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60659 Shultz

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Investigator: Rebecca Leigh Shultz (WEIN 10127262)
Pages filed sequentially in binder 60659

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R. Leigh Shultz
30 Nov 2001

Subject: Initiation of Bulk and Solution Stability Studies for L-221869 Tartrate and Besylate Salts

A stock solution of the L-tartaric acid salt (NB72061-16) was made by dissolving 7.64 mg of the salt in 7.64 mL water (note water solubility greater than 1 mg/mL). Similarly, a stock solution of the besylate salt (NB72061-22) was made by dissolving 7.23 mg of the salt in 7.23 mL of water (note water solubility greater than 1 mg/mL). Aliquots of each of these stock solutions were used to make HPLC samples of the two salts in water (0.1 mg/mL) to check the salts by HPLC. The salts were run in the sample set 60659.106.seq using the original L-221869 method L221869_method1.mth.

The stock solutions prepared above were used to make stability samples for the two salts. For each salt, 54 samples were prepared. One set of vials was made for each of three time points (1, 2, and 4 weeks). Within each time point, a set of vials was placed in each of 3 stability stations: –20 °C (samples R), 40 °C (samples C), and 80 °C (samples F). In each station at each time point, samples were prepared at pH 2, 4, 6, 8, 10, and in water (concentration of salt ca. 0.1 mg/mL). The vials were crimped with Teflon-lined caps, labeled as *60659-106 Tar* or *60659-~~101~~ Bs*, and placed in the stability stations at 12:00 pm. Bulk stability was also initiated for both salts. Nine vials were prepared for each salt according to the table below. The vials were placed in the appropriate stability stations at 12:00 pm. -106 Bs typo RLS

Sample (Tar)	Conditions	Mass salt (mg)	Sample (Bs)	Conditions	Mass salt (mg)
60659-106R1	–20 °C 1 wk	1.07	60659-1061R1	–20 °C 1 wk	2.00
60659-106 R2	–20 °C 2 wks	1.69	60659-106 R2	–20 °C 2 wks	1.18
60659-106 R4	–20 °C 4 wks	1.36	60659-106 R4	–20 °C 4 wks	1.56
60659-106 D1	40/75 1wk	1.15	60659-106 D1	40/75 1wk	1.49
60659-106 D2	40/75 2 wk	1.42	60659-106 D2	40/75 2 wk	1.14
60659-106 D4	40/75 4 wk	1.28	60659-106 D4	40/75 4 wk	1.01
60659-106 F1	80 °C 1 wk	1.22	60659-106 F1	80 °C 1 wk	1.82
60659-106 F2	80 °C 2 wks	1.42	60659-106 F2	80 °C 2 wks	2.66
60659-106 F4	80 °C 4 wks	1.44	60659-106 F4	80 °C 4 wks	1.60

The remaining Tar and Bs stock solutions were frozen to prevent decomposition.

Rebecca Leigh Shultz
30 Nov 2001

RLS 30 Nov 2001

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
03 Dec 2001

Subject: Bulk stability of L-221869 free base (4-week time point)

Samples 60659-93 A4-R4 (7 samples) were removed from the stability stations at 10:30 am. All of the samples appeared unchanged (white powder) except for sample F4, which had turned yellow. Each of the samples was dissolved in 3.0 mL of 0.1% phosphoric acid; this solution was in turn diluted with 16 mL of 0.1% phosphoric acid (total volume = 19mL, approximate concentration of free base = 0.1 mg/mL). An aliquot of each solution was placed in an HPLC vial and assayed in the sample set 60659.107.seq using the method L221869_method1.mth. Each sample was assayed twice.

Subject: Bulk and solution stability of L-221869 tosylate and HCl salts (2-week time point)

The bulk and solution stability samples (labeled R2, C2 (solution) or D2 (bulk), and F2) for the tosylate and the HCl salts of L-221869 were removed from the stability stations (–20 °C, 40 °C (40/75 for bulk), and 80 °C) at 10:30 am and allowed to come to ambient temperature. The caps of the solution samples were removed and replaced with PP/Al caps. The bulk samples, which did not appear to have changed over the 2-week time period (except for HCl F2, which had yellowed), were dissolved in 3.0 mL of 0.1% phosphoric acid; these solutions were then further diluted with 16 mL of 0.1% phosphoric acid. Aliquots of each of these solutions were transferred to HPLC vials for analysis along with solution stability samples. They were assayed in the sample set 60659.107.seq using the method L221869_method1.mth. Each sample was analyzed once.

Rebecca Leigh Shultz
03 Dec 2001

RLS 03 Dec 2001

COUNTERSIGNATURE 21 Jan 2002
DATE

R. Leigh Shultz
04 Dec 2001

Subject: Solution stability of L-221869-000R (4-week data)

The four-week solution stability samples (60659-93) were removed from the stability stations at 10:00 am and were allowed to come to ambient temperature. Seven samples were removed from each of five stations (–20, 5, 25, 40, and 80 °C). Upon examination, sample 60659-93 R4 pH=W was observed to be in a cracked vial (discovered before the sample melted completely). In order to salvage the sample, the solution was transferred to a new vial as the sample melted. The samples were assayed in the sample set 60659.108.seq using the method L221869_method1.mth. Each sample was assayed twice.

Subject: Evaluation of thermal effects on vials and HPLC caps

The blank vials which had been placed in the stability stations on 05 Nov 2001 (60659-93) were removed after 4 weeks and weighed to determine if the mass of the vials had changed on storage. The conditions, intial weights, and final weights are shown in the table below.

Vial label	Conditions	Mass (initial) (g)	Mass (final) (g)	Change (g)
Blank vial A	5 °C	6.25912	6.25932	+0.00020
Blank vial C	40 °C	6.20152	6.17624	–0.02528
Blank vial D	40 °C/75%RH	6.26689	6.28318	+0.01629
Blank vial E	60 °C	6.21773	6.17478	–0.04295
Blank vial F	80 °C	6.21650	6.16892	–0.04758
Blank vial G	25 °C/60%RH	6.26962	6.28055	+0.01093
Blank vial R	–20 °C	6.20560	6.20658	+0.00098

These results indicate that vials kept in a humid environment (–20 °C, 5 °C, 25 °C/60% RH, or 40 °C/75% RH) gain weight, most likely due to absorption of water by the cap liner or the label. Vials kept at elevated temperatures (40, 60, 80 °C) at ambient RH show weight loss, probably due to evaporation of plasticizers in the vial caps and labels. These results suggest that total vial weights (including sample) should not be used to assess weight changes of samples on stability.

HPLC vials capped with the Al/PP crimp caps were also removed from the stability stations (7) after 4 weeks. Each vial had initially contained 1 mL of water, and the liquid level had been marked on the outside of the vial. The loss of liquid over the four-week period was evaluated visually, as shown in the table below.

Vial label	Conditions (t = 4 wks)	Liquid level
Blank vial A	5 °C	No change
Blank vial C	40 °C	~ 40 % loss
Blank vial D	40 °C/75%RH	< 5 % loss
Blank vial E	60 °C	100 % loss
Blank vial F	80 °C	100 % loss
Blank vial G	25 °C/60%RH	< 5 % loss
Blank vial R	–20 °C	No change

These results indicate that the Al/PP caps are not sufficient for stability studies due to sample loss via evaporation.

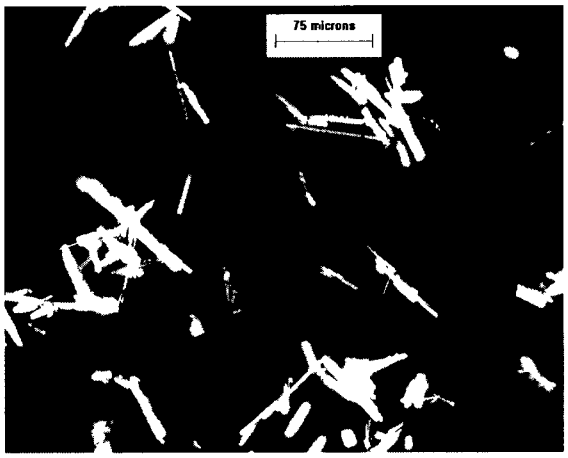
Rebecca Leigh Shultz
04 Dec 2001

21 Jan 2002
COUNTERSIGNATURE DATE

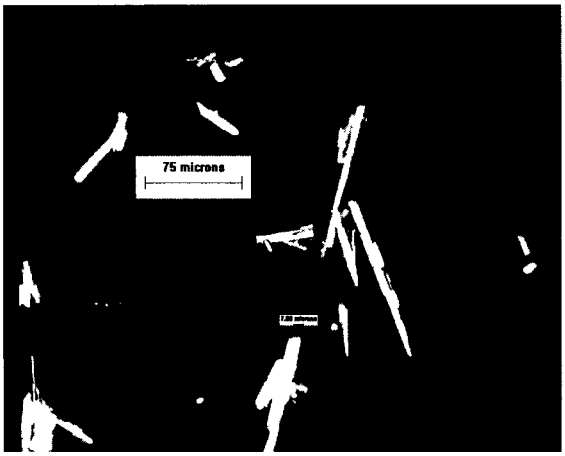
R. Leigh Shultz
04 Dec 2001

Subject: Initial physical characterization of L-224715-000T001

The DP-IV antagonist L-224715 was received from Basic Chemistry (D. Kim) as the free base. This material is a white powder. It was analyzed by microscopy to determine particle size and morphology and to assess crystallinity. Two images at 200X magnification are shown below. In each image, the large bar is 75 microns in length. In the second image, the smaller bar is 7.5 microns in length.



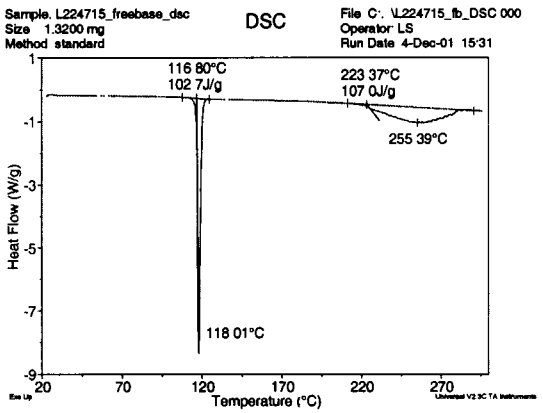
L-224715-000T001: free base (image 1)



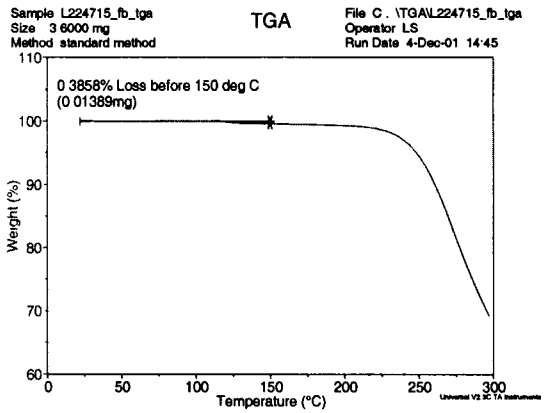
L-224715-000T001: free base (image 2)

The particles are birefringent under polarized light. Most are between 50-100 microns in length, though there are smaller particles observed. The aspect ratio of the particles is ca. 10:1 (needles). The birefringence suggests crystallinity, which will have to be confirmed by XRPD.

The thermal properties of the material were probed using DSC and TGA. The data are shown in the two figures below.



DSC of L-224715-000T001 (25-300 °C, 10 °C/min)



TGA of L-224715-000T001 (25-300 °C)

DSC results suggest a crystalline material (single polymorph) with a melting point of 118.01 °C (102.7 J/g). The endotherm above 223 °C is most likely due to decomposition of the sample. This is consistent with the TGA results, which show weight loss of ca. 30% beginning around 225 °C. TGA also indicates that the solid contains 0.39% volatiles (Basic Chemistry indicated that the sample contained 0.4 % 2-propanol).

Rebecca Leigh Shultz 04 Dec 2001

COUNTERSIGNATURE DATE

21 Jan 2002

R. Leigh Shultz
06 Dec 2001

Subject: Hygroscopicity of L-224715-000T001 at 25 °C

A small sample of L-224715-000T001 (ca. 7-8 mg) was placed in the clean, tared microbalance pan of the VTI to measure the hygroscopicity of the solid at 25 °C from 5-95 %RH. The method std25A.mth was used, which incorporates a drying step at 40 °C. The sample was left to run overnight.

Subject: Photostability of L-221869-000R (crystalline free base)

The photostability samples for L-221869-000R (60659-94, 12 samples) were removed from the freezer and allowed to thaw. The dark samples were wrapped with aluminum foil to prevent exposure, and all 12 samples were placed in the Rayonet irradiator for 17 hours (fluorescent light).

Subject: Initiation of bulk stability for L-224715-000T001 (free base)

Samples of L-224715-000T001 (Basic Chemistry) were weighed into tared scintillation vials to initiate bulk stability studies of the free base according to the table below.

Sample	Conditions	Mass L224715 (mg)	Cap
60659-110 A1	5 °C, 1 week	1.17	closed
60659-110 A2	5 °C, 2 weeks	1.11	closed
60659-110 A4	5 °C, 4 weeks	1.81	closed
60659-110 C1	40 °C, 1 week	1.58	closed
60659-110 C2	40 °C, 2 weeks	1.50	closed
60659-110 C4	40 °C, 4 weeks	1.28	closed
60659-110 D1	40 °C/75%RH, 1 week	1.14	open
60659-110 D2	40 °C/75%RH, 2 weeks	1.15	open
60659-110 D4	40 °C/75%RH, 4 weeks	1.76	open
60659-110 E1	60 °C, 1 week	1.06	closed
60659-110 E2	60 °C, 2 weeks	1.15	closed
60659-110 E4	60 °C, 4 weeks	1.60	closed
60659-110 F1	80 °C, 1 week	1.10	closed
60659-110 F2	80 °C, 2 weeks	1.21	closed
60659-110 F4	80 °C, 4 weeks	1.02	closed
60659-110 G1	25 °C/60%RH, 1 week	1.47	open
60659-110 G2	25 °C/60%RH, 2 weeks	1.24	open
60659-110 G4	25 °C/60%RH, 4 weeks	1.45	open
60659-110 R1	–20 °C, 1 week	1.17	closed
60659-110 R2	–20 °C, 2 weeks	1.68	closed
60659-110 R4	–20 °C, 4 weeks	1.40	closed

The vials were placed in the stability stations at 3:00 pm (each set of vials was placed in a 50-mL beaker with a green tape label).

Rebecca Leigh Shultz
06 Dec 2001

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
06 Dec 2001

Subject: Initiation of solution stability for L-224715-000T001 (free base)

A sample (12.91 mg) of L-224715-000T001was weighed into a tared 20-mL scintillation vial. Water (HPLC grade, 12.91 mL) was added via pipet to make a 1.0 mg/mL stock solution of L-224715. This stock solution was used to prepare all solution stability and photostability samples discussed below. The buffers used were 20mM sodium acetate (pH = 4.02), 20 mM sodium phosphate (2 buffers, pH = 6.04 and pH = 7.97), and 20 mM sodium carbonate (pH = 9.95). For stability at pH 2, 0.01N HCl was used.

Four sets of samples were prepared: a photostability set, labeled 60659-110 L (light-exposed samples, pH 2, 4, 6, 8, 10, and water) and 60659-110 D (dark control samples, pH 2, 4, 6, 8, 10, and water). An aliquot of the stock solution (0.10 mL) was added to each HPLC vial, followed by 0.90 mL of the appropriate buffer or water. The vials (12 total) were crimp-capped with Teflon-lined caps and placed in the freezer (–20 °C).

Three additional sets of samples were prepared. The sets were identical to each other and are intended to represent three different stability timepoints (1, 2, and 4 weeks). Each set contains vials to be put in each of five stability stations: –20 °C freezer (R), 5 °C refrigerator (A), 25 °C/60%RH oven (G), 40 °C/75%RH oven (C), and 80 °C/ambient RH oven (F). The set in each stability station contains six vials: pH's 2, 4, 6, 8, 10, and plain water. All of these samples were prepared in a fashion analogous to the photostability samples described above using the 1 mg/mL stock solution of L-224715 (90 vials total). The vials were labeled with the notebook page 60659-110, a letter indicating the stability station, a number indicating the timepoint (1, 2, or 4), and the pH buffer used. They were crimp-capped with Teflon-lined caps to prevent evaporation and placed in the stability stations at 3:00 pm.

Additional samples were prepared, again using the stock solution of L-224715 (0.1 mL) and an appropriate diluent (0.9 mL). Two of the samples were diluted with 0.1 N HCl, while two others were diluted with 0.1 N NaOH. The other two vials were diluted with 3% w/v H_2O_2. The two peroxide samples were placed in the 25 °C/60%RH oven. The remaining vials (HCl and NaOH) were placed in the 80 °C oven. These vials will be left overnight and will be used to evaluate the applicability of the HPLC method for L-221869 to L-224715.

A fresh sample of L-224715-000T in water (0.1 mg/mL) was prepared from the stock solution and was assayed immediately by HPLC along with a sample of the cyclic amine fragment of the molecule (NB32073-11). These two samples were assayed in the set 60659.110.seq using the current method for L-221869, L221869_method1.mth.

