Justification
Section III.F.1. Updated HPLC chromatographic parameters (Water in the Mobile Phase was substituted by Phosphate Buffer pH 3.0)	To improve separation of the Impurity and therefore the chromatographic performance
Section III.F.2. Added new reagents	Due to changes in the Impurity HPLC conditions
Section III.F.3. Updated the reagent preparation	Due to changes in the Impurity HPLC conditions
Section III.F.6. Added 2 injections of diluent before the system suitability	To equilibrate better the system
Section III.F.7. Updated the RRT of impurities	Due to changes in the Impurity HPLC conditions
Section III.I. Added disintegration parameters	Based on USP General Chapter <701>
Chromatograms: Updated the impurity profile chromatogram	Due to changes in the Impurity HPLC conditions

Revision 02, Effective date: 10/06/11

Proposed Change	Justification
Section III.F.1. & III.F.3. Buffer Molarity updated to 0.05M	Typo error during the preparation of the revision 01

Revision 03, Effective date: 11/01/11

Proposed Change	Justification
Section III.C.5, III.F.4 and III.G.4. Added statement of standard stability	Based on the standards stability study during the method validation
Section III.C.8. Added table of possible peaks observed in the assay sample chromatograms	Based on the assay specificity study during the method validation
Section III.F.7.1. Table of degradation	Based on the impurity

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

impurities was updated with the RRF, RRT and LOQ values	specificity study during the method validation
Section III.F.7.2. Added LOQ value in the text	Based on the impurity method validation
Section III.G.4.2. "mg" in the denominator of the formula was removed	As the 100 in that formula refers to 100%
Section III.G.6.4. Added statement of possible peak observed in the dissolution sample chromatograms	Based on the dissolution specificity study during the method validation

Revision 04, Effective date: 11/07/11

Proposed Change	Justification
Section III.F.2. HPLC grade replaced by ACS grade or equivalent for Phosphoric acid	To be able to use ACS quality reagent in the mobile phase preparation
Section III.G.6.1. Additional cleaning procedure of the dissolution baths was added before the initiation of Nitroglycerin dissolution to avoid cross contamination	Corrective action of the LIR # 11-065

Revision 05, Effective date: 12/14/11

Proposed Change	Justification
Section III.C.6. Added statement about assay sample stability	Based on stability validation studies (Report No. ARVR-11-0175)
Section III.F.5. Added statement about impurity sample stability	Based on stability validation studies (Report No. ARVR-11-0175)
Section III.G.6.2. Added statement about dissolution sample stability	Based on stability validation studies (Report No. ARVR-11-0175)

Revision 06, Effective date: 12/22/11

Proposed Change	Justification
Section VI. Name of Nitroglycerin Blends updated from Final Blend for Nitroglycerin Tablets to Nitroglycerin Tablets Blend	To match the master batch records updates
Spec-R199412, R199424 & R199415 for Nitroglycerin Blends: The names of Nitroglycerin Blends were updated from Final Blend for Nitroglycerin Tablets to	To match the master batch records updates

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Nitroglycerin Tablets Blend	
Spec-R199417, R199426 & R199419 for Nitroglycerin Sublingual Tablets: Tablets description was updated	Word "embossed" was replaced by "engraved"

Revision 07, Effective date: 06/01/12

Proposed Change	Justification
Section I. PURPOSE. List of TESTS readjusted and TESTs names were updated	To comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method"
Section III. PROCEDURES. Added EHS statements	To comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.A: Appearance was renamed as Description	To comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.B: Identification. Content was reorganized	To harmonize all STM's Tests
Section III.C. Assay. Section divided in subsections. Added the Factors to be used in Empower Calculations	To facilitate the understanding and comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.D: Blend Uniformity. Section divided in subsections. Added the Factors to be used in Empower Calculations	To facilitate the understanding and comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.E. Renamed to Uniformity of Dosage Units (Content Uniformity). Section was divided in subsections. Added the Factors to be used in Empower Calculations	To facilitate the understanding and comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.F: Renamed to Degradation Products. Section was divided in subsections. Added the Factors to be used in Empower Calculations	To comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.G: Dissolution. Section was divided in subsections. Added the Factors to be used in Empower Calculations	To comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.H. Water Determination was renamed to Water Content and divided in subsections	To facilitate the understanding and comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method
Section III.I: Disintegration. Section was divided in subsections	To facilitate the understanding and comply with the new revision of SOP AR-0051"How to Prepare a Standard Test Method

