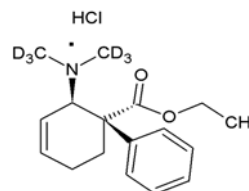


Certified Reference Material - Certificate of Analysis

Tilidine-D₆, Primary Standard

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP



Catalog Number:	T-069
Lot:	FE06051302
Retest:	May 2018
Description:	Tilidine-D ₆ HCl in Methanol. Nominal concentration is adjusted for HCl content.
Packaging:	Solution in 2 mL amber USP Type I glass ampoule containing not less than 1 mL of certified solution.
Storage:	Store unopened in freezer (-10 °C to -25 °C). See Stability Section for short-term storage.
Shipping:	Ship cold. See Stability Section.
Intended Use:	This Certified Reference Material is suitable for the <i>in vitro</i> identification, calibration, and quantification of the analyte(s) in analytical and R&D applications. Not suitable for human or animal consumption.
Instructions for Use:	Users should quantitatively transfer desired volume using established good laboratory practices to spike into matrix or to dilute to the desired concentration. Each ampoule is intended for one-time use.
Regulatory:	USDEA Exempt Canadian TK # 61-727 Safety: Danger. See Safety Data Sheet

- Retest Date - stability studies ongoing. Certificate of Analysis will be updated upon completion of retest.
- Ampoules are overfilled to ensure a minimum 1 mL volume can be transferred when using a 1 mL Class A volumetric pipette.
- For MS Applications, we advise laboratories not to mix lots during a single sequence.
- For quantitative applications, the minimum sample size for intended use is 1 µL.

Analyte	Certified Concentration Value
Tilidine-D ₆	100.0 ± 0.5 µg/mL
<ul style="list-style-type: none"> Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the mass balance purity factor, material density, balance, and weighing technique. This standard is prepared gravimetrically and mass results are reported on the conventional basis for weighing in air. Nominal concentration is calculated based on: the actual measured mass; Mass Balance Purity Factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20 °C. Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics. Nominal concentration is adjusted for HCl content. No adjustment required before use. Additional certification information available upon request. 	

Metrological Traceability

- This standard has been prepared and certified under the ISO Guide 34, ISO/IEC 17025, ISO 9001 and ISO 13485 standards. This standard meets the requirements of a Certified Reference Material and a Primary Standard as defined by ISO and is traceable to the SI and higher order standards through an unbroken chain of comparisons.
- This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.1% relative uncertainty. Balance calibration adjustments are performed weekly utilizing the balance's internal adjustment mechanism. Calibration verifications are performed pre-use. Weigh tapes from the calibration verification are included in the production batch record for this standard. Production data package available upon request.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- Concentration is verified against an independently prepared calibration solution gravimetrically prepared.
- Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of this COA. The density and material Mass Balance Purity Factor is traceable to the SI and higher order reference standards through mass measurement and instrument qualification and calibrations.

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration/retest date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.




Darron Ellsworth, Quality Assurance Manager

April 21, 2015

Date

Solution Standard Verification

Concentration accuracy and within- and between-bottle homogeneity are analytically verified against an independently prepared calibration solution.

Solution standard verification demonstrates confirmation that the specified requirements for the Primary Standard have been fulfilled and validated under ISO 13485.

Standard Solution Assay Parameters				Calibration Curve	
Analysis Method:	HPLC/UV			Calibration Curve:	Linear Regression
Column:	Betasil Phenyl, 3 µm, 4.6 x 150 mm			Number of Points:	4
Mobile Phase:	Acetonitrile:: 10 mM KH ₂ PO ₄ (30::70)			Linearity (r):	0.999
Flow Rate:	1 mL/min				
Wavelength:	215 nm				

Standard Solution	Lot Number	Verified Concentration (µg/mL)		%RSD - Homogeneity	
		Actual Results	Acceptance Criteria	Actual Results	Acceptance Criteria
New Lot	FE06051302	98.71	± 3%	0.5	≤ 3%

- Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.
- Within-sample and between-sample homogeneity of the New Lot is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. Multiple samples pulled from across the lot using a random stratified sampling plan were analyzed to verify homogeneity. % RSD results shown above for the New Lot demonstrate ampoule-to-ampoule homogeneity.