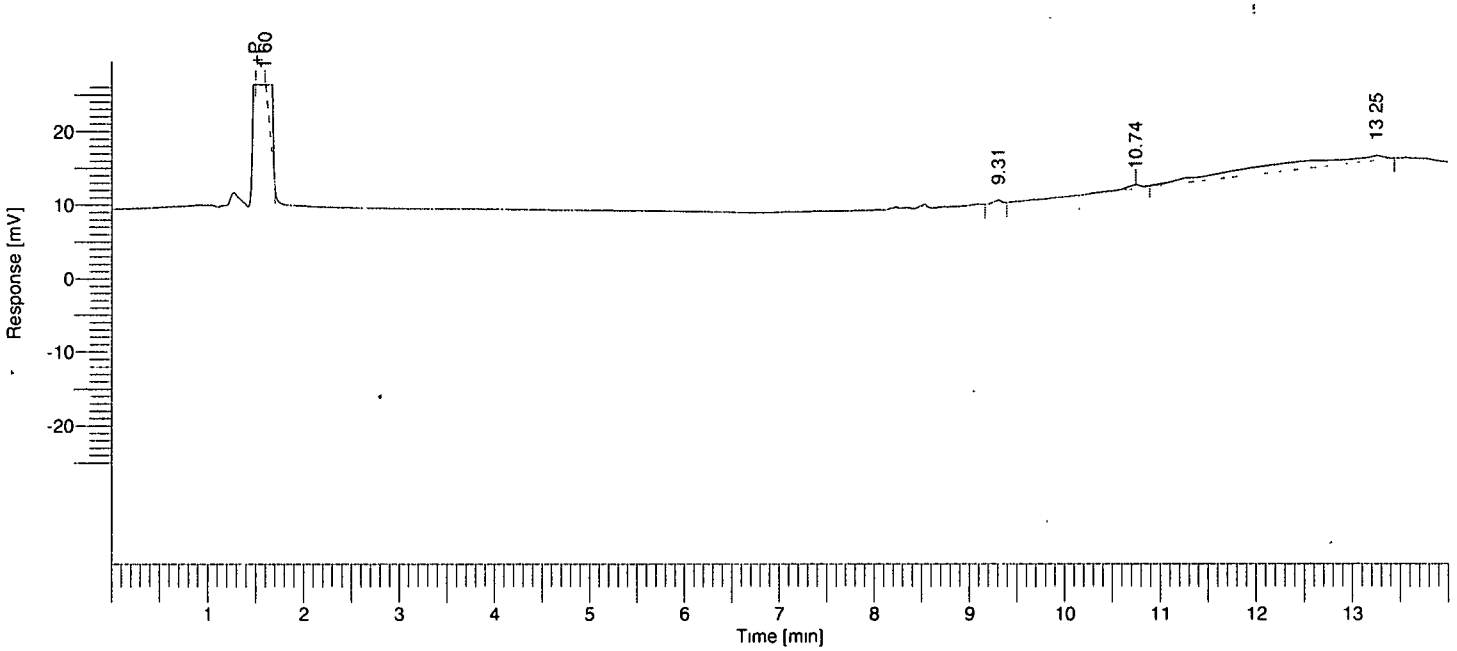
The stock solution of L-224715 was frozen for future use. Note that preparation of these samples showed that the solubility of L-224715 in water is greater than 1 mg/mL; the solubility across the pH range 2-10 is greater than 0.1 mg/mL.

Rebecca Leigh Shultz
06 Dec 2001

COUNTERSIGNATURE 21 Jan 2002 DATE

Software Version	: 6.2.0.0.0:B27	Date	: 12/6/01 5:03:03 PM
Reprocess Number	: ry80m-108-pelc: 136		
Sample Name	: cyclic amine fragment	Data Acquisition Time	: 12/6/01 4:20:38 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

Result File : D:\Projects\L224715\RawData\60659.110.002.rst
Sequence File : D:\Sequences\L224715\60659.110.idx



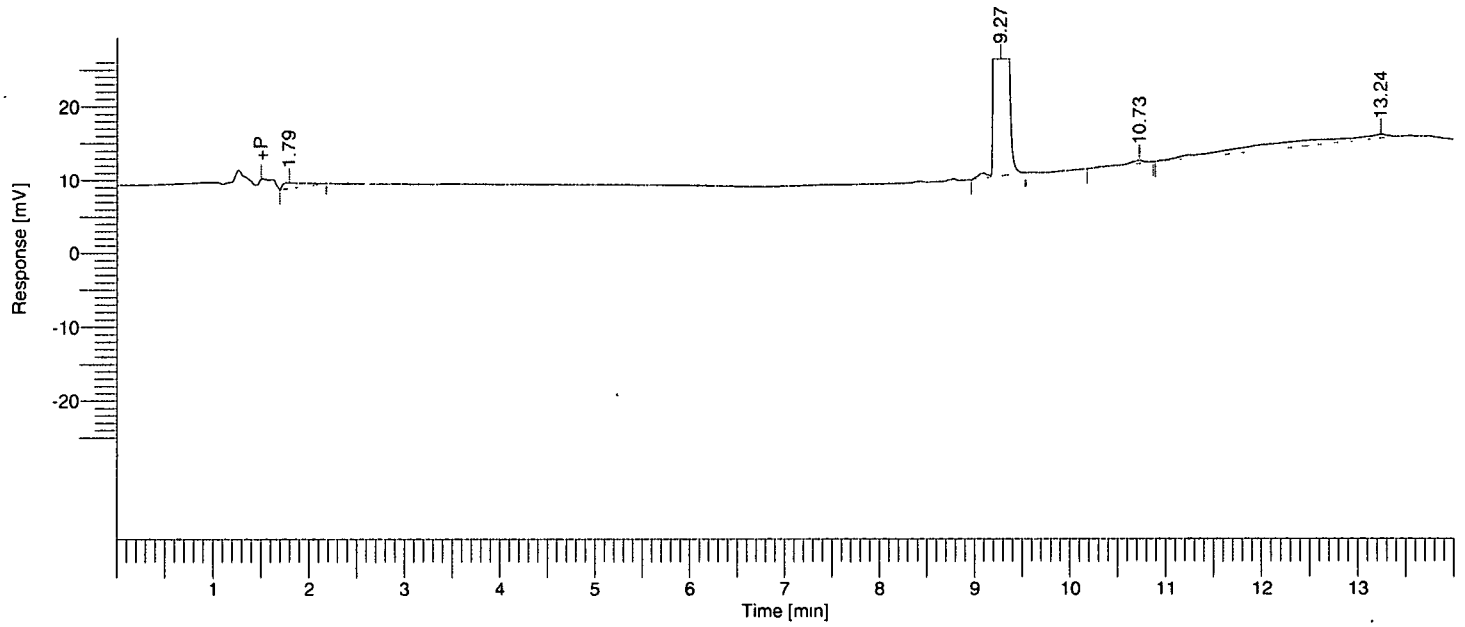
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.598	0.18	825677.27	121102.50	78.97
3		10.742	1.22	14212.82	1036.09	1.36
4		13.247	1.50	205624.04	1221.29	19.67
				1045514.13	123359.89	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/6/01 5:03:03 PM
Reprocess Number	: ry80m-108-pelc: 137		
Sample Name	: L224715-000T	Data Acquisition Time	: 12/6/01 4:40:11 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

Result File : D:\Projects\L224715\RawData\60659.110.003.rst
Sequence File : D:\Sequences\L224715\60659.110.idx



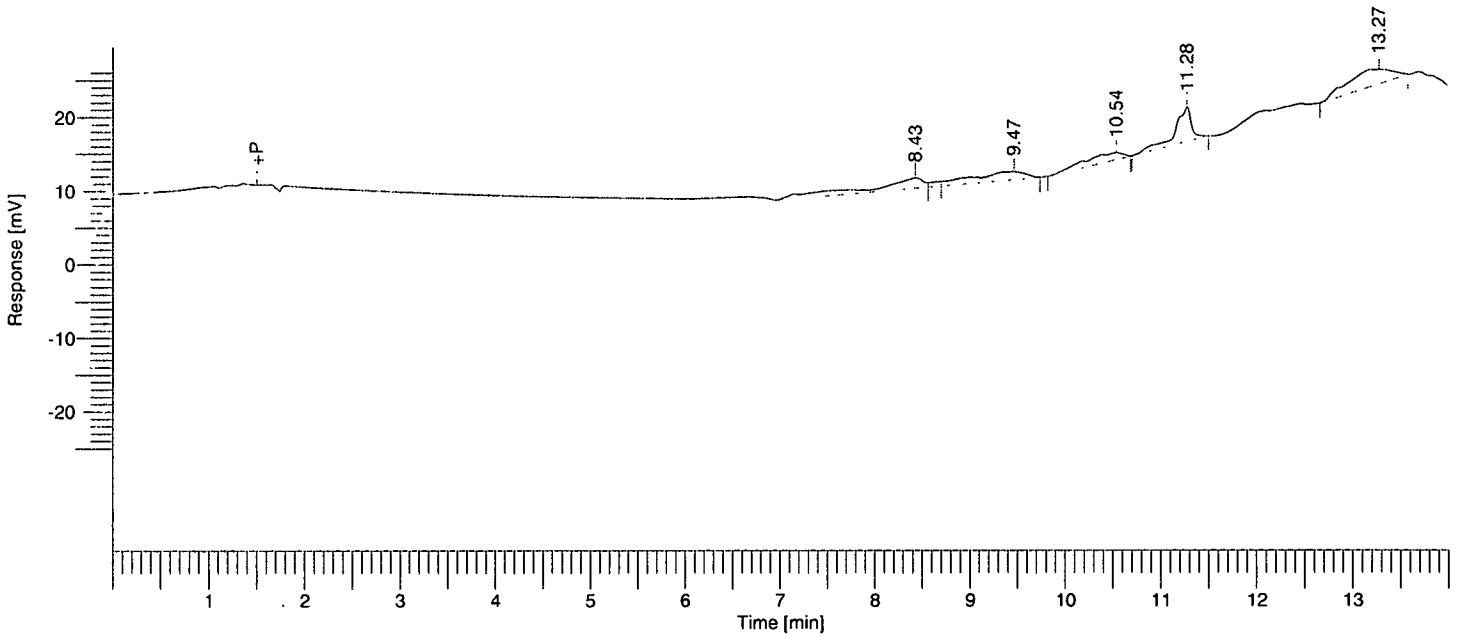
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.794	0.20	23607.54	1452.92	0.53
2		9.274	1.05	4327518.38	827594.00	96.25
4		13.238	1.50	145182.76	1043.54	3.23
				4496308.68	830090.46	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/6/01 5:03:02 PM
Reprocess Number	: ry80m-108-pelc: 135		
Sample Name	: water	Data Acquisition Time	: 12/6/01 4:01:07 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/1	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L224715\RawData\60659.110.001.rst
Sequence File : D:\Sequences\L224715\60659.110.idx



Single Injection Report

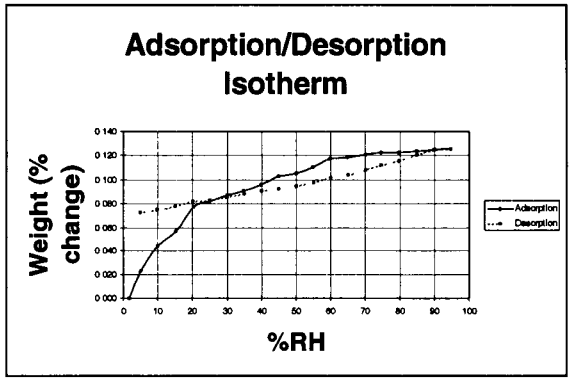
Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		8.429	0.96	113397.75	2681.96	21.82
2		9.465	1.07	89365.33	2009.54	17.19
3		10.540	1.20	62106.22	1737.39	11.95
4		11.276	1.28	112238.05	9168.55	21.60
5		13.272	1.50	142622.36	4324.58	27.44
				519729.70	19922.02	100.00

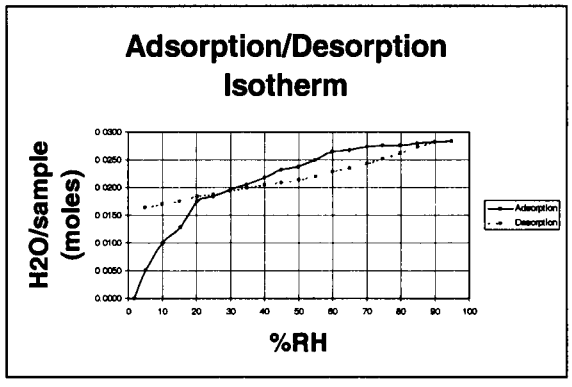
R. Leigh Shultz
07 Dec 2001

Subject: Hygroscopicity of L-224715-000T001

The VTI run on the free base of L-224715 was finished by 8:00 am, so the solid was removed from the pan and saved for analysis. The data are shown below and appear after this page as supplementary material.



1. %wt change versus %RH for L-224715-000T



2. Moles H_2O versus %RH for L-224715-000T

The free base L-224715-000T is non-hygroscopic, gaining only 0.125% water over the entire RH range. See NB60659-113 for physical characterization of the solid remaining after the VTI run was complete.

Subject: HPLC analysis of stressed samples of L-224715

The fresh sample of L-224715 in water was analyzed by HPLC using the established method for L-221869. In this method, L-224715 elutes at 9.27 minutes. The samples stressed in acid, base, and peroxide were removed from the stability stations at 8:00 am. The acid and base samples were neutralized by the addition of an 0.1 mL aliquot of 1N HCl or NaOH. The six samples were then assayed by HPLC in the sequence 60659.112.seq using the method L221869_method1.mth. Aging in 0.1N HCl causes a small amount of hydrolysis (area % parent 90.20), with the hydrolysis products appearing at 8.45 min and 1.64 min (carboxylate and amine, respectively). The acid degredates are well-separated by this method. Aging in 0.1N NaOH produces a significant amount of hydrolysis (area % parent 0.60), with the same hydrolysis products present. Additionally, other degredates are present at 10.63, 10.99, and 12.08 min. These may be due to de-amination. Aging in peroxide produces no degredates, but the parent peak appears to have a shoulder on its left side (loss of F?).

Rebecca Leigh Shultz
07 Dec 2001

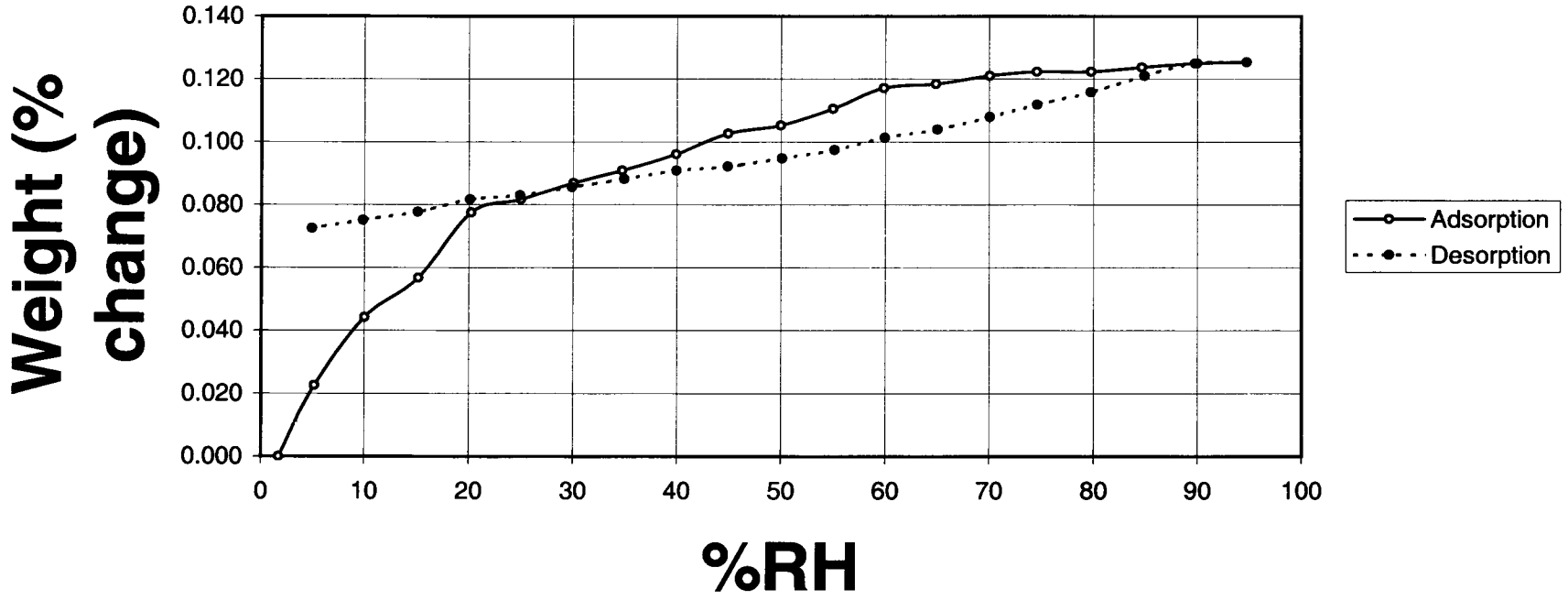
Kathy Rhodes 21 Jan 2002
COUNTERSIGNATURE DATE

File Name	L715fb25.lsh		
Experiment	Step Isotherm		
Operator	LS		
Experiment ID	L715fb25		
Sample Name	L224715 free base		
Sample Lot #	L-224715-000T001		
Notes	DP-IV		
	NB60659-110		
Drying Temp	40 ºC		
Heating Rate	5 ºC/min		
Max Drying Time	60 min		
Equil Crit	0.0100 wt % in	2.00 min	
Expt Temp	25 ºC		
Max Equil Time	180 min		
Equil Crit	0.0100 wt % in	5.00 min	
RH Steps	5 to 95 in 5% steps		
Data Logging Interval	2.00 min or	0.0100 wt %	
Expt Started	12/06/2001		
Run Started	11:47:06		