Revision 08, Effective date: 09/10/12

Proposed Change	Justification
Section III. EHS Safety Statements: Removed #	As this statement is repeated

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

2	
Section III.D. 2. Sample preparation procedure was updated to include the weight of the caps in the initial and final weights of the sample vial. Also added in the procedure statement to rinse the inside portion of the cap.	Corrective action of Even Reference Form WES/E-12/033

Revision 09, Effective date: 05/16/13

Proposed Change	Justification
Section III.C.3.1. Added stability statement for Mobile Phase	Stability study demonstrated and recorded in ARVR-13-0105
Section III.F.3.2 & III.F.3.3. Added stability statement for Mobile Phase	Stability study demonstrated and recorded in ARVR-13-0105
Section III.G.3.2. Added stability statement for Mobile Phase	Stability study demonstrated and recorded in ARVR-13-0105

Revision 10, Effective date: MAR 1 4 2014

Proposed Change	Justification
Section III.F.7.1. Added new identified degradation compound	Based on conclusions from studies conducted to identify the unknown impurity at RRT 1.02. Refer to Reports: -ARVR-14-0055 "Structure Elucidation and relative response Factor Determination of the Peak at RRT=1.02 in Nitroglycerin Sublingual Tablets" -DEREK Evaluation of an identified impurity from Gad Consulting services from January 31, 2014
Spec-R199417, R199426 & R199419 :Nitroglycerin Sublingual Tablets, 0.3mg, 0.4mg and 0.6mg, respectively: Included the degradation compound 1-Chloro-2,3-Dinitro-2,3-propanediol	Added new identified degradation compound under Specification section of the Related Compounds. Refer to Reports listed above.

VI. SPECIFICATION AND ANALYTICAL REPORTS

For Specification Sheets, see the list below:

1. Nitroglycerin Sublingual Tablets Blend, 0.3 mg, Spec - R199412
2. Nitroglycerin Sublingual Tablets Blend, 0.4 mg, Spec - R199424
3. Nitroglycerin Sublingual Tablets Blend, 0.6 mg, Spec - R199415
4. Nitroglycerin Sublingual Tablets, 0.3 mg, Spec - R199417
5. Nitroglycerin Sublingual Tablets, 0.4 mg, Spec - R199426
6. Nitroglycerin Sublingual Tablets, 0.6 mg, Spec - R199419

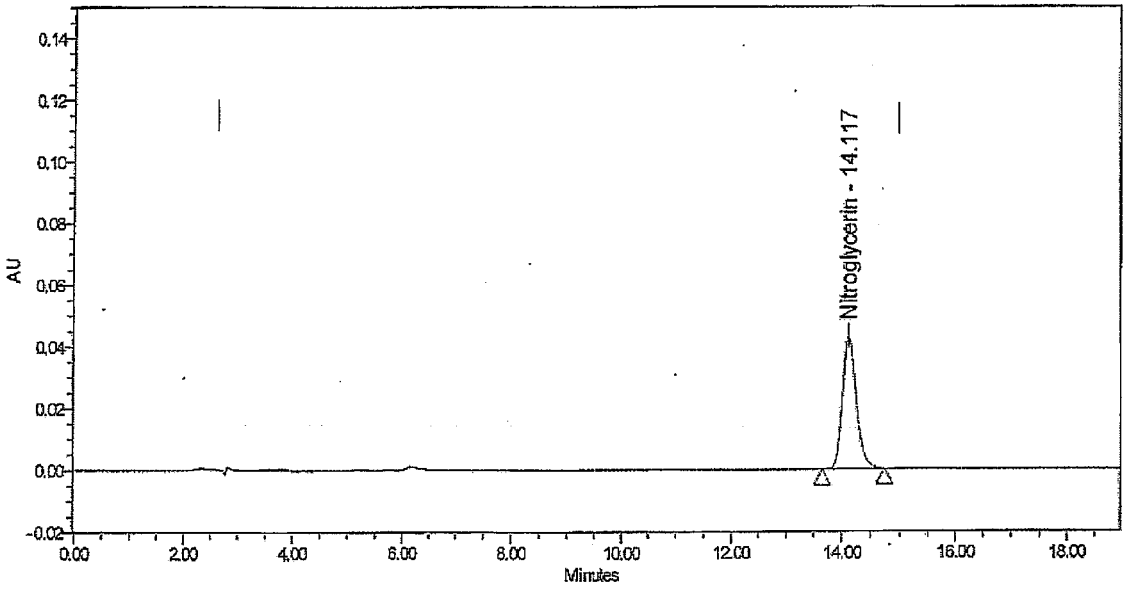
Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Example of Nitroglycerin assay standard solution