Analyte Certification - Mass Balance Purity Factor

Each analyte is thoroughly identified and characterized using an orthogonal approach. A mass balance purity factor is assigned incorporating chromatographic purity and residual impurities. The mass balance purity factor and salt adjustment are utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

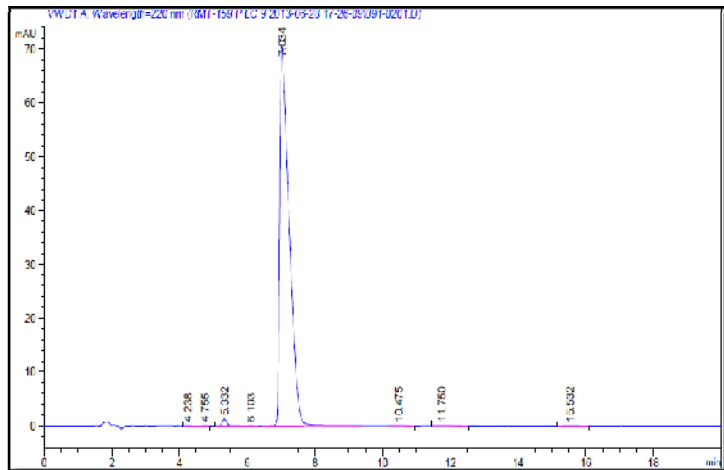
Material	Lot Number	CAS Number	Chemical Formula	Molecular Weight (salt)	Molecular Weight (base)	Salt Adjustment
Tilidine-D ₆ HCl	FC06171301	NA	C ₁₇ D ₆ H ₁₇ NO ₂ •HCl	315.87	279.40	1.131
Material Characterization Summary						
Analytical Test			Method		Results	
Primary Chromatographic Purity by HPLC/UV Analysis			SP10-0102		98.9%	
Secondary Chromatographic Purity LC/MS Analysis			SP10-0107		98.4%	
Identity by ¹ H-NMR Analysis			USP <761>, SP10-0116		Consistent with Structure	
Identity by LC/MS Analysis			SP10-0107		Consistent with Structure	
Isotopic Purity and Distribution by LC/MS SIM or High Resolution MS			SP10-0107		0.00% D ₀ vs D ₆	
					0.00% D ₀	0.00% D ₄
					0.00% D ₁	0.88% D ₅
					0.00% D ₂	98.31% D ₆
					0.05% D ₃	0.76% D ₇
Residual Solvent Analysis by GC/FID Headspace			AM1087 ¹		0.31%	
Residual Water Analysis by Karl Fischer Coulometry			AM1346 ¹		0.78%	
Inorganic Content by Microash Analysis			SP10-0135		< 0.2%	
Mass Balance Purity Factor					97.85%	

- The primary chromatographic purity is calculated as the average of two independently performed analyses utilizing two different methods. Acceptance criteria requires the purity values to be within 0.5% of each other.
- The primary chromatographic purity value is used to calculate the Mass Balance Purity Factor.
- A secondary chromatographic purity method is utilized as a control.
- Mass Balance Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].
- Mass Balance Purity Factor does not include adjustment for chiral and/or isotopic purity.