Elap Time min	Weight mg	Weight % chg	Samp Temp deg C	Samp RH %	H2O/sample
62.5	7.612	0.000	25.44	1.74	0.0000
72.2	7.614	0.023	25.29	5.17	0.0051
84.0	7.615	0.044	25.27	10.01	0.0100
92.6	7.616	0.057	25.26	15.16	0.0128
123.6	7.618	0.078	25.25	20.22	0.0175
137.1	7.618	0.082	25.25	24.99	0.0185
152.6	7.618	0.087	25.24	30.03	0.0197
167.6	7.619	0.091	25.24	34.72	0.0206
189.6	7.619	0.096	25.24	39.92	0.0218
228.1	7.620	0.103	25.24	44.91	0.0232
244.1	7.620	0.105	25.24	49.96	0.0238
263.1	7.620	0.111	25.23	54.91	0.0250
278.6	7.621	0.117	25.23	59.86	0.0265
292.6	7.621	0.118	25.23	64.85	0.0268
309.6	7.621	0.121	25.23	70.05	0.0274
325.6	7.621	0.122	25.23	74.60	0.0277
344.1	7.621	0.122	25.23	79.77	0.0277
363.1	7.621	0.124	25.23	84.70	0.0280
378.1	7.621	0.125	25.23	89.97	0.0283
391.1	7.621	0.125	25.23	94.76	0.0284
401.1	7.621	0.125	25.23	89.80	0.0283
410.6	7.621	0.121	25.23	84.96	0.0274
424.6	7.621	0.116	25.23	79.76	0.0262
438.6	7.620	0.112	25.23	74.61	0.0253
454.1	7.620	0.108	25.23	70.04	0.0244
465.1	7.620	0.104	25.23	64.99	0.0235
484.1	7.620	0.101	25.23	59.93	0.0229
507.1	7.619	0.097	25.23	55.06	0.0220
517.6	7.619	0.095	25.23	50.08	0.0215
531.1	7.619	0.092	25.23	44.90	0.0209

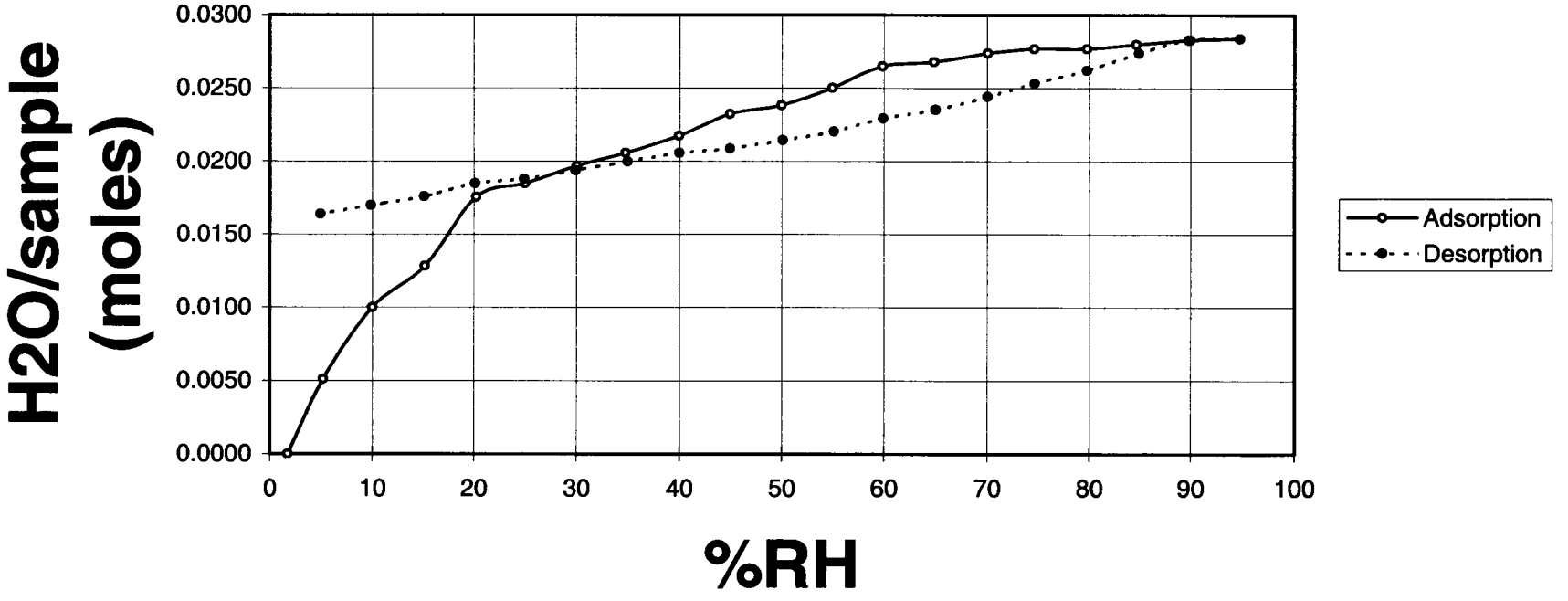
551.6	7.619	0.091	25.23	39.96	0.0206
589.6	7.619	0.088	25.23	34.93	0.0200
637.6	7.618	0.086	25.24	29.91	0.0194
659.1	7.618	0.083	25.24	24.91	0.0188
675.6	7.618	0.082	25.24	20.11	0.0185
692.1	7.618	0.078	25.24	15.12	0.0176
700.6	7.617	0.075	25.24	9.86	0.0170
709.1	7.617	0.072	25.24	4.90	0.0164

Adsorption/Desorption Isotherm



12/07/2001

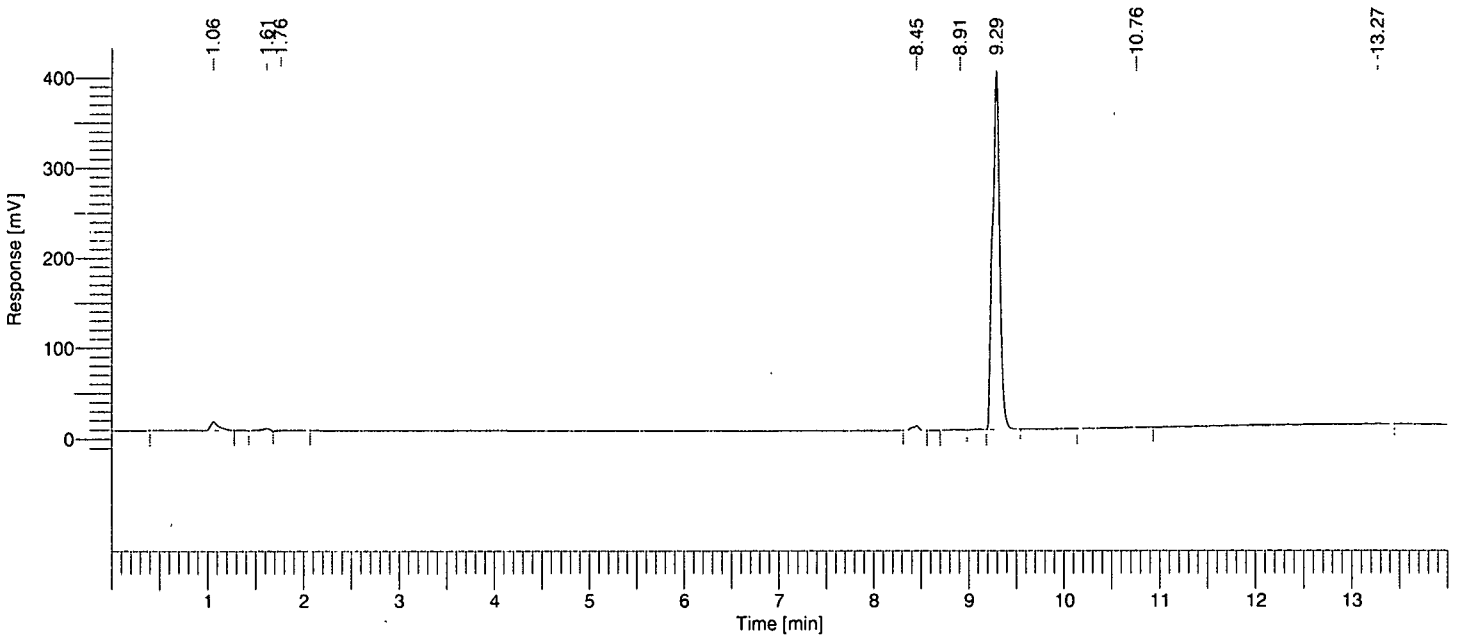
Adsorption/Desorption Isotherm



12/07/2001

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:04 PM
Reprocess Number	: ry80m-108-pelc: 139		
Sample Name	: 0.1N HCl 80C A	Data Acquisition Time	: 12/7/01 9:20:30 AM
trument Name	: PELC	Channel	: A
ck/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

Result File : D:\Projects\L224715\RawData\60659.112.002.rst
Sequence File : D:\Sequences\L224715\60659.112.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

ak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.058	1.00	128020.45	19500.35	2.83
2		1.614	1.53	36128.22	5294.17	0.80
3		1.760	1.66	15827.21	1243.29	0.35
4		8.453	7.99	47779.18	10015.82	1.06
6		9.292	8.78	4078128.45	774595.50	90.18
7		10.761	10.17	15219.70	971.47	0.34
8		13.270	12.54	201102.96	1180.07	4.45
				4522206.17	812800.68	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:05 PM
Reprocess Number	: ry80m-108-pelc: 140		
Sample Name	: 0.1N HCl 80C B	Data Acquisition Time	: 12/7/01 9:40:03 AM
trument Name	: PELC	Channel	: A
ck/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

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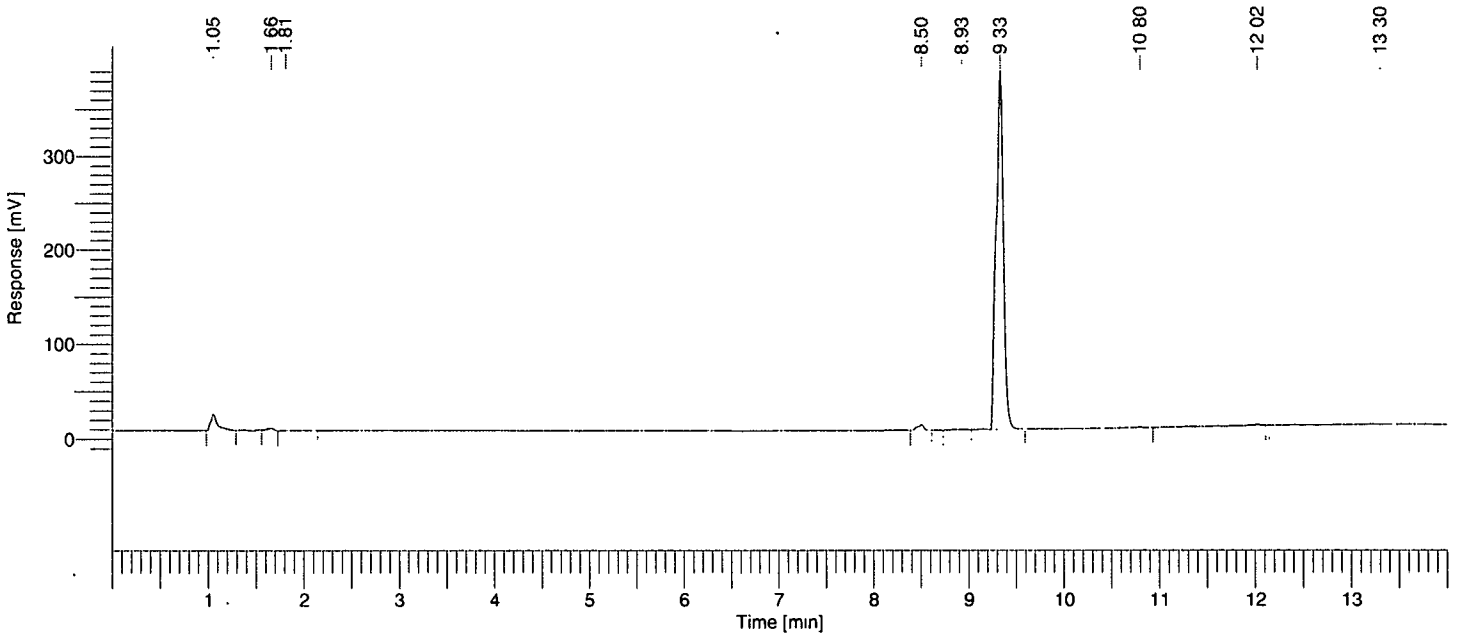
Sequence File : D:\Sequences\L224715\60659.112.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

ak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.052	1.00	167378.22	33947.35	3.85
2		1.657	1.58	21328.13	4357.78	0.49
3		1.807	1.72	13005.80	978.21	0.30
4		8.505	8.08	53832.15	11391.89	1.24
5		8.929	8.49	10311.35	984.97	0.24
6		9.330	8.87	3926511.95	744493.59	90.22
8		12.018	11.42	72738.96	3324.15	1.67
9		13.297	12.64	86829.72	1094.28	2.00
				4351936.27	800572.23	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:06 PM
Reprocess Number	: ry80m-108-pelc: 141		
Sample Name	: 0.1N NaOH 80C A	Data Acquisition Time	: 12/7/01 9:59:35 AM
ıtrument Name	: PELC	Channel	: A
ck/Vial	: 1/4	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 4		

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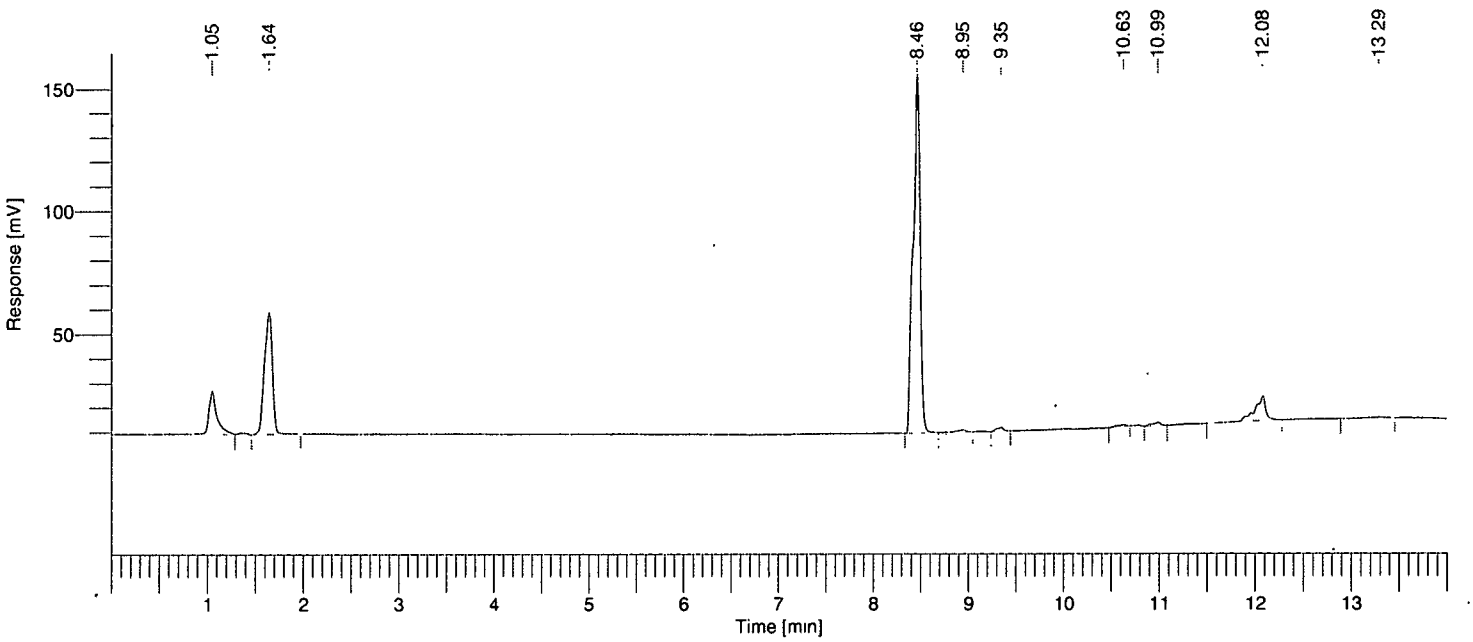

Single Injection Report

Rebecca Leigh Shultz
NB 60659

ak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.049	1.00	196268.96	34531.93	8.15
2		1.642	1.57	560287.22	96960.25	23.28
3		8.462	8.07	1464170.50	284395.59	60.82
5		9.346	8.91	14547.91	3091.47	0.60
7		10.989	10.48	15996.67	2549.50	0.66
8		12.079	11.52	155940.95	20087.49	6.48
				2407212.20	441616.24	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:06 PM
Reprocess Number	: ry80m-108-pelc: 142		
Sample Name	: 0.1N NaOH 80C B	Data Acquisition Time	: 12/7/01 10:19:07 AM
trument Name	: PELC	Channel	: A
ck/Vial	: 1/5	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 5		