SAMPLE INFORMATION

Sample Name:	STD 1-SS	Acquired By:	lkhondoker
Sample Type:	Standard	Date Acquired:	8/25/2011 5:56:26 PM EDT
Vial:	1	Acq. Method Set:	Nitroglycerin Assay WLC06
Injection #:	1	Date Processed:	8/26/2011 7:53:50 AM EDT
Injection Volume:	20.00 ul	Processing Method:	Nitroglycerin Assay_1
Run Time:	19.0 Minutes	Channel Name:	A1100 DAD AU Ch1
Sample Set Name:	082511_ Assay_Lc06	Proc. Chnl. Descr.:	DAD AU Ch 1 Sample 220, Bw 8

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Units	Area
1	Nitroglycerin	14.117	mg/ml	724180

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Example of Nitroglycerin Impurity Profile solution

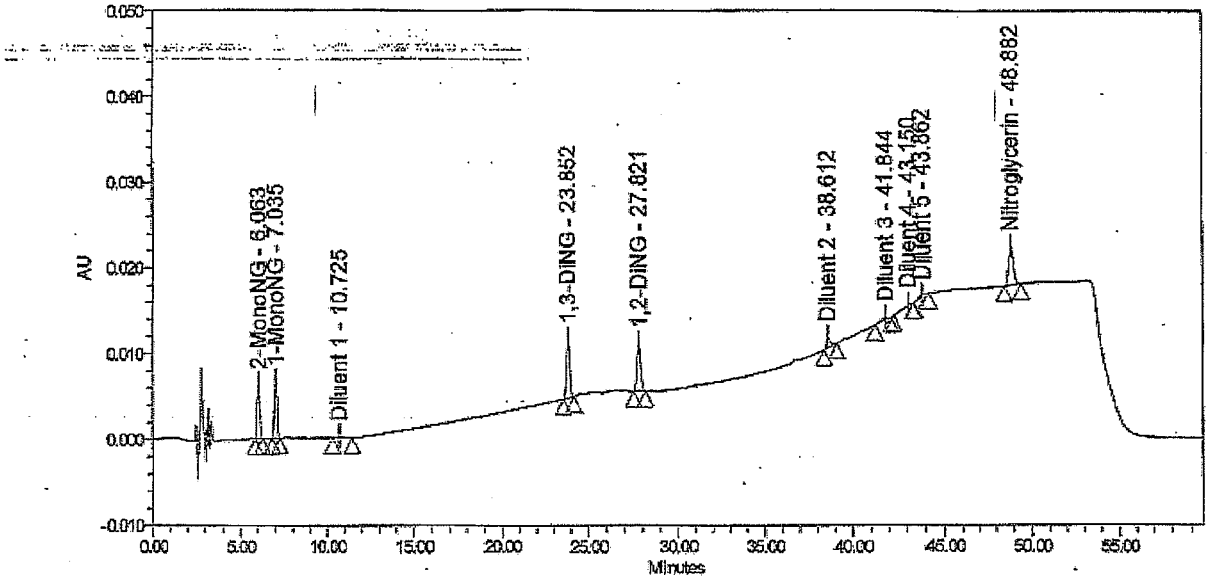
Empower 2

Individual Chromatograms

SAMPLE INFORMATION

Sample Name:	Profile	Acquired By:	oridge
Sample Type:	Unknown	Date Acquired:	10/5/2011 2:44:04 PM EDT
Vial:	3	Acq. Method Set:	Nitrog Diss WLC05
Injection #:	1	Date Processed:	10/5/2011 3:57:35 PM EDT
Injection Volume:	20.00 ul	Processing Method:	Nitroglycerin RC_922411A
Run Time:	60.0 Minutes	Channel Name:	A1100 DAD AU Ch1
Sample Set Name:	100511_40m_gradLC05	Proc. Chnl. Descr.:	DAD AU Ch 1 Sample 215, Bw 8