¹ Validated analytical method

Spectral and Physical Data

HPLC/UV



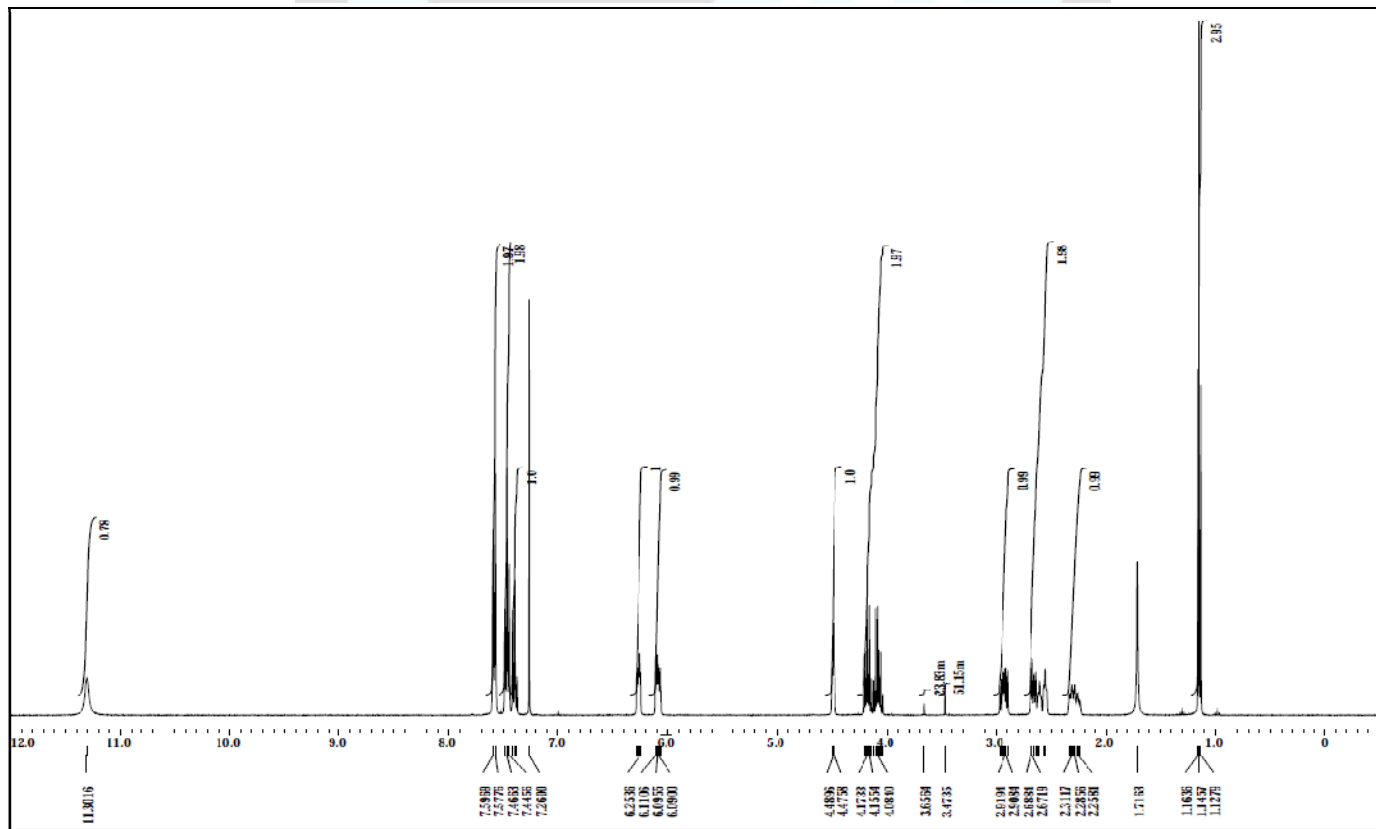
Column: Betasil Phenyl, 5 μ m, 4.6 x 150 mm
Mobile Phase: Acetonitrile::10 mM KH_2PO_4 (30::70)
Flow Rate: 1.0 mL/min
Wavelength: 220 nm

Data File Name: RMT-159 P LC 9 2013-06-28 17-26-09\091-0201.D
Instrument: LC#9
Sample Name: FC06171301
Acquired: June 28, 2013

Peak #	Ret Time	Area	Height	Area %
1	4.24	0.24	0.02	0.02
2	4.76	0.27	0.04	0.02
3	5.33	10.90	1.27	0.80
4	6.10	0.17	0.02	0.01
5	7.03	1348.96	70.55	98.93
6	10.75	1.00	0.06	0.07
7	11.75	1.09	0.06	0.08
8	15.53	0.98	0.05	0.07

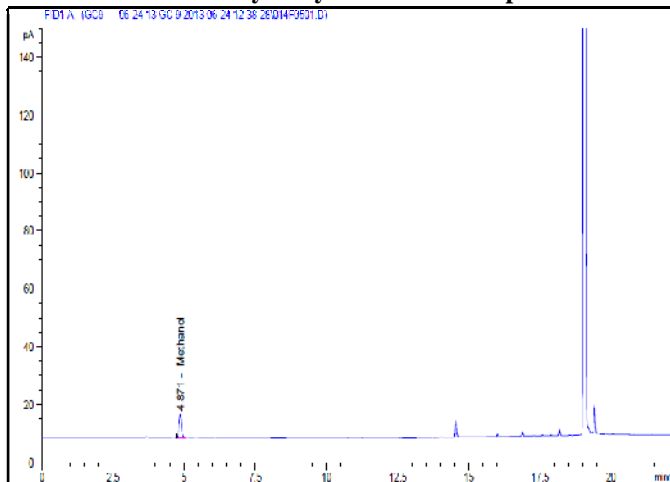
1H NMR

Instrument: JEOL ECS 400
Solvent: $CDCl_3$



Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



Column: DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program: 40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas: Helium
Flow Rate: 2.0 mL/min
Detector Heater Temp: 250°C
Injector: Headspace Sampler
HS Oven Temp: 60°C
Vial Equilibration: 10 minutes