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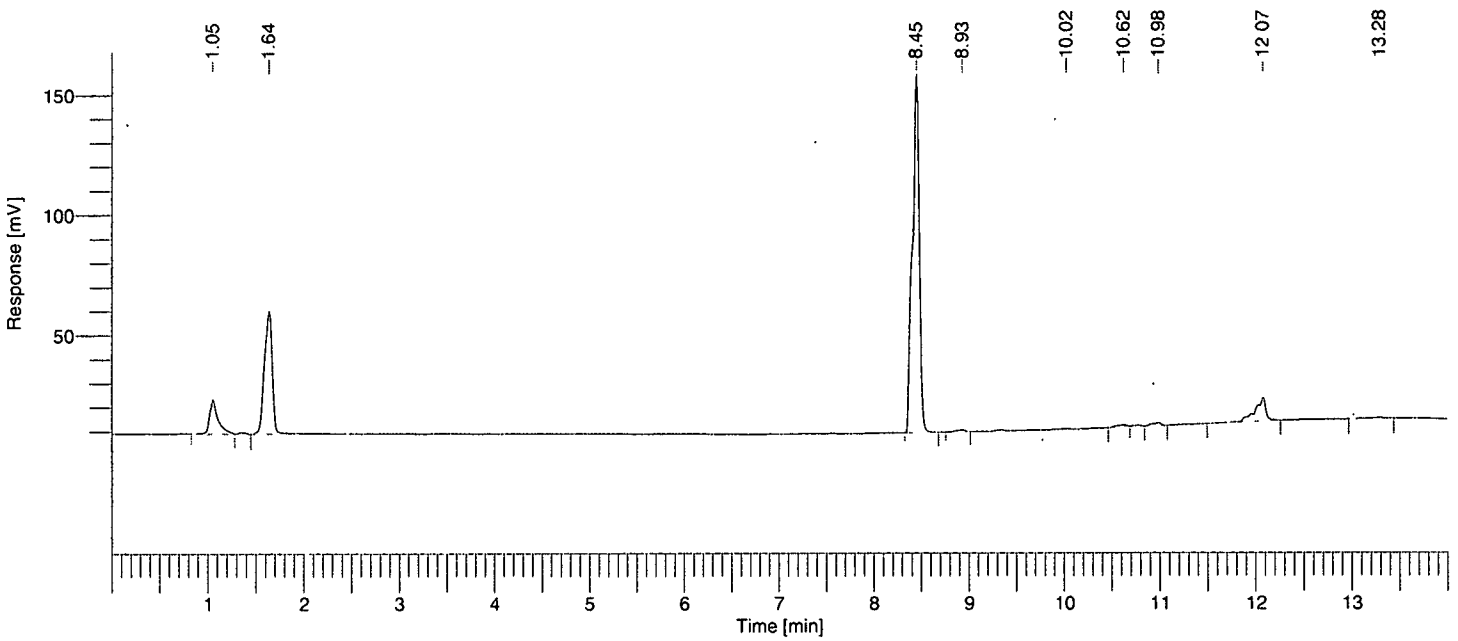
Sequence File : D:\Sequences\L224715\60659.112.idx



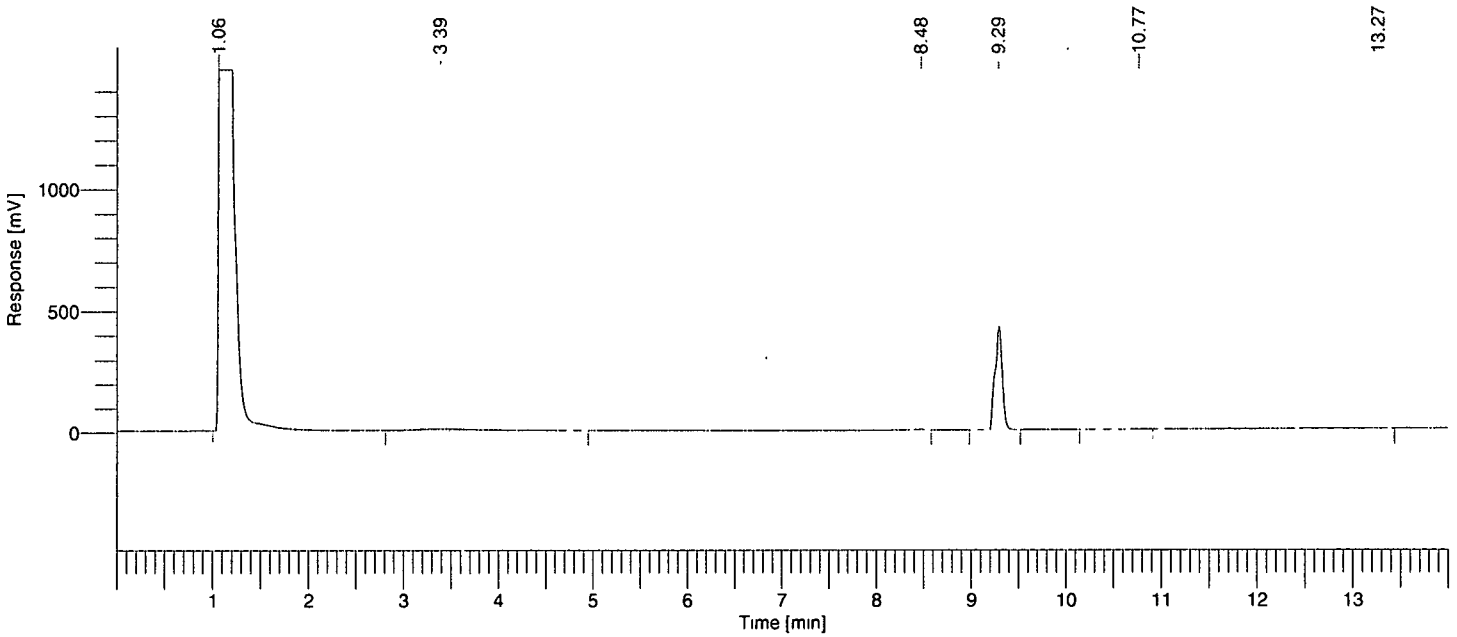
Single Injection Report

Rebecca Leigh Shultz
NB 60659

ak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.052	1.00	166603.00	27447.94	7.07
2		1.638	1.56	558786.19	99368.77	23.71
3		8.447	8.03	1468885.57	291409.73	62.34
7		10.983	10.44	15705.42	2528.58	0.67
8		12.074	11.48	146427.50	19300.70	6.21
				2356407.69	440055.72	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:07 PM
Reprocess Number	: ry80m-108-pelc: 143		
Sample Name	: 3% H2O2 25C A	Data Acquisition Time	: 12/7/01 10:38:39 AM
trument Name	: PELC	Channel	: A
ck/Vial	: 1/6	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 6		

Result File : D:\Projects\L224715\RawData\60659.112.006.rst
Sequence File : D:\Sequences\L224715\60659.112.idx



Single Injection Report

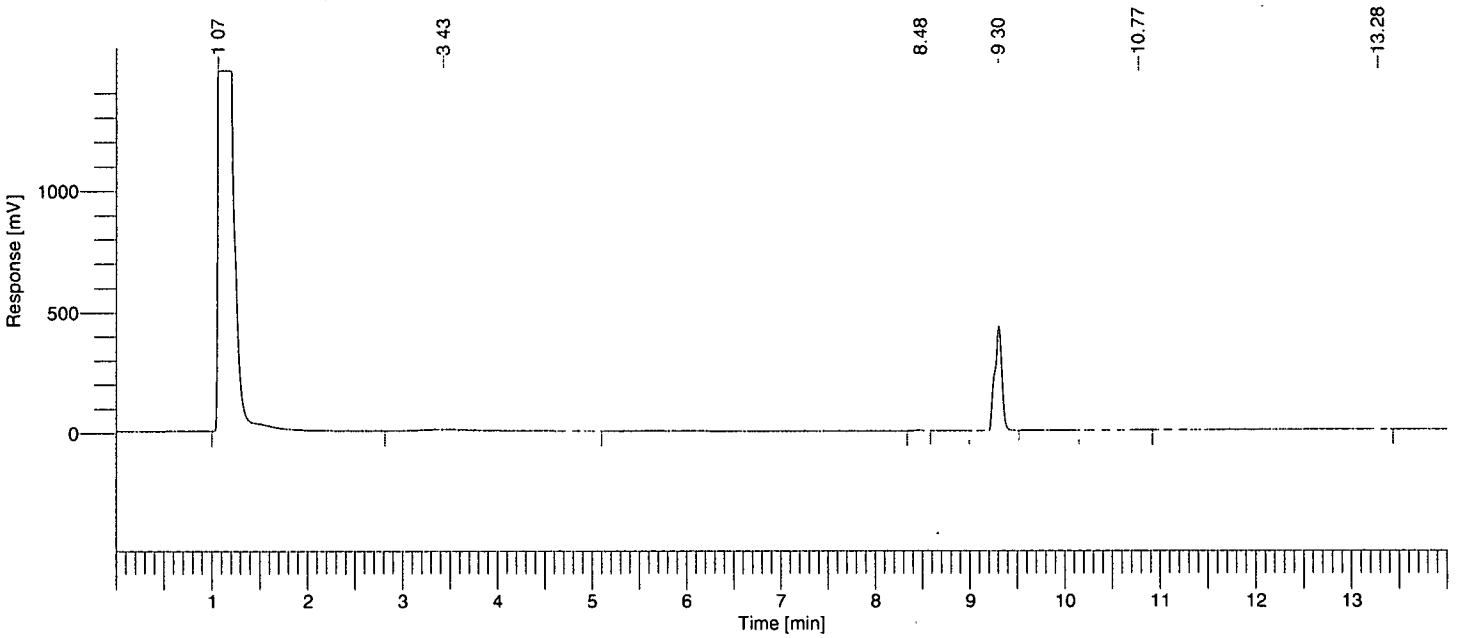
Rebecca Leigh Shultz
NB 60659

ak Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1	1.063	1.00	35027926.08	2.88e+06	87.05
2	3.388	3.19	667727.64	13914.93	1.66
3	8.476	7.97	32215.07	7014.52	0.08
4	9.293	8.74	4369306.93	832462.66	10.86
5	10.767	10.13	10417.32	766.09	0.03
6	13.270	12.48	133434.93	982.58	0.33
			40241027.98	3.74e+06	100.00

Warning -- Signal level out-of-range in peak

Software Version	: 6.2.0.0.0:B27	Date	: 12/7/01 2:17:07 PM
Reprocess Number	: ry80m-108-pelc: 144		
Sample Name	: 3% H2O2 25C B	Data Acquisition Time	: 12/7/01 10:58:11 AM
trument Name	: PELC	Channel	: A
ck/Vial	: 1/7	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 7		

Result File : D:\Projects\L224715\RawData\60659.112.007.rst
Sequence File : D:\Sequences\L224715\60659.112.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

ak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.067	1.00	35006377.82	2.88e+06	87.04
2		3.430	3.22	663416.52	13545.27	1.65
3		8.480	7.95	33148.55	7310.75	0.08
4		9.299	8.72	4374963.24	843563.67	10.88
5		10.772	10.10	10992.20	788.79	0.03
6		13.275	12.45	130249.48	950.14	0.32
				40219147.80	3.75e+06	100.00

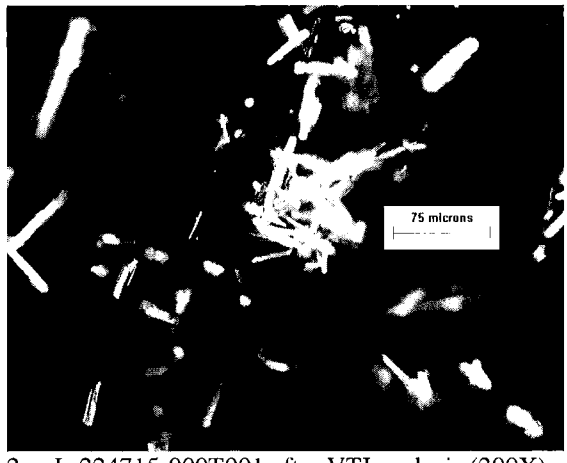
Warning -- Signal level out-of-range in peak

R. Leigh Shultz
07 Dec 2001

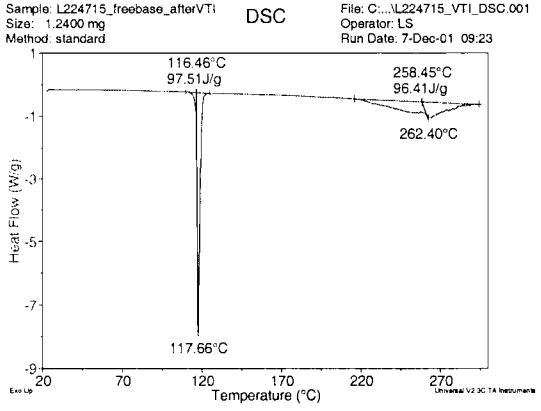
Subject: Physical Characterization of L-224715 Post-VTI

The solid remaining after the VTI run was analyzed by DSC (10 °C/min), TGA, and microscopy. Microscope images are shown below (200X magnification), followed by DSC and TGA traces.

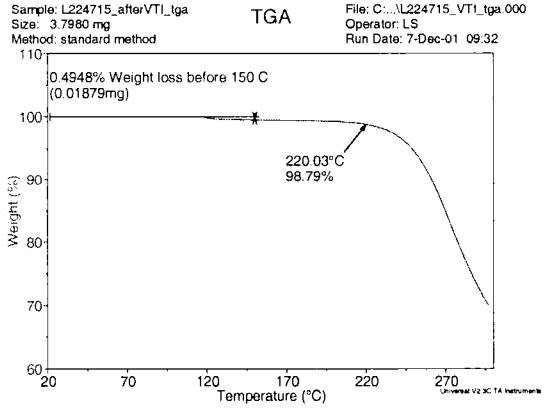
1. L-224715-000T001 after VTI analysis (200X)



2. L-224715-000T001 after VTI analysis (200X)



3. DSC of L-224715-000T001 after VTI analysis



4. TGA of L-224715-000T001 after VTI analysis

Microscopy shows that the morphology and crystallinity of the solid are unchanged. The crystals are still birefringent with an average length of 50-100 microns. DSC shows a slight lowering of the melting point (117.66 °C versus 118.01 °C, probably not significant), and the heat of fusion dropped from 102.7 J/g to 97.51 J/g (probably not significant due to small sample size). The melting endotherm does not appear to have broadened significantly. TGA shows a weight loss of 0.4948% before 150 °C (compared to 0.3858% in the original solid), which is consistent with the VTI data (0.072% water weight retained upon completion of the VTI run).

Rebecca Leigh Shultz

07 Dec 2001

COUNTERSIGNATURE DATE

R. Leigh Shultz
07 Dec 2001

Subject: Analysis of photostability samples of L-221869-000R (free base)

The irradiation of the samples was complete by 8:30 am, but the samples were allowed to remain in the dark until they could be analyzed. They were removed from the Rayonet at 1:30 pm and analyzed in the HPLC sample set 60659.114.seq using the method L221869_method1.mth. Each sample was injected once.

Subject: Solution and bulk stability of L-221869 tartrate and besylate salts (1-week time point)

The 1-week solution and bulk samples of L-221869 tartrate (18 solution samples, 3 bulk) and besylate (18 solution samples, 3 bulk) were removed from the stability stations at 1:00 pm and allowed to come to ambient temperature. No changes were observed in the bulk solids at this time point. The bulk samples were each dissolved in 3.0 mL of 0.1% phosphoric acid; these solutions were then diluted with 16.0 mL additional 0.1% phosphoric acid to bring the concentration of each sample to ca. 0.1 mg/mL. An aliquot of each solution was placed in an HPLC vial, and the bulk samples were analyzed with the solution samples in the sequence 60659.114.seq using the method L221869_method1.mth. Each sample was assayed once.

Rebecca Leigh Shultz
07 Dec 2001

RLS 07 Dec 2001

Countersignature Date 21 Jan 2002

R. Leigh Shultz
11 Dec 2001

Subject: Equilibrium Solubility of L-221869 Tartrate and Besylate Salts

Samples of the tartrate (NB72061-34) and besylate (NB72061-32) salts were weighed into test tubes for solubility measurements, as detailed in the table below. An aliquot of solvent was placed in each tube. In each of the four cases, the solid dissolved almost immediately (within 1 minute of solvent addition). The tubes were capped and placed on the rotator overnight.

Salt	Mass of salt (mg)	Solvent	$pH_{initial}$	Vol added (mL)	Min. solubility (mg/mL)
Tartrate (72061-34)	9.73	Water	5.85	0.500	19.46
Tartrate (72061-34)	4.13	0.01N HCl ~~Water~~	~~5.85~~ 2.17	0.413	10.0
Besylate (72061-32)	9.99	Water ~~0.01N HCl~~	~~2.17~~ 5.85	0.500	19.98
Besylate (72061-32)	3.37	0.01N HCl	2.17	0.337	10.0

typo RLS 12Dec2001

Rebecca Leigh Shultz
12 Dec 2001

RLS 12 Dec 2001

Cathy Fredes 21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
12 Dec 2001

Subject: Solution photostability of L-224715-000T001

The solution photostability samples previously prepared (60659-111) were removed from the freezer and allowed to warm to ambient temperature. The six dark samples, labeled 60659-110 D, were wrapped in aluminum foil and placed in the Rayonet irradiator along with the six light samples (60659-110 L). The timer was set for 17 hours of exposure, and the unit was turned on and left to expose the samples overnight (fluorescent light). The temperature inside the Rayonet during exposure was measured to be 29 °C.

Subject: HPLC analysis of L-221869 besylate and tartrate solubility samples

The four solubility samples for the besylate and tartrate salts were removed from the rotator and transferred to centrifuge vials. The pH of each solution was measured. An aliquot of each solution was then diluted to 10.00 mL with water to produce a solution of appropriate concentration for HPLC quantitation.

Salt	Solvent	pH_{final}	Aliquot used (mL)
Besylate	water	6.17	0.050
Besylate	0.01N HCl	2.20	0.100
Tartrate	water	3.62	0.050
Tartrate	0.01N HCl	3.02	0.100

These samples were analyzed by HPLC along with four standard solutions of the tosylate salt for calibration (60659-103) in the sample set 60659.116.seq using the method L221869_method1.mth.

Subject: Determination of stoichiometry for L-221869 tartrate salt

In order to determine how many tartrate counterions are present in the salt per molecule of L-221869 parent, solutions of the tartrate salt of known concentration (0.10 mg/mL and 0.050 mg/mL) were made using a previously made stock solution. These samples were also analyzed in the sample set 60659.116.seq and will be calibrated with the tosylate salt standards in the sample set.

Rebecca Leigh Shultz
12 Dec 2001

RLS 12 Dec 2001

COUNTERSIGNATURE 21 Jan 2002 DATE

R. Leigh Shultz
13 Dec 2001

Subject: Photostability of L-224715-000T001

The photostability samples (60659-111, 116) were removed from the Rayonet irradiator at 8:30 am after 17 hours of exposure. The Teflon caps were removed and replaced with Al/PP caps, and the samples were assayed in the HPLC sample set 60659.117.seq using the method L221869_method1.mth.