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Height	Area
1	2-MonoNG	6.063	6312	64582
2	1-MonoNG	7.035	6579	66853
3	Diluent 1	10.725	146	5130
4	1,3-DING	23.852	6897	91358
5	1,2-DING	27.821	5617	81119
6	Diluent 2	38.612	1255	17274
7	Diluent 3	41.844	329	7756
8	Diluent 4	43.150	254	5132

	Name	RT	Height	Area
9	Diluent 5	43.862	219	5540
10	Nitroglycerin	48.882	4578	84578

Title: Nitroglycerin Sublingual Tablets, 0.3 mg, 0.4 mg & 0.6 mg	Test Method #:STM-AR-FP-0164

Example of Nitroglycerin Dissolution Standard

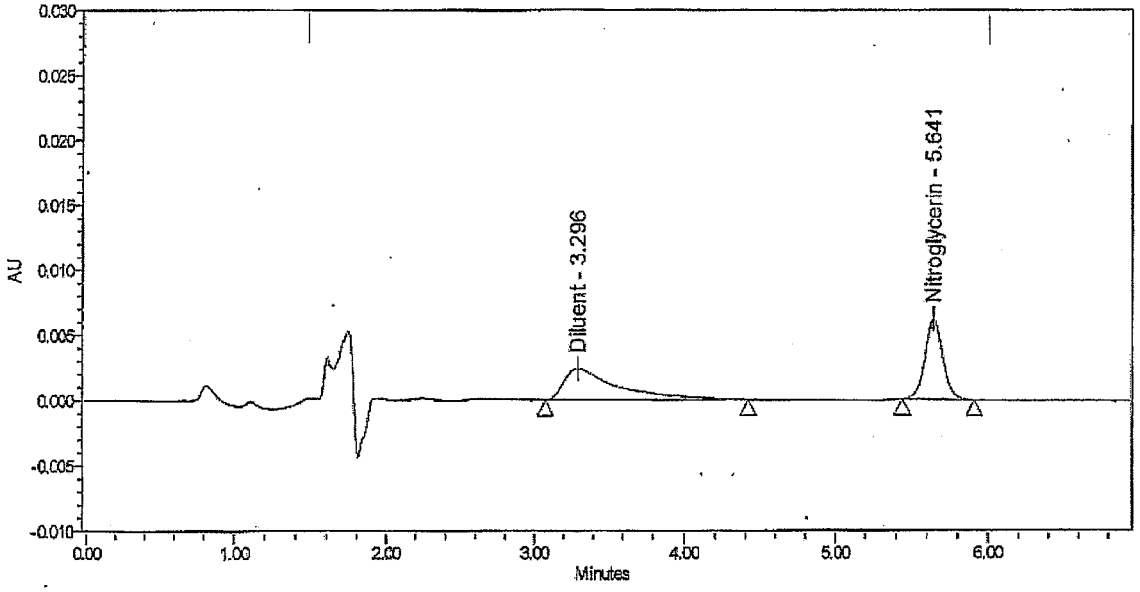
Empower 2

Individual Chromatograms

SAMPLE INFORMATION

Sample Name:	Std	Acquired By:	bguo
Sample Type:	Standard	Date Acquired:	7/29/2011 3:21:30 PM EDT
Vial:	100	Acq. Method Set:	Nitrog Diss WLC17 35D
Injection #:	2	Date Processed:	7/29/2011 4:19:10 PM EDT
Injection Volume:	80.00 ul	Processing Method:	Nitroglycin Diss WLC23
Run Time:	7.0 Minutes	Channel Name:	A1100 DAD AU Ch1
Sample Set Name:		Proc. Chnl. Descr.:	DAD AU Ch 1 Sample 220, Bw 8

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Height	Area	% Area	Int Type	USP Resolution	USP Tailing	USP Plate Count
1	Diluent	3.296	2382	59352	53.75	BB		2.859	633
2	Nitroglycerin	5.641	6198	51062	46.25	BB	6.208848e+000	1.051	9482

Watson

Document Information

Document Details:

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Effective: 29 Sep 2011 11:24:09 GMT -06:00
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Any Approval signatures that have been applied to this document are shown below:

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Decision : Approved
Decision Date : 29 Sep 2011 11:06:56 GMT -06:00
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Purpose : Revise STM
Meaning Of Signature : Document Approval