Data File Name: GC 9 2013-06-24 12-38-28\014F0501.D
Instrument: GC#9
Sample Name: FC06171301
Acquired: June 24, 2013

Peak	Compound	Area	Weight %
1	Methanol	49.77	0.31
2	NMP	NA	NA
Total			0.31

Spectral and Physical Data (cont.)

LC/MS

Column: Acquity UPLC BEH C18, 1.7 μ m, 2.1 x 50 mm

Mobile Phase: A:: 0.1% Formic Acid in Water

B:: Acetonitrile

Gradient:

Time (mins)	%A	%B
0.0	90	10
6.0	50	50
7.1	90	10
8.0	90	10

Flow Rate: 0.4 mL/min

Scan Range: 100-1200 amu

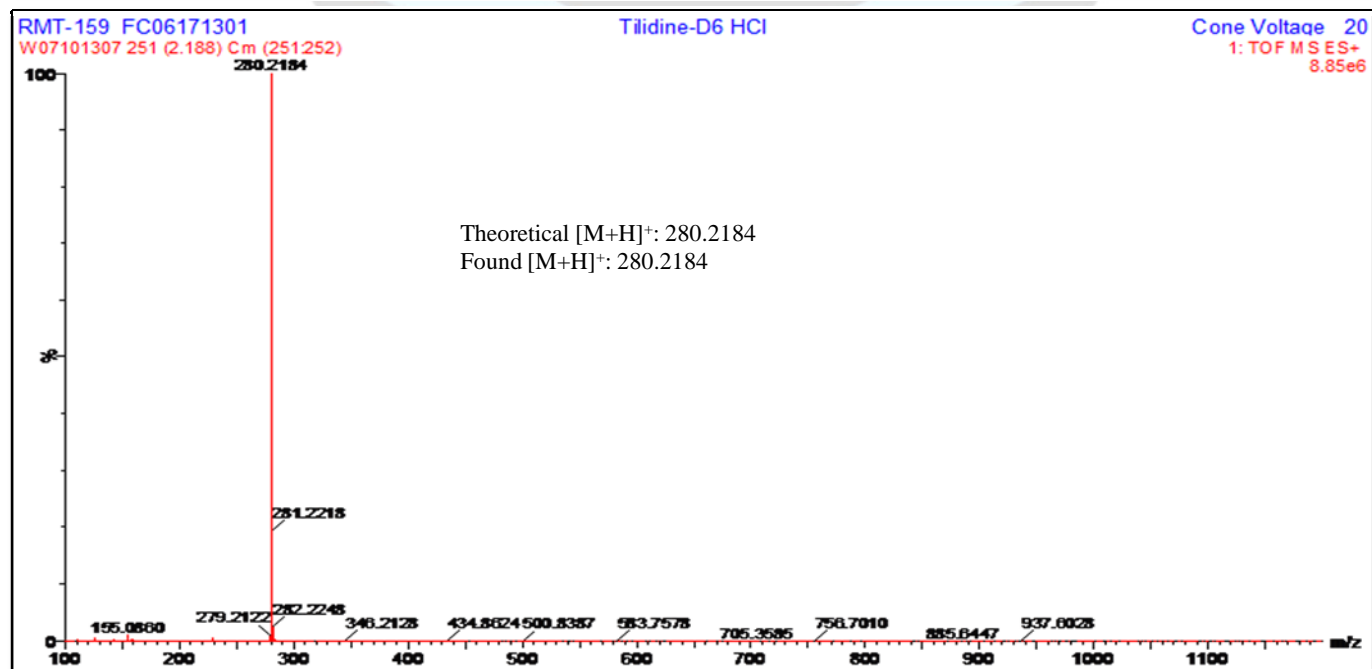
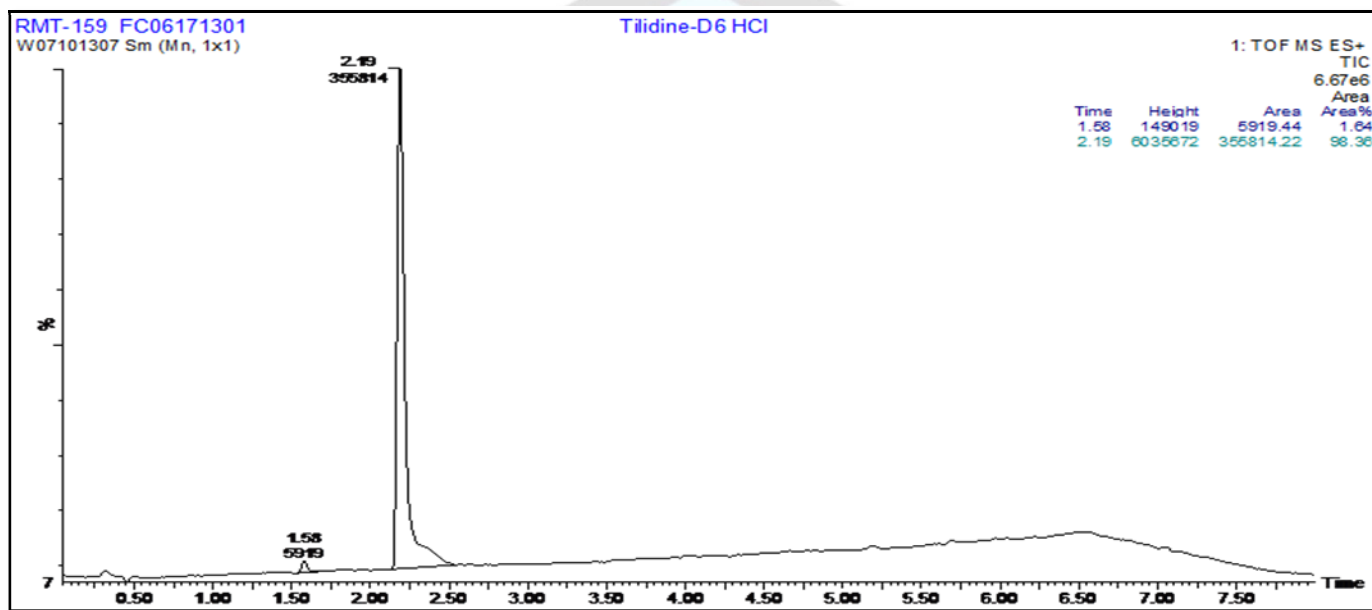
Ionization: Electrospray, Positive Ion