Subject: Solution and bulk stability of L-224715-000T001 (one week time point)

The one week bulk (7 vials) and solution (30 vials) stability samples (60659-111) were removed from the stability stations at 8:30 am and allowed to come to ambient temperature. The Teflon caps were removed from the solution samples and were replaced with Al/PP caps. Each of the bulk samples was dissolved in 3.0 mL of 0.1% phosphoric acid; these solutions were further diluted with 16.0 mL 0.1% phosphoric acid. An aliquot of each of the dilute solutions was placed in an HPLC vial for analysis. The solution and bulk samples were assayed in the sample set 60659.117.seq using the method L221869_method1.mth.

Rebecca Leigh Shultz
13 Dec 2001

RLS 13 Dec 2001

COUNTERSIGNATURE 21 Jan 2002 DATE

R. Leigh Shultz
13 Dec 2001

Subject: XRPD analysis of L-224715-000T001

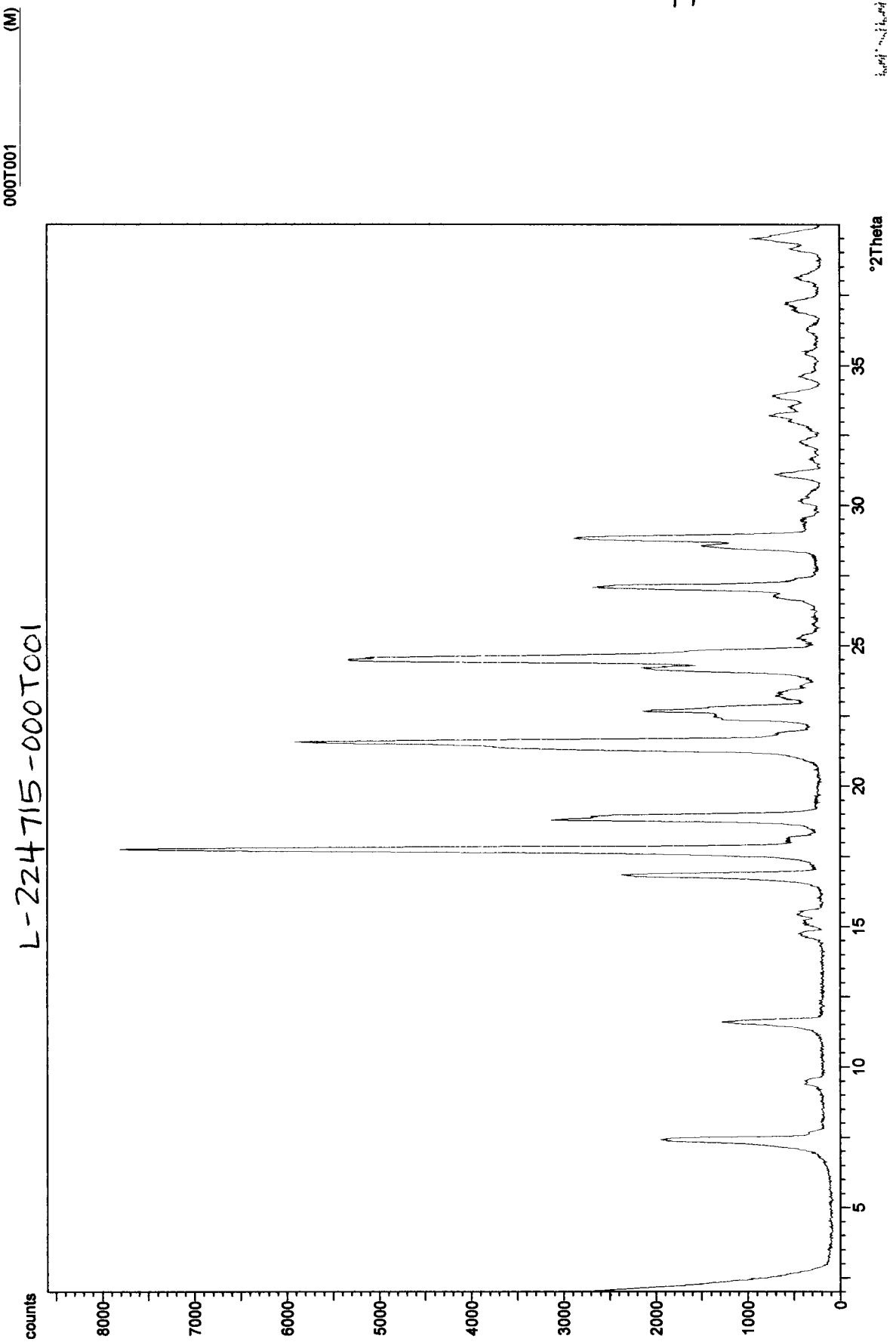
A sample of L-224715-000T001 was transferred to WP for X-ray powder diffraction. The solid was loaded into a low-background sample holder, and the standard procedures (in written form next to X-ray machine) were used to obtain the powder pattern of the sample. Flat stage optics were used with the power settings at 40kV and 40mA. The pattern obtained in a scan from 0 to 40 °2theta is attached as supplementary material

The XRPD pattern shows a highly crystalline material with no amorphous halo.

Rebecca Leigh Shultz
13 Dec 2001

21 Jan 2002
COUNTERSIGNATURE DATE

NB60659 pg 118
supplemental data



Rebecca Leigh Shuler 13 Dec 2001

R. Leigh Shultz
14 Dec 2001

Subject: Solution and bulk stability of L-221869 Tartrate and Besylate salts (2 week time point)

The two-week solution and bulk stability samples (3 bulk vials, 18 solution vials for each salt) were removed from the stability stations at 9:30 am and were allowed to come to ambient temperature. The Teflon caps on the solution samples were replaced with Al/PP crimp caps. The bulk samples were dissolved in 3.0 mL 0.1% phosphoric acid each and further diluted with 16 mL of 0.1% phosphoric acid. An aliquot of each of these dilute solutions was transferred to an HPLC vial for analysis. The bulk and solution samples were analyzed in the sample set 60659.119.seq using the method L221869_method1.mth.

Subject: Equilibrium solubility of L-224715-000T001

Samples of the crystalline free base L-224715-000T001 were weighed into each of 7 test tubes for solubility measurements, as detailed in the table below. An aliquot of solvent (0.50 mL) was placed in each tube. None of the solids dissolved completely with gentle agitation. The tubes were capped and placed on the rotator to equilibrate at 3:30 pm.

Solvent	Initial pH of solvent	Mass L-224715-000T001 (mg)
Water	6.47	9.55
0.9 % NaCl	5.80	9.91
0.01 N HCl	2.01	10.30
20 mM sodium acetate	4.02	14.56
20 mM sodium phosphate	6.05	9.82
20 mM sodium phosphate	7.92	10.75
20 mM sodium carbonate	10.02	12.10

Subject: Standard solutions of L-224715 for HPLC calibration

In order to quantitate solubility, standard solutions of L-224715-000T were made in 0.1% phosphoric acid. A sample of the free base (5.85 mg) was weighed into a tared 10-mL volumetric flask and diluted to the mark with 0.1% phosphoric acid. This stock solution was used to make several other standards, as detailed in the table below.

Standard	Volume stock (mL)	Total volume (mL)	[L224715-000T] (mg/mL)
A	3.42	10.00	0.200
B	2.56	10.00	0.150
C	1.71	10.00	0.100
D	0.855	10.00	0.050
E	0.171	10.00	0.010

In addition, a standard was made using 1.00 mL of standard E in 10.00 mL, resulting in a standard that is 0.0010 mg/mL in free base. These standards were placed in the refrigerator for storage until they are needed.

Rebecca Leigh Shultz
14 Dec 2001

21 Jan 2002
COUNTERSIGNATURE DATE

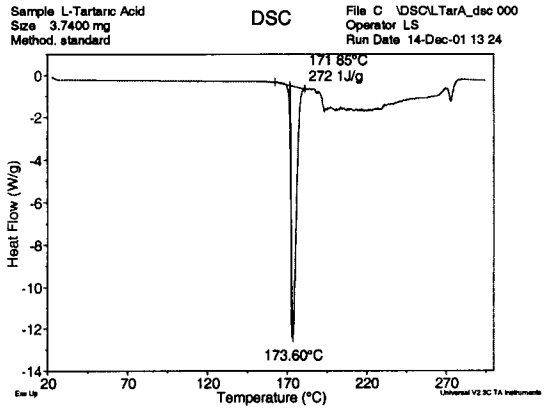
R. Leigh Shultz
14 Dec 2001

Subject: Dynamic vapor sorption analysis of commercial L-tartaric acid

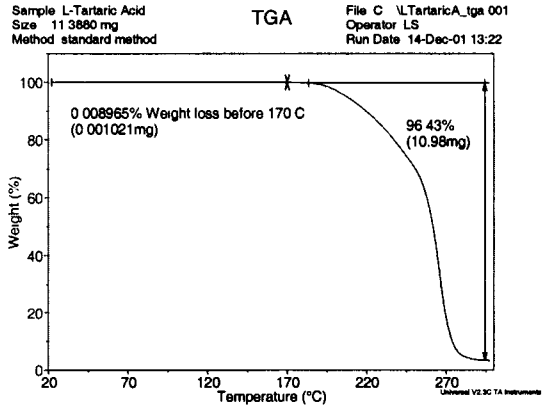
In order to determine the hygroscopicity of L-tartaric acid, a sample was placed on the VTI microbalance pan and run at 25 °C using the method std25A.mth. The data will be analyzed when the run in completed.

Subject: Thermal behavior of commercial L-tartaric acid

In order to determine the melting point of the L-tartaric acid, a DSC was run on a small sample. The results, shown below in Figure 1, indicate that the material is pure and is a single polymorph which melts at 173.6 °C (272.1 J/g). TGA indicates that the solid retains no volatiles (arguing against hygroscopicity) and decomposes completely to volatiles by 300 °C (96.43% weight loss).



1. DSC (10 °C/min) of L-tartaric acid (Fisher)



2. TGA of L-tartaric acid (Fisher)

Rebecca Leigh Shultz
14 Dec 2001

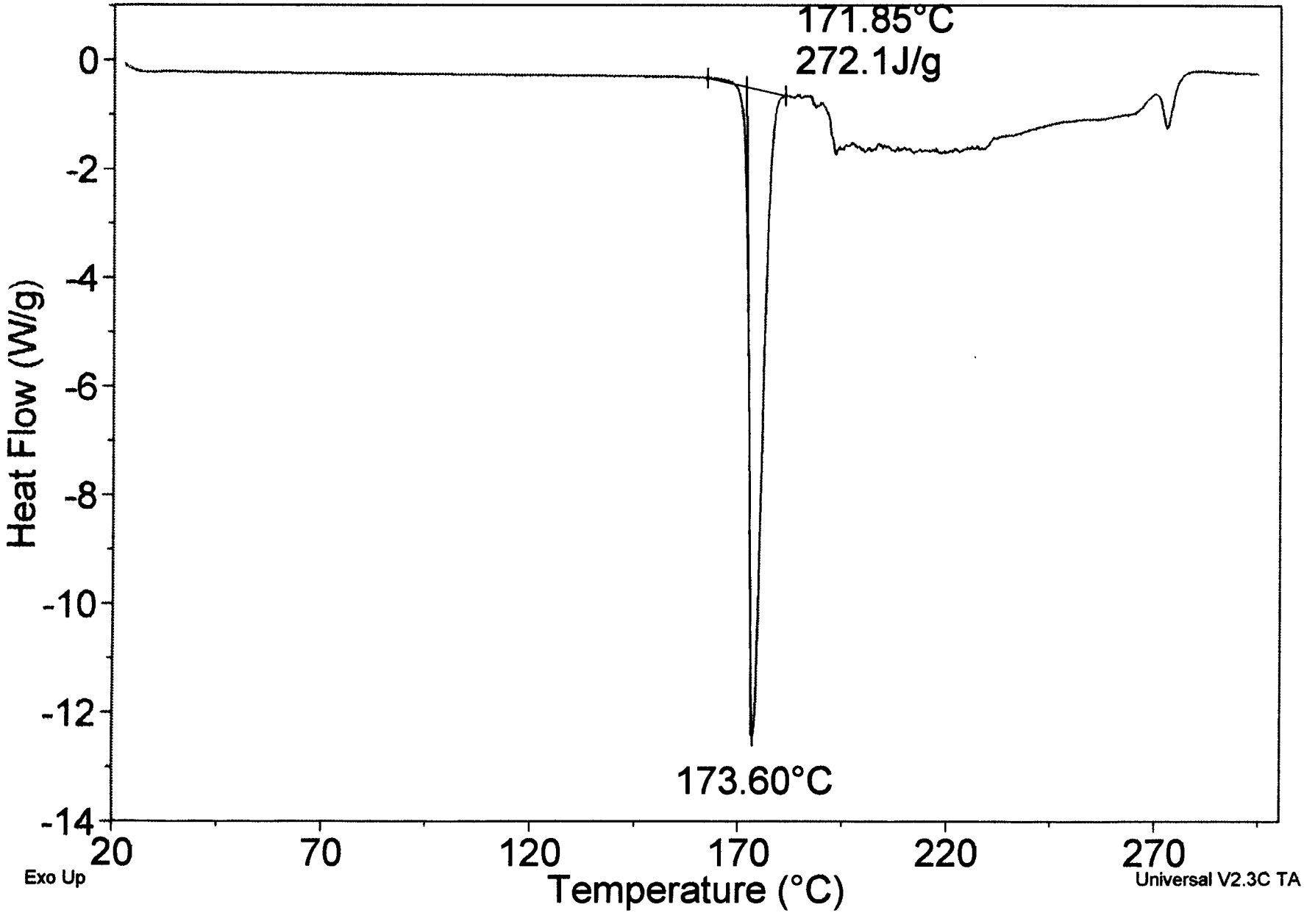
RLS 14 Dec 2001

Cathy Rhodes 21 Jan 2002
COUNTERSIGNATURE DATE

Sample: L-Tartaric Acid
Size: 3.7400 mg
Method: standard

DSC

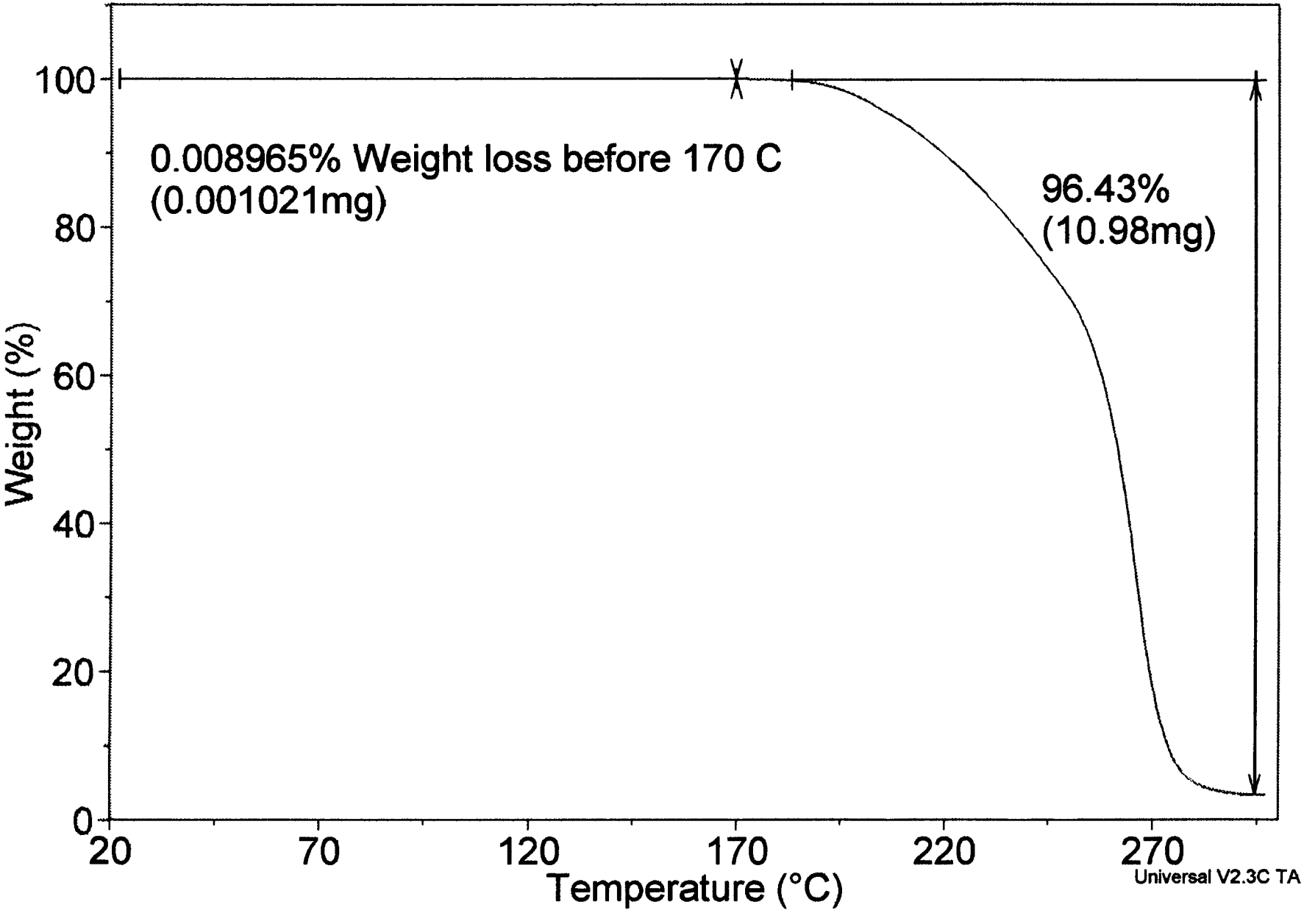
File: C:...\DSC\LTarA_dsc.000
Operator: LS
Run Date: 14-Dec-01 13:24



Sample: L-Tartaric Acid
Size: 11.3880 mg
Method: standard method

TGA

File: C:...\LTartaricA_tga.001
Operator: LS
Run Date: 14-Dec-01 13:22



R. Leigh Shultz
15 Dec 2001

Subject: Equilibrium solubility of L-224715-000T001

The solubility samples (60659-119) were removed from the rotator at 11:00 am. Each of the seven tubes still had solid remaining in it. The solution and some solid from each tube were transferred via Pasteur pipet to a 1.5-mL centrifuge tube, and all of the tubes were centrifuged (10,000 rpm) for 10 minutes to settle the solid from the supernatant. The final pH of each solution was measured using the pH meter (glass electrode), and the pH's of the solutions are reported in the table below. Two dilutions of each sample were done to ensure that each solubility could be measured on the LC. The samples labeled "A" contained 0.10 mL of supernatant from each sample diluted to 5.0 mL with water. The samples labeled "B" contained 0.25 mL of supernatant from each sample diluted to 5.0 mL with water. Six standard solutions (60659-119) and the 14 solubility samples were analyzed by HPLC in the sample set 60659.121.seq using the method L221869_method1.mth.

Solvent	Initial pH	Final pH
Water	6.47	9.37
Saline	5.80	9.31
0.01N HCl	2.01	8.27
20 mM sodium acetate	4.02	8.10
20 mM sodium phosphate	6.05	8.13
20 mM sodium phosphate	7.92	8.97
20 mM sodium carbonate	10.02	10.07

The final pH values of all of the samples are higher than the intial pH's, indicating that the drug likely neutralized the acidic solutions. The solubility samples will need to be analyzed for degradation.

Rebecca Leigh Shultz
15 Dec 2001

RLS 15 Dec 2001

Kathy Rhodes 21 Jan 2002
COUNTERSIGNATURE DATE

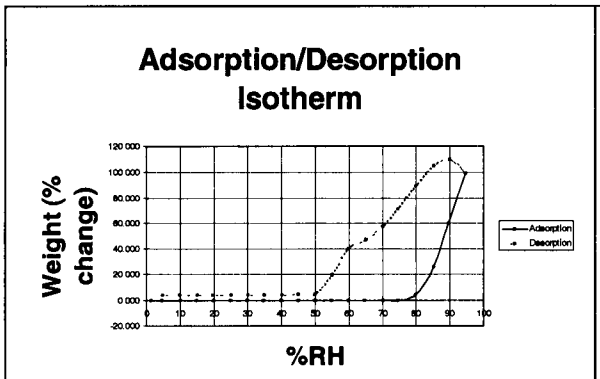
R. Leigh Shultz
17 Dec 2001

Subject: Solution and bulk stability of L-221869 HCl and tosylate salts (four-week time point)

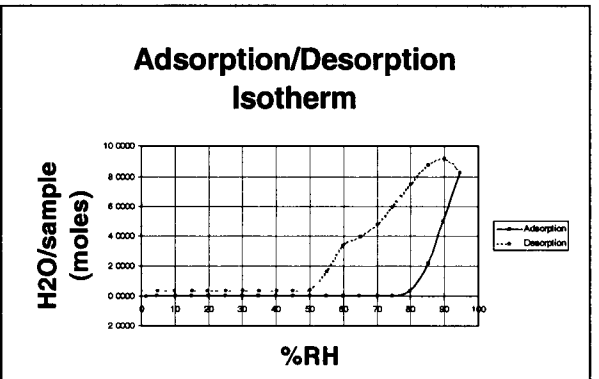
The solution (18 samples for each salt) and bulk (3 samples for each salt) stability samples (60659-101 HCl and OTs) were removed from the stability stations at 9:00 am and allowed to equilibrate at ambient temperature. The solution samples were transferred to clean vials that were compatible with the PE HPLC. The bulk samples were dissolved in 3.0 mL 0.1% phophoric acid each. These solutions were further diluted with 16 mL of 0.1% phosphoric acid each. An aliquot (1.0 mL) from each of the six solutions was transferred to an HPLC vial for analysis. Both the bulk and solution samples were assayed by HPLC in the sample set 60659.122.seq using the method L221869_method1.mth.

Subject: Hygroscopicity of L-tartaric acid (commercial)

Dynamic vapor sorption analysis was used to measure the hydroscopicity of commercial L-tartaric acid (Fisher). The data are shown graphed below, and raw data in table format follow this page as supplementary material.



Vapor sorption isotherm (25 °C) for L-tartaric acid



Vapor sorption isotherm (25 °C) for L-tartaric acid

The data show that L-tartaric acid is very hygroscopic above 80 %RH, absorbing more than 100% of its weight in water. Upon lowering the humidity, the water is retained until 50 %RH, and the material retains ca. 4.3% water weight upon completion of the experiment (equivalent to 0.36 mol water).

Rebecca Leigh Shultz
17 Dec 2001

RLS 17 Dec 2001

Cynthia Peterson 21 Jan 2002
COUNTERSIGNATURE DATE

Supplement to
60659-122

File Name	LTarAcid.lsh		
Experiment	Step Isotherm		
Operator	LS		
Experiment ID	LTarAcid		
Sample Name	L-Tartaric Acid, commercial		
Sample Lot #	Fisher Lot no.975716		
Notes			
	NB60659-119		
Drying Temp	40 ºC		
Heating Rate	5 ºC/min		
Max Drying Time	60 min		
Equil Crit	0.0100 wt % in	2.00 min	
Expt Temp	25 ºC		
Max Equil Time	180 min		
Equil Crit	0.0100 wt % in	5.00 min	
RH Steps	5 to 95 to 5 in 5%RH steps		
Data Logging Interval	2.00 min or	0.0100 wt %	
Expt Started	12/14/2001		
Run Started	13:16:13		

Elap Time min	Weight mg	Weight % chg	Samp Temp deg C	Samp RH %	H2O/sample
58.0	23.999	0.000	25.46	1.46	0.0000
132.1	24.002	0.013	25.26	4.62	0.0011
153.1	24.002	0.014	25.26	10.05	0.0012
178.1	24.002	0.015	25.25	14.87	0.0012
186.1	24.002	0.015	25.25	19.87	0.0013
195.1	24.002	0.015	25.25	24.88	0.0012
204.1	24.002	0.015	25.25	29.97	0.0013
213.1	24.002	0.015	25.25	34.81	0.0013
222.1	24.002	0.015	25.25	39.88	0.0013
231.1	24.003	0.015	25.25	44.95	0.0013
240.1	24.003	0.015	25.25	49.90	0.0013
251.1	24.003	0.016	25.25	54.86	0.0014
260.1	24.003	0.017	25.25	59.80	0.0014
271.1	24.003	0.018	25.25	64.74	0.0015
282.1	24.004	0.021	25.25	69.99	0.0017
291.1	24.006	0.029	25.24	74.57	0.0024
475.1	24.997	4.158	25.24	79.74	0.3467
659.1	30.318	26.329	25.24	85.07	2.1954
843.1	38.319	59.669	25.24	89.52	4.9754
1029.1	47.766	99.036	25.23	94.52	8.2579
1213.1	50.360	109.844	25.23	89.88	9.1592
1397.1	49.183	104.939	25.22	85.15	8.7501
1583.1	45.468	89.461	25.22	80.01	7.4596
1767.1	41.111	71.303	25.22	74.59	5.9455
1953.1	37.704	57.107	25.22	70.02	4.7618
2139.0	35.312	47.143	25.22	65.11	3.9309
2324.6	33.632	40.142	25.22	59.86	3.3472
2510.6	28.698	19.579	25.21	54.93	1.6326
2651.8	25.067	4.451	25.22	49.95	0.3711
2662.7	25.056	4.407	25.22	45.06	0.3674
2676.3	25.045	4.361	25.23	40.12	0.3637
2687.1	25.041	4.343	25.23	35.02	0.3621
2698.1	25.038	4.330	25.22	30.06	0.3611
2709.1	25.035	4.318	25.22	25.08	0.3600
2715.6	25.033	4.308	25.22	19.77	0.3592
2724.1	25.031	4.299	25.22	15.10	0.3585
2731.6	25.029	4.294	25.22	9.91	0.3581
2739.6	25.029	4.291	25.22	4.86	0.3578

Rebecca Segal Shelley
17 Dec 2001

R. Leigh Shultz
20 Dec 2001

Subject: Bulk and solution stability of L-224715-000T001 (two-week time point)

The two week samples for L-224715-000T (60659-110, 111) were removed from the stability stations at 10:00 am and allowed to come to ambient temperature. The solution vials (30 samples) were decrimped, and the solutions were transferred to screw-cap vials for HPLC analysis. No physical changes were observed in any of the bulk samples; each sample was dissolved in 3.0 mL 0.1% phosphoric acid. These solutions were further diluted with 16 mL 0.1% phosphoric acid and shaken to mix. An aliquot of each of these solutions was placed in an HPLC vial for analysis. The solution and bulk samples were assayed in the sample set 60659.123.seq using the method L221869_method1.mth.

Rebecca Leigh Shultz
20 Dec 2001

BSS 20 Dec 2001

COUNTERSIGNATURE 21 Jan 2002
DATE

R. Leigh Shultz
28 Dec 2001

Subject: Solution and bulk stability of L-221869 tartrate and besylate salts (4 week samples)

The four week solution and bulk stability samples for the besylate and tartrate salts were removed from the stability stations at 10:30 am and transferred to the freezer for storage until they could be analyzed by HPLC.

Rebecca Leigh Shultz
28 Dec 2001

RLS 28 Dec 2001

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
02 Jan 2002

Subject: Solution and bulk stability of L-221869 tartrate and besylate salts (4-wk time point)

The 4-wk samples for L-221869 tartrate and besylate were removed from the freezer at 1:30 pm (see 60659-124) and were allowed to come to ambient temperature. Five of the bulk stability samples appeared unchanged; however, the tartrate F4 bulk sample had yellowed slightly. Each bulk sample was dissolved in 3.0 mL 0.1% phosphoric acid; these solutions were then diluted with 15.0 mL 0.1% phosphoric acid. An aliquot of each of these solutions was then transferred to an HPLC vial for analysis. The bulk samples were analyzed with the solution samples in the sample set 60659.125.seq using the method L221869_method1.mth.

Rebecca Leigh Shultz

02 Jan 2002

02 Jan 2002

RLS

Kathl. Rhodes 25 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
03 Jan 2002

Subject: Bulk and solution stability of L-224715-000T001 (four-week timepoint)

The four-week bulk and solution samples (60659-110, 111) were removed from the stability stations at 9:30 am. The bulk sample F4 (80 °C) had yellowed slightly, but all of the other samples appeared unchanged. All of the samples were placed in the freezer (-20 °C) until HPLC analysis could be done.

Subject: Stoichiometry of L-221869 tartaric acid salt

HPLC data from two different concentration of the tartaric acid salt were analyzed against known standards of the tosylate salt (see 60659-116) to determine the stoichiometry of the tartaric acid salt. The data are shown below.

Sample	[salt] (mg/mL)	[869] (mg/mL)	Theo [869], 1:1 salt (mg/mL)	Theo [869], 2:1 salt (mg/mL)
A	0.1	0.065	0.072	0.084
B	0.05	0.033	0.036	0.042

The data indicate that the tartaric acid salt of L-221869 is a 1:1 salt. This conclusion is supported by solution ^{1}H NMR data (M. Palucki). The native pH of a concentrated solution of L-221869 tartrate in water is 3.62 (60659-116), suggesting the presence of a free carboxylic acid group consistent with a 1:1 salt. The correct conversion factor for the tartrate salt is therefore [L-221869] = 0.722[L-221869 tartrate].

Subject: Liberation of L-221869 free base from fumarate salt

A sample of the fumarate salt (L-221869-003X005, 125.70 mg) was weighed into a tared scintillation vial. This corresponds to 109.36 mg of the free base (2.81 x 10-4 mol free base). 0.28 mL of 1 N NaOH will be needed to neutralize this amount of salt. 1.1 mL of water and 3.0 mL of methylene chloride were added to the vial, followed by 0.28 mL of 1 N NaOH. The vial was shaken, and the organic layer was transferred to a new vial. The extraction was repeated with 2 x 3 mL of methylene chloride. The collective methylene chloride solution was dried with molecular sieves and transferred into a 10-mL pear-shaped flask. The volatiles were removed under reduced pressure to leave an oil, which was dissolved in 2 mL 2-propanol and filtered at 0.2 microns into a clean vial. The 2-propanol solution was seeded with L-221869 (JR_C065_C), and the volume of the solution was reduced by half under a stream of nitrogen. The vial was capped and left on the bench at ambient temperature overnight.

Rebecca Leigh Shultz
03 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
04 Jan 2002

Subject: Bulk and solution stability of L-224715-000T001 (4 week time point)

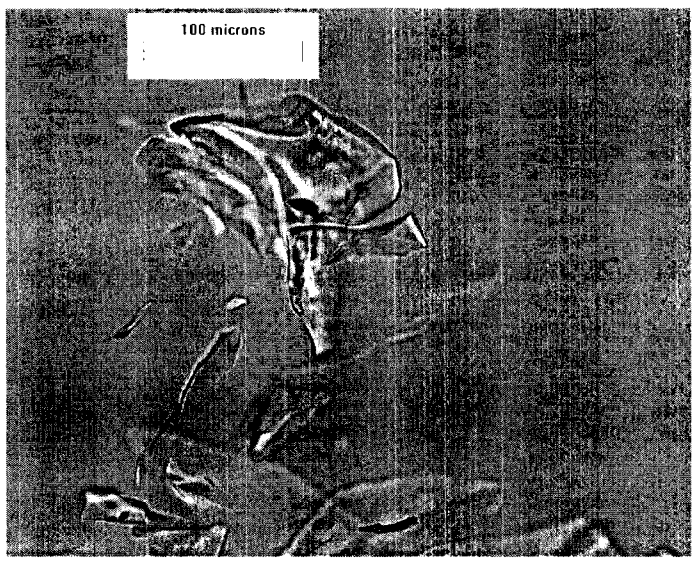
The 4-week bulk and solution samples were removed from the freezer, where they had ben stored since their removal from the stability stations (24 hours). They were allowed to come to ambient temperature. The bulk samples (7) were dissolved in 3.0 mL 0.1% phosphoric acid each; each of these solutions was further diluted with 16 mL of 0.1% phosphoric acid. An aliquot of each of these dilute solutions was placed in an HPLC vial for analysis. The bulk and solution samples were analyzed in the sample set 60659.127.seq using the method L221869_method1.mth.

Subject: Crystallization of L-221869 free base

After sitting overnight, the 2-propanol solution of L-221869 free base had some crystals on the walls of the vial. The volume of the solution was again reduced under a stream of nitrogen, producing a white solid, which was dried for ca. 6 hours in the vacuum oven at 30 °C.

Subject: Initial physical assessment of L-383548 (PPAR)

A microscope image (200X magnification) was obtained of the sodium salt of L-383548-001X001.



The salt is amorphous, showing no defined morphology or birefringence. The average particle size is between 100 – 200 μm.

Rebecca Leigh Shultz
04 Jan 2002

COUNTERSIGNATURE DATE

R. Leigh Shultz
07 Jan 2002

Subject: Crystallization of L-221869 free base

After drying, the vial was weighed (tare = 4.86653 g; weight = 4.93756 g; 71 mg total yield (65%)). Analysis of the white solid by microscopy indicated a birefringent, crystalline material.

Subject: Vapor sorption analysis of L-221869 tartrate salt at 40 °C

In order to determine the hygroscopicity of the tartrate salt, a VTI run was set up using the template std40. Approximately 5 mg of material from the second batch of tartrate was used (NB72061-34). The instrument was left to run overnight.

Subject: Initial chemical stability assessment of L-383548 (PPAR)

A stock solution of L-383548 was made by dissolving a sample of L-383548-001X001 (1.02 mg) in 1.00 mL of water. Aliquots of this stock solution were added to each of 8 vials, according to the table below, to assess the gross chemical stability of L-383548 and to aid in HPLC method development.

Solvent	[L-383548-001X] (mg/mL)	Conditions	Observations
0.1 N NaOH	0.1	80 °C, 18 hours	clear solution
0.1 N NaOH	0.1	80 °C, 18 hours	clear solution
0.1 N HCl	0.1	80 °C, 18 hours	cloudy suspension
0.1 N HCl	0.1	80 °C, 18 hours	cloudy suspension
3% w/v H_2O_2	0.1	25 °C, dark, 18 hours	cloudy suspension
3% w/v H_2O_2	0.1	25 °C, dark, 18 hours	cloudy suspension
water	0.1	immediate assay	clear solution
water	0.1	immediate assay	clear solution

An HPLC method was created (based on the method used for the PPAR compound L-114902) for analysis of L383548 samples.