Data File Name: W07101307

Instrument: Waters XEVO G2 QTOF

Sample Name: FC06171301

Acquired: July 10, 2013



Spectral and Physical Data (cont.)

Isotopic Purity and Distribution by High Resolution LC/MS

Column: Acquity UPLC BEH C18, 1.7 μm, 2.1 x 50 mm

Flow Rate: 0.4 mL/min

Mobile Phase: A:: 0.1% Formic Acid in Water

Scan Range: 274-281 amu

B:: Acetonitrile

Ionization: Electrospray, Positive Ion

Gradient:

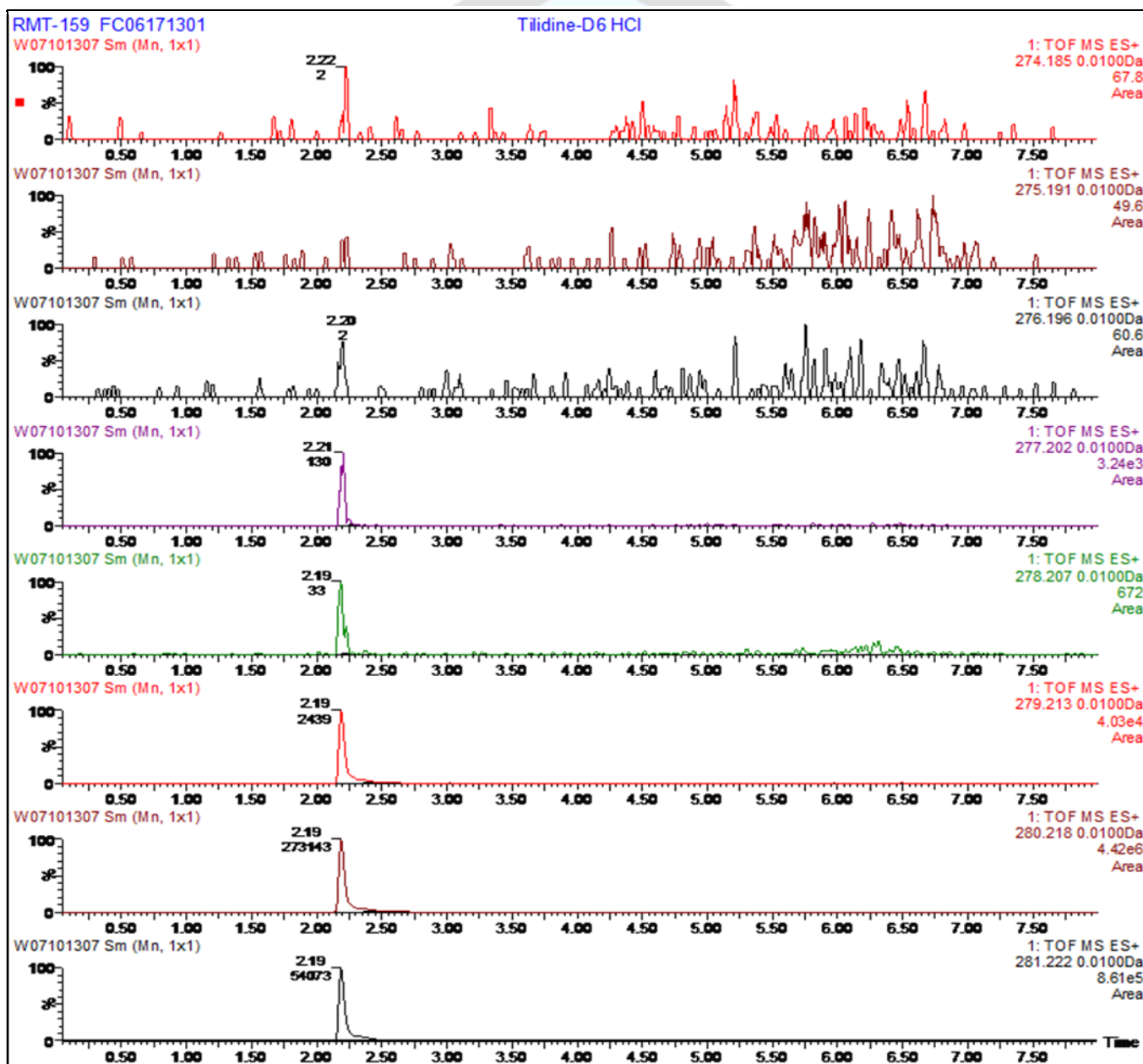
Time (mins)	%A	%B
0.0	90	10
6.0	50	50
7.1	90	10
8.0	90	10

Data File Name: W07101307

Instrument: Waters XEVO G2 QTOF

Sample Name: FC06171301

Acquired: July 10, 2013



Stability

Short term stability studies have been performed under accelerated conditions for periods up to four weeks. Short term data is utilized to predict long term stability and to support transport conditions and normal laboratory use. Real-time stability studies are performed at the recommended storage conditions over the life of the product.

Short Term Stability : A summary of accelerated stability findings for this product is listed below.		
Storage Condition	Mean Kinetic Temperature (MKT)	Time Period/Result
Freezer	-15°C	No decrease in purity was noted after four weeks.
Refrigerator	4°C	
Room Temperature	21°C	
40°C	40°C	0.7% decrease in purity was noted after four weeks.
Transport/Shipping : Ship cold.		
Short Term Storage: Stability data supports short term storage for no more than 3 months at Refrigerate Conditions.		
Long Term Stability: Long term stability has been assessed for freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 46 months has been established for a related product (T-068, Tilidine HCl) through real-time stability studies.		

COA Revision History

Revision No.	Date	Reason for Revision
00	August 19, 2013	Initial version
01	September 23, 2013	Updated Stability Section with 4-week study data, including changing Shipping conditions to 'Ship Cold.'
02	December 30, 2014	Revised Retest Date from August 2016 to May 2018.
		Added minimal sample size statement to front page.
		Changed Results for Primary Chromatographic Purity by HPLC/UV from 99.0% to 98.9% in table on page two.
03	April 21, 2015	Updated Safety Section from "Flammable, Poison" to "Danger. See Safety Data Sheet"