Method name:	L383548_method1
Column:	Symmetry C18 3 micron, 100 x 4.6 mm
Mobile Phase:	A = 0.1% phosphoric acid, pH = 2.17
	B = acetonitrile
Program:	5 min, isocratic 50% A:50% B
	7 min, linear gradient to 5% A:95% B
Flow Rate:	1.0 mL/min
Injection delay:	5 minutes

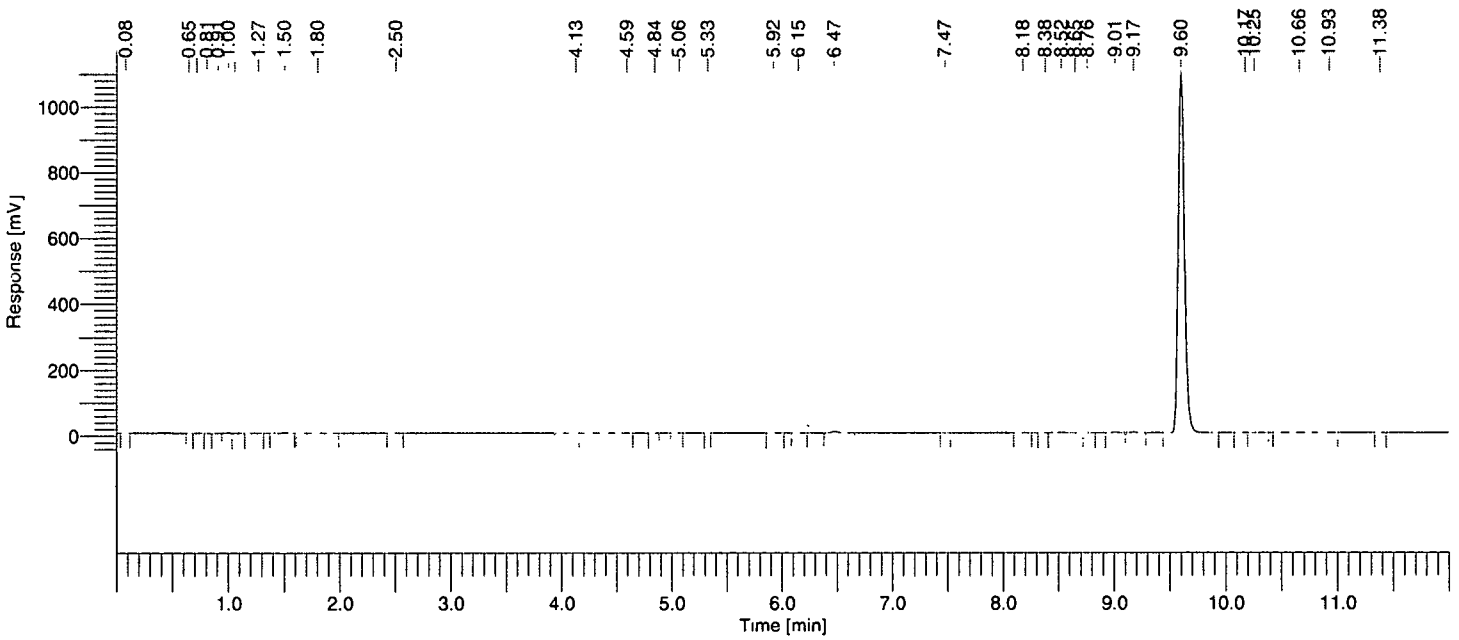
This method was used to analyze the two fresh samples of L-383548 in water (sequence 60659.128.seq). The parent elutes at 9.60 minutes using this method, with 98.80 area % purity (sample 1).

Rebecca Leigh Shultz
07 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

Software Version	: 6.2.0.0.0:B27	Date	: 1/7/02 4:26:59 PM
Reprocess Number	: ry80m-108-pelc: 493		
Sample Name	: L383548_wtr_fresh_1	Data Acquisition Time	: 1/7/02 4:13:36 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L383548\RawData\60659.128.003.rst
Sequence File : D:\Sequences\L383548\60659.128.seq
Sample Notes:
Initial method for L-383548, based on method for L-114902
Column: Symmetry C18 3 micron, 100mm



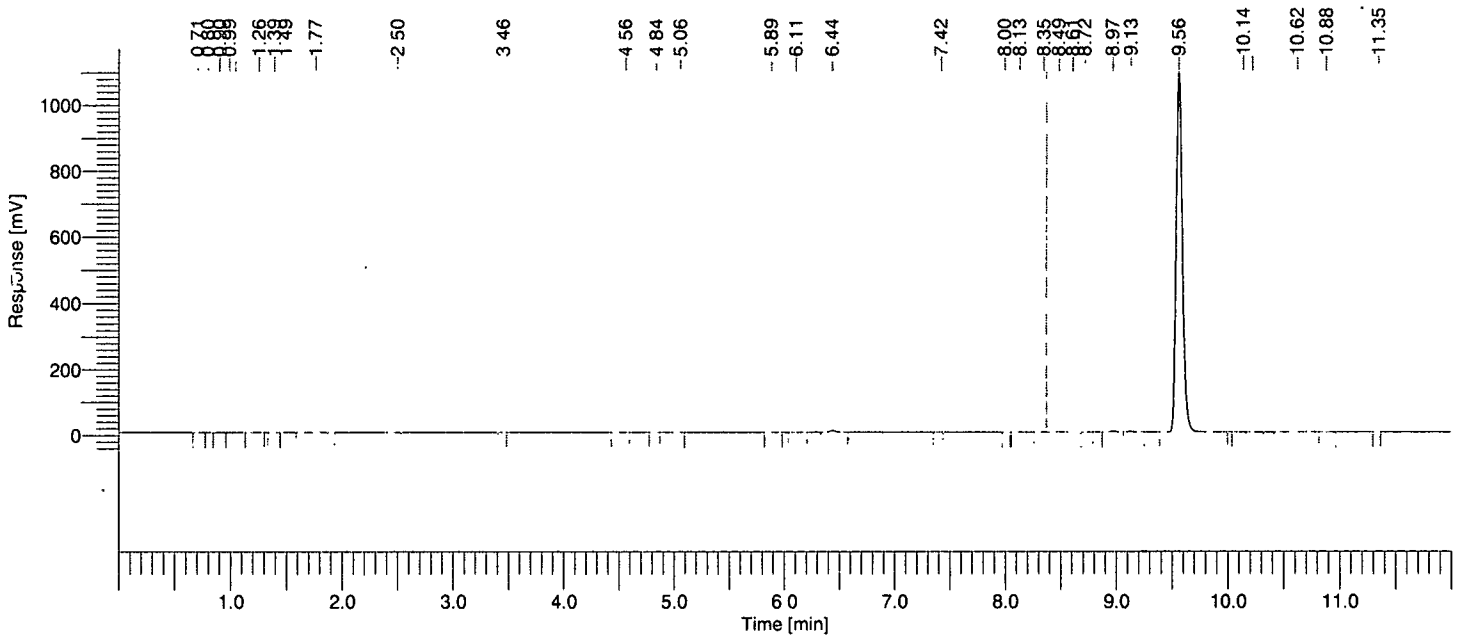
Single Injection Report

Rebecca Leigh Shultz
IB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
19		6.472	80.13	38002.64	8769.60	0.45
23		8.523	105.51	14009.78	3262.57	0.17
26		9.007	111.51	19591.48	5030.76	0.23
27		9.171	113.53	17893.58	4438.28	0.21
28		9.598	118.82	8273061.24	2.13e+06	98.80
31		10.657	131.93	10808.90	915.67	0.13
				8373367.62	2.16e+06	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/7/02 5:04:17 PM
Reprocess Number	: ry80m-108-pelc: 494		
Sample Name	: L383548_wtr_fresh_2	Data Acquisition Time	: 1/7/02 4:31:08 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L383548\RawData\60659.128.004.rst
Sequence File : D:\Sequences\L383548\60659.128.seq
Sample Notes:
Initial method for L-383548, based on method for L-114902
Column: Symmetry C18 3 micron, 100mm



Single Injection Report

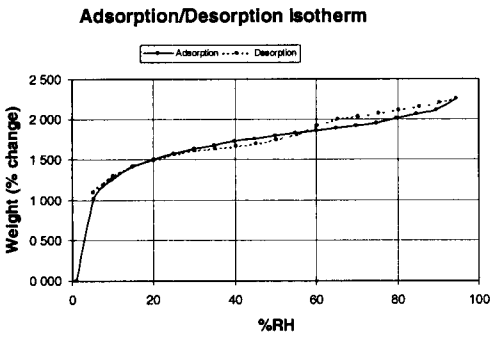
Rebecca Leigh Shultz
JB 60659

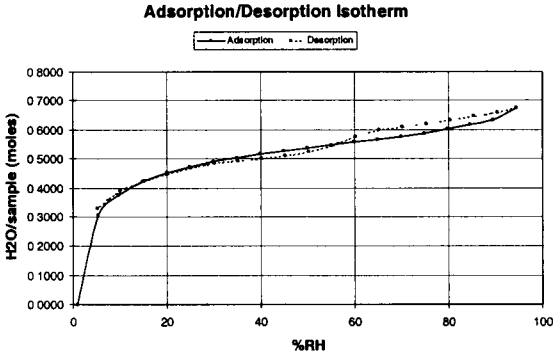
Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
17		6.437	9.10	38050.90	8828.73	0.46
22		8.487	12.00	14188.17	3258.45	0.17
25		8.971	12.68	19514.09	5042.32	0.23
26		9.134	12.91	17967.53	4466.30	0.21
27		9.563	13.52	8270930.03	2.13e+06	98.93
				8360650.71	2.15e+06	100.00

R. Leigh Shultz
08 Jan 2002

Subject: Hygroscopicity of L-221869 tartrate salt at 40 °C

The VTI run (NB60659-128) on the tartrate salt of L-221869 (NB72061-34) had finished, so the material was recovered and the data analyzed. The adsorption/desorption isotherms appear below, and full-size graphs follow this page as supplementary material.





The VTI results are nearly identical to the vapor sorption results from the tartrate salt at 25 °C (data from Y. Wang, Physical Measurements). The data suggest the existence of a stable hemihydrate for the tartrate salt. Revisiting the results obtained for the stoichiometry of the tartrate salt (NB60659-126) and recalculating the concentration of L-221869 parent using the conversion factor for a 1:1 tartrate hemihydrate (FB = 0.710·Tar, MW(tartrate salt) = 548.441) give the following results:

Calculation error noted and corrected 17 Jan 2002 RLS

Sample	[salt] (mg/mL)	[869] (mg/mL)	Theo [869], 1:1 salt (mg/mL)	Theo [869], 2:1 salt (mg/mL)
A	0.1	~~0.071~~ 0.065	~~0.072~~ 0.071	0.084
B	0.05	~~0.036~~ 0.033	0.036	0.042

suggest
These results ~~confirm~~ that the tartrate salt exists as a hemihydrate under ambient conditions.
Results are likely lower than theoretical values due to inaccuracy in [salt]. RLS

Subject: Initial chemical stability assessment of L-383548-001X001

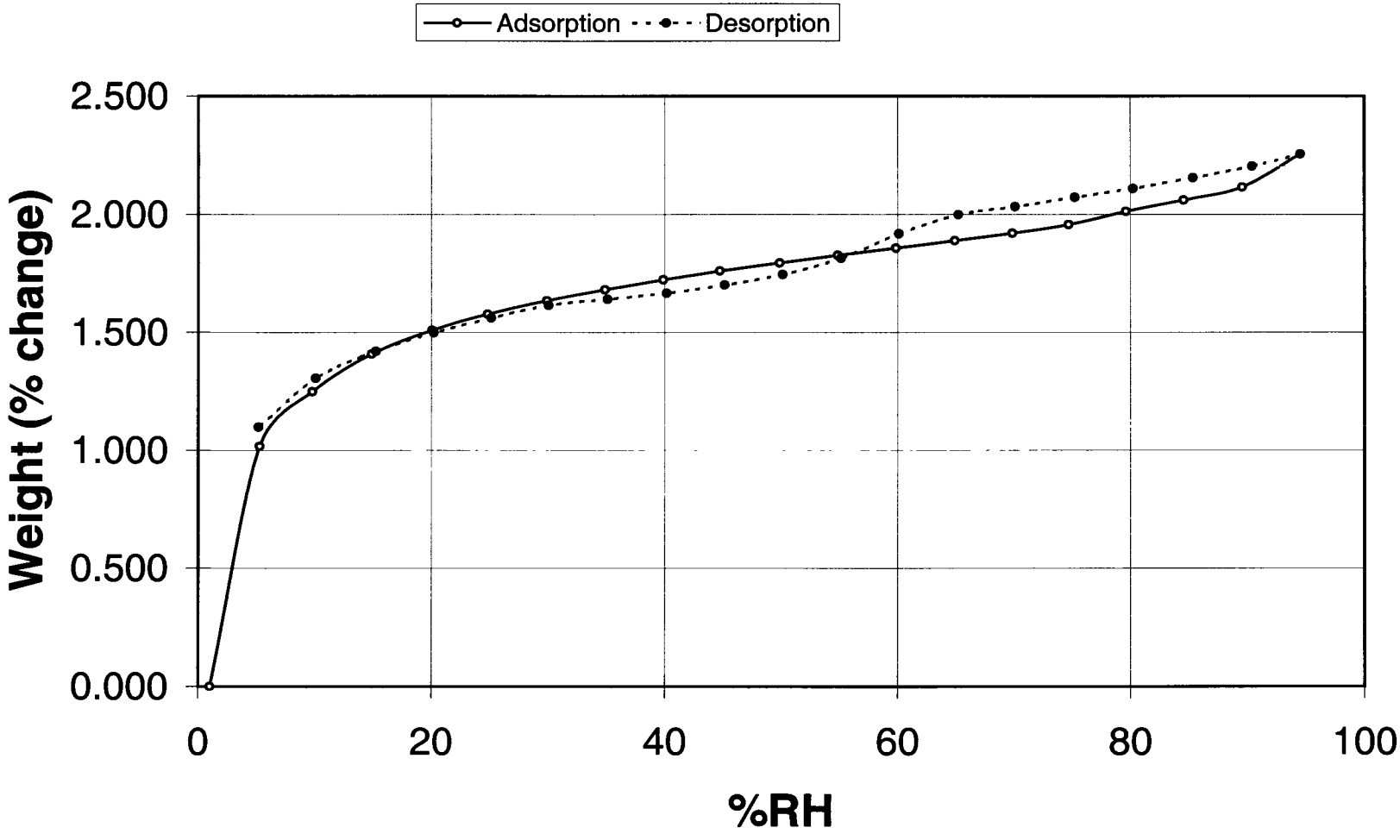
The six stressed samples of L-383548 (NB60659-128) were removed from the stability stations at 10:30 am. The samples in 0.1 N NaOH were neutralized by the addition of 0.09 mL 1 N HCl, causing a small amount of solid to precipitate. The samples in 0.1 N HCl were neutralized by the addition of 0.09 mL 1 N NaOH, dissolving the precipitate already in the vials. These observations suggest that the solubility of L-383548 is pH-dependent. The samples were analyzed by HPLC in the sample set 60659.129.seq using the method L383548_method1 described on page 128 of this notebook.

The HPLC results for the stressed samples indicate that L-383548 is relatively stable (very few degradates) under stress in acid and base. The samples in peroxide do show some degradation (one major product). This compound does not have the liabilities for development that L-114902 did.

Rebecca Leigh Shultz
08 Jan 2002

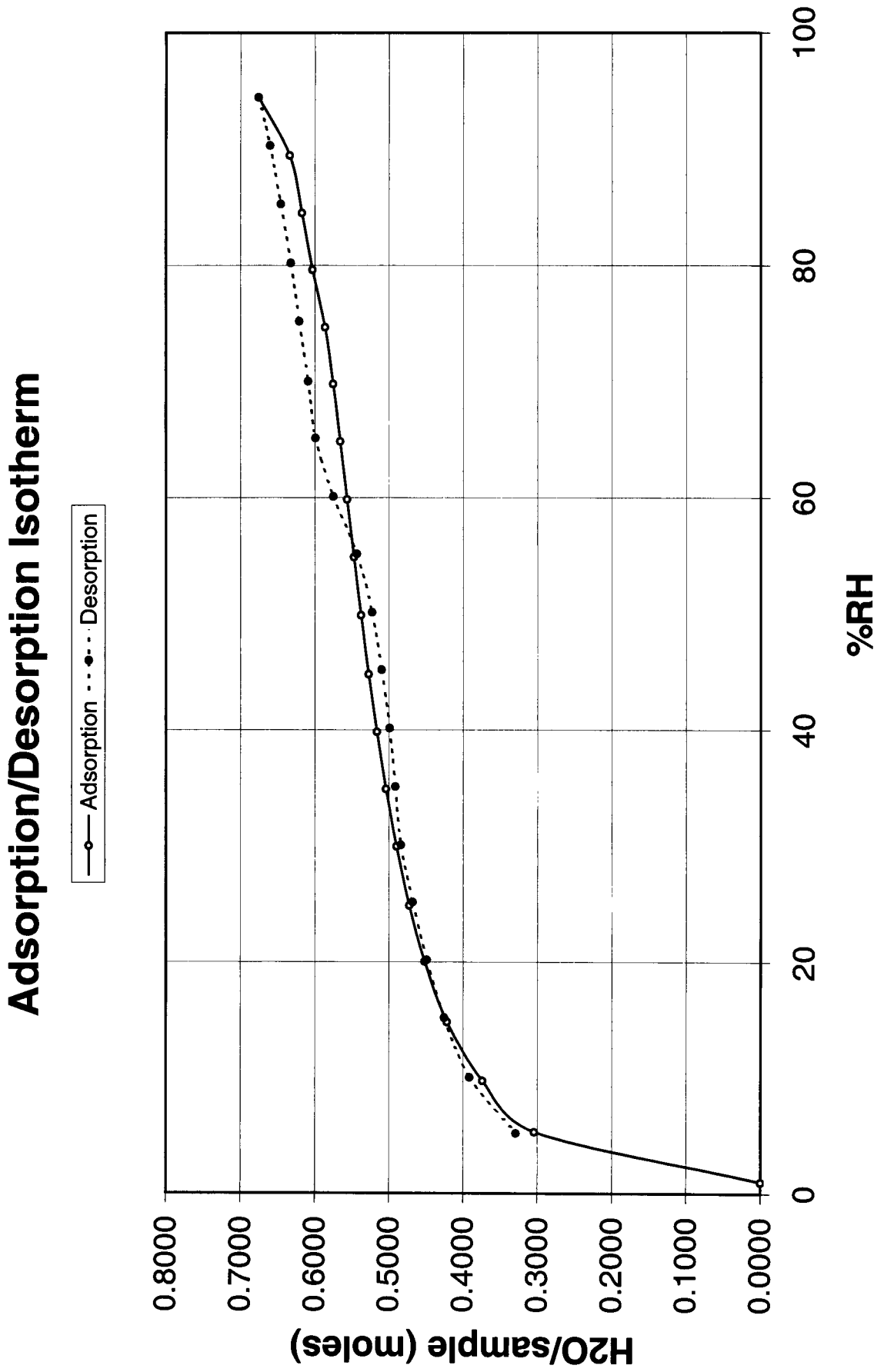
21 Jan 2002
COUNTERSIGNATURE DATE

Adsorption/Desorption Isotherm



60659-129

60659-129


Adsorption/Desorption Isotherm
Adsorption
Desorption
H2O/sample (moles)
0.8000
0.7000
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
%RH
0
20
40
60
80
100

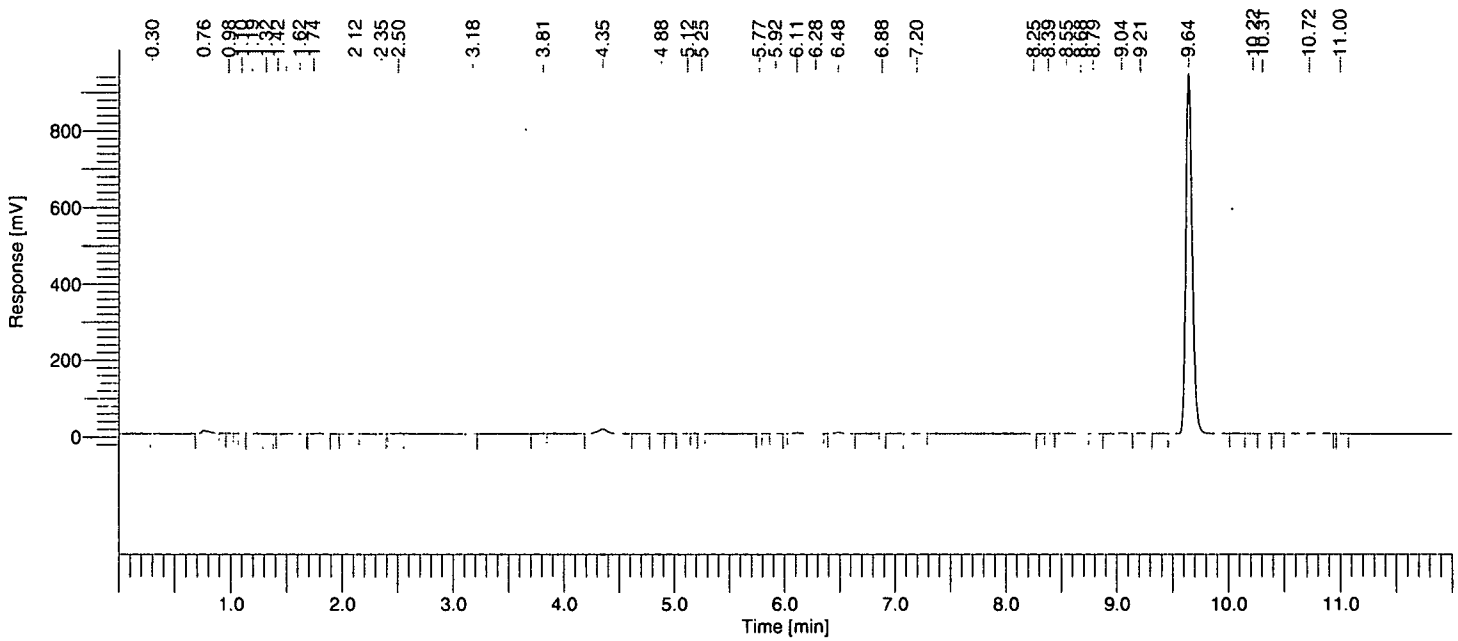
60659-129

File Name	869Tar40.lsh		
Experiment	Step Isotherm		
Operator	LS		
Experiment ID	869Tar40		
Sample Name	L-221869 tartrate		
Sample Lot #	batch 2 (72061-34)		
Notes	DP-IV		
	NB60659-128		
Drying Temp	60 ºC		
Heating Rate	5 ºC/min		
Max Drying Time	60 min		
Equil Crit	0.0100 wt % in	2.00 min	
Expt Temp	40 ºC		
Max Equil Time	180 min		
Equil Crit	0.0100 wt % in	5.00 min	
RH Steps	5 to 95 to 5 in 5% steps		
Data Logging Interval	2.00 min or	0.0100 wt %	
Expt Started	01/07/2002		
Run Started	14:24:22		

Elap Time min	Weight mg	Weight % chg	Samp Temp deg C	Samp RH %	H2O/sample
123.6	5.074	0.000	40.50	1.01	0.0000
181.8	5.126	1.016	40.13	5.30	0.3043
199.8	5.138	1.248	40.12	9.76	0.3740
218.6	5.146	1.408	40.12	14.87	0.4219
235.6	5.151	1.508	40.12	20.07	0.4520
245.2	5.155	1.577	40.12	24.87	0.4726
256.6	5.157	1.634	40.12	29.94	0.4898
269.6	5.160	1.682	40.12	34.89	0.5039
282.6	5.162	1.723	40.12	39.86	0.5163
295.6	5.164	1.760	40.12	44.81	0.5276
308.7	5.166	1.794	40.12	49.91	0.5376
323.7	5.167	1.827	40.12	54.89	0.5475
338.7	5.169	1.857	40.12	59.87	0.5565
355.7	5.170	1.888	40.12	64.90	0.5659
372.7	5.172	1.920	40.12	69.91	0.5754
385.7	5.174	1.956	40.12	74.73	0.5862
398.7	5.177	2.013	40.12	79.64	0.6032
411.7	5.179	2.060	40.12	84.56	0.6173
426.7	5.182	2.115	40.12	89.52	0.6339
443.0	5.189	2.255	40.12	94.51	0.6758
455.2	5.186	2.204	40.12	90.39	0.6604
468.3	5.184	2.155	40.12	85.34	0.6457
483.3	5.181	2.109	40.11	80.24	0.6321
496.3	5.180	2.072	40.11	75.25	0.6209
513.3	5.178	2.032	40.11	70.14	0.6091
528.3	5.176	1.999	40.11	65.20	0.5990
544.5	5.172	1.918	40.11	60.12	0.5749
557.3	5.167	1.814	40.11	55.17	0.5435
572.3	5.163	1.745	40.11	50.17	0.5228
585.3	5.161	1.701	40.11	45.20	0.5099
598.3	5.159	1.666	40.11	40.16	0.4992
611.3	5.158	1.640	40.11	35.10	0.4915
624.4	5.156	1.615	40.11	30.07	0.4839
635.4	5.154	1.561	40.11	25.17	0.4679
652.4	5.150	1.498	40.11	20.22	0.4490
669.4	5.147	1.420	40.11	15.22	0.4254
680.6	5.141	1.306	40.11	10.05	0.3913
695.2	5.130	1.098	40.11	5.20	0.3291

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:12 PM
Reprocess Number	: ry80m-108-pelc: 495		
Sample Name	: stressed 0.1 N NaOH 80C A	Data Acquisition Time	: 1/8/02 11:14:57 AM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L383548\RawData\60659.129.002.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



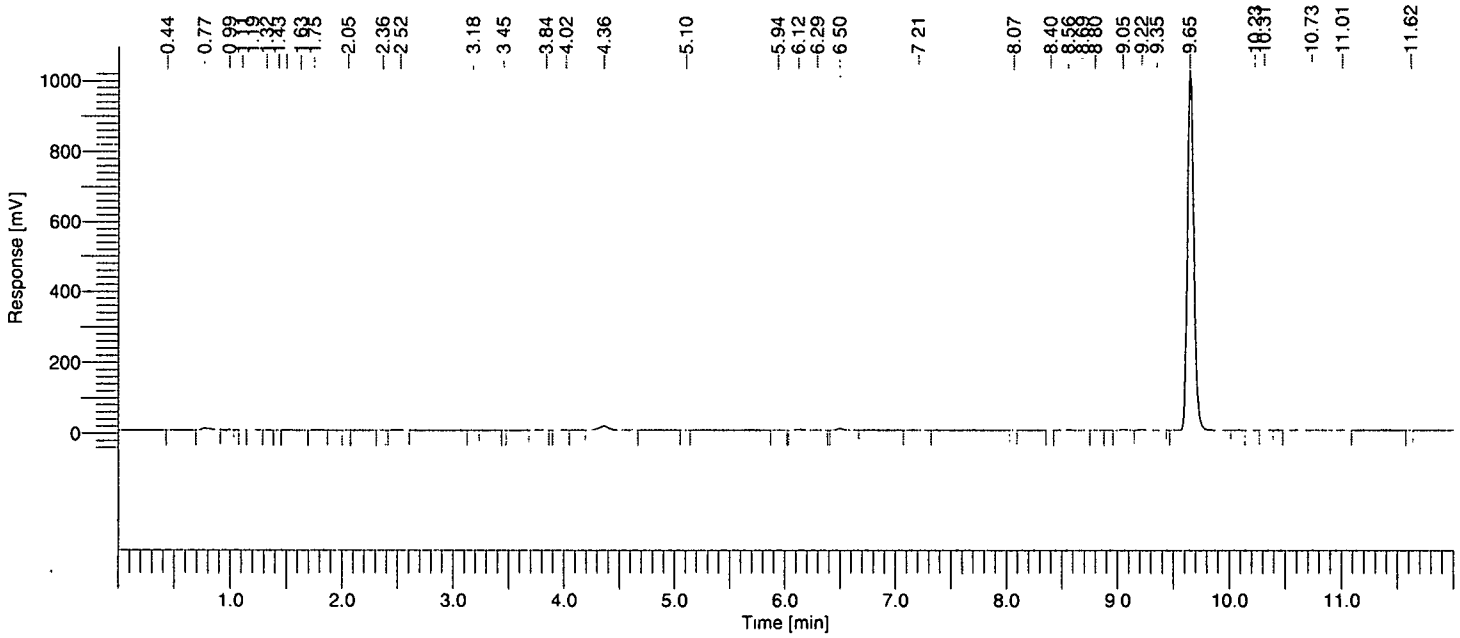
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
2		0.759	2.55	69357.19	15924.10	0.92
16		4.350	14.63	140823.76	23354.75	1.86
22		6.111	20.55	20496.32	4433.19	0.27
24		6.485	21.81	30465.02	6956.05	0.40
32		9.042	30.41	16251.75	4134.08	0.21
33		9.212	30.99	14184.26	3660.02	0.19
34		9.643	32.43	7272987.71	1.83e+06	96.15
				7564566.00	1.89e+06	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:13 PM
Reprocess Number	: ry80m-108-pelc: 496		
Sample Name	: stressed 0.1 N NaOH 80C B	Data Acquisition Time	: 1/8/02 11:32:29 AM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

Result File : D:\Projects\L383548\RawData\60659.129.003.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



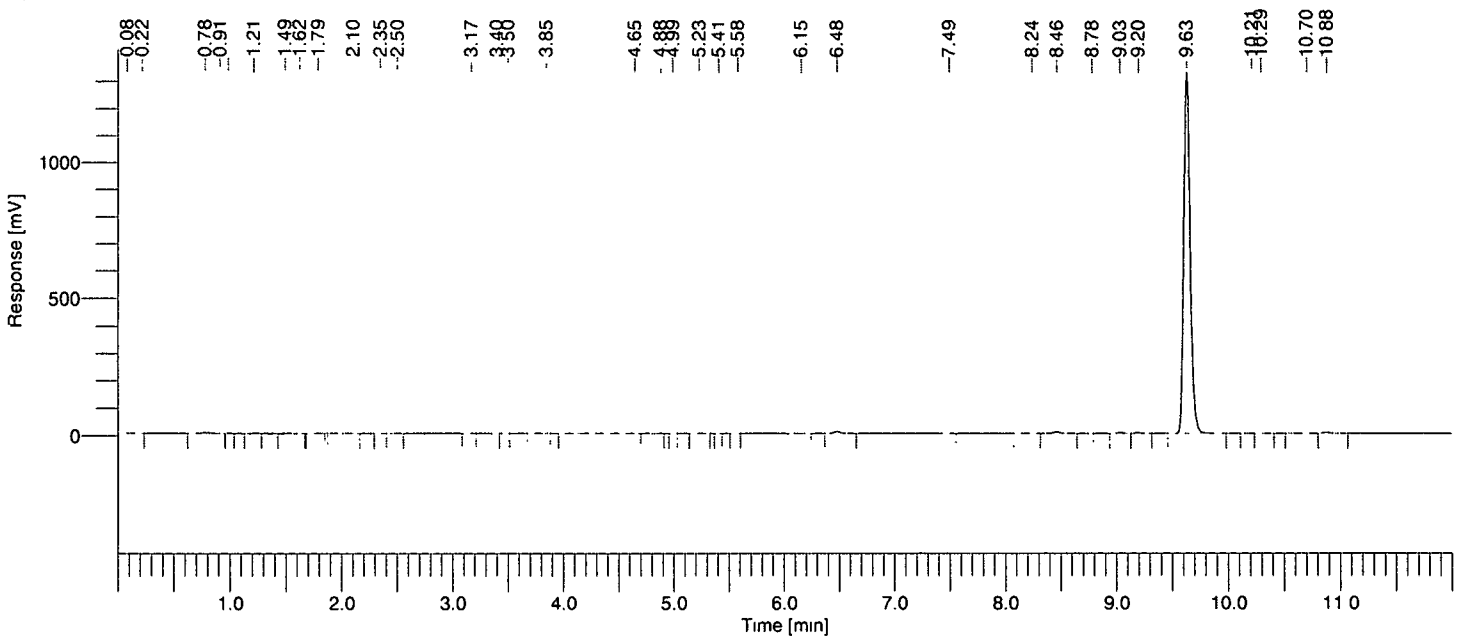
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
2		0.767	1.75	53971.74	11165.52	0.66
18		4.360	9.96	146536.08	23359.80	1.78
21		6.122	13.98	21946.94	4511.67	0.27
23		6.496	14.84	39682.33	9014.21	0.48
27		8.558	19.55	10143.08	2371.24	0.12
30		9.048	20.66	18334.44	4562.48	0.22
31		9.216	21.05	16841.76	4097.40	0.20
33		9.647	22.03	7921486.67	1.99e+06	96.14
36		10.735	24.52	10884.31	887.49	0.13
				8239827.34	2.05e+06	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:14 PM
Reprocess Number	: ry80m-108-pelc: 497		
Sample Name	: stressed 0.1 N HCl 80C A	Data Acquisition Time	: 1/8/02 11:50:01 AM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/4	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

Result File : D:\Projects\L383548\RawData\60659.129.004.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



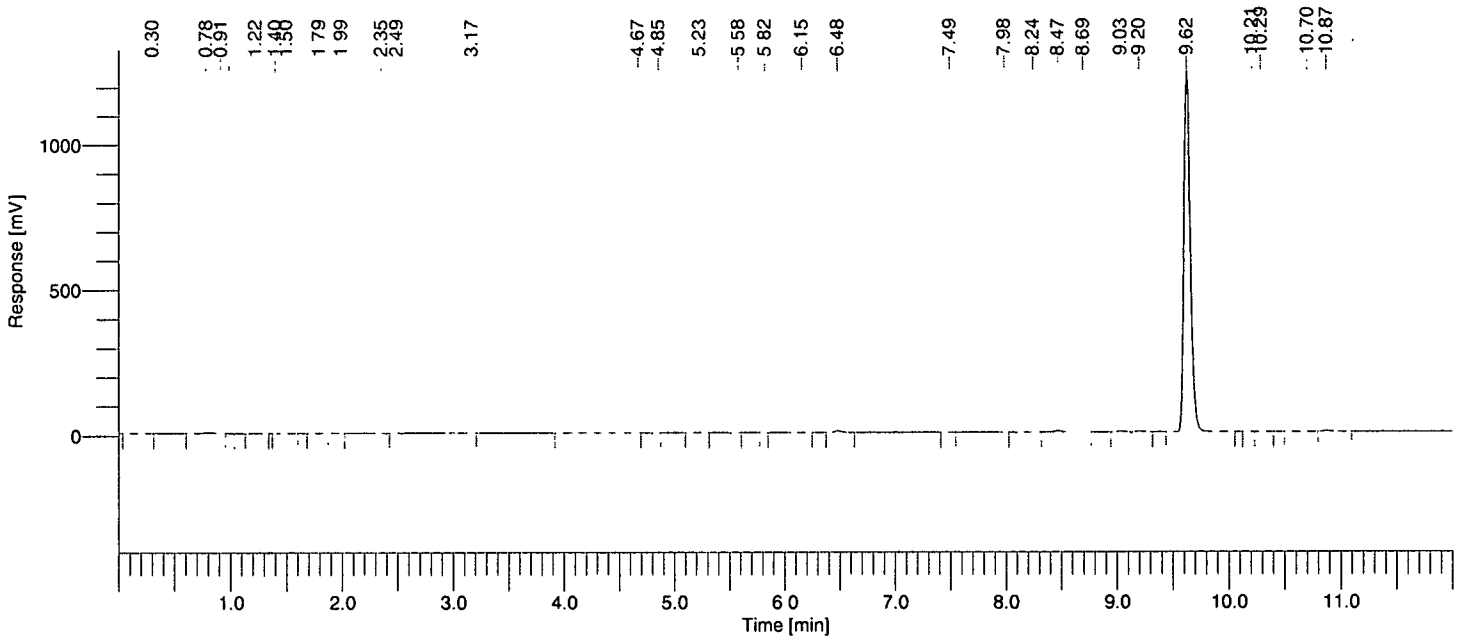
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
3		0.778	9.66	38310.54	6666.39	0.36
24		6.479	80.41	59631.78	13523.86	0.57
27		8.464	105.04	58391.98	11480.76	0.56
29		9.030	112.06	21656.26	5481.14	0.21
30		9.197	114.13	20130.85	4931.42	0.19
31		9.628	119.48	10265771.48	2.58e+06	97.77
35		10.879	135.00	36116.17	8134.66	0.34
				10500009.06	2.63e+06	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:15 PM
Reprocess Number	: ry80m-108-pelc: 498		
Sample Name	: stressed 0.1 N HCl 80C B	Data Acquisition Time	: 1/8/02 12:07:33 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/5	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 4		

Result File : D:\Projects\L383548\RawData\60659.129.005.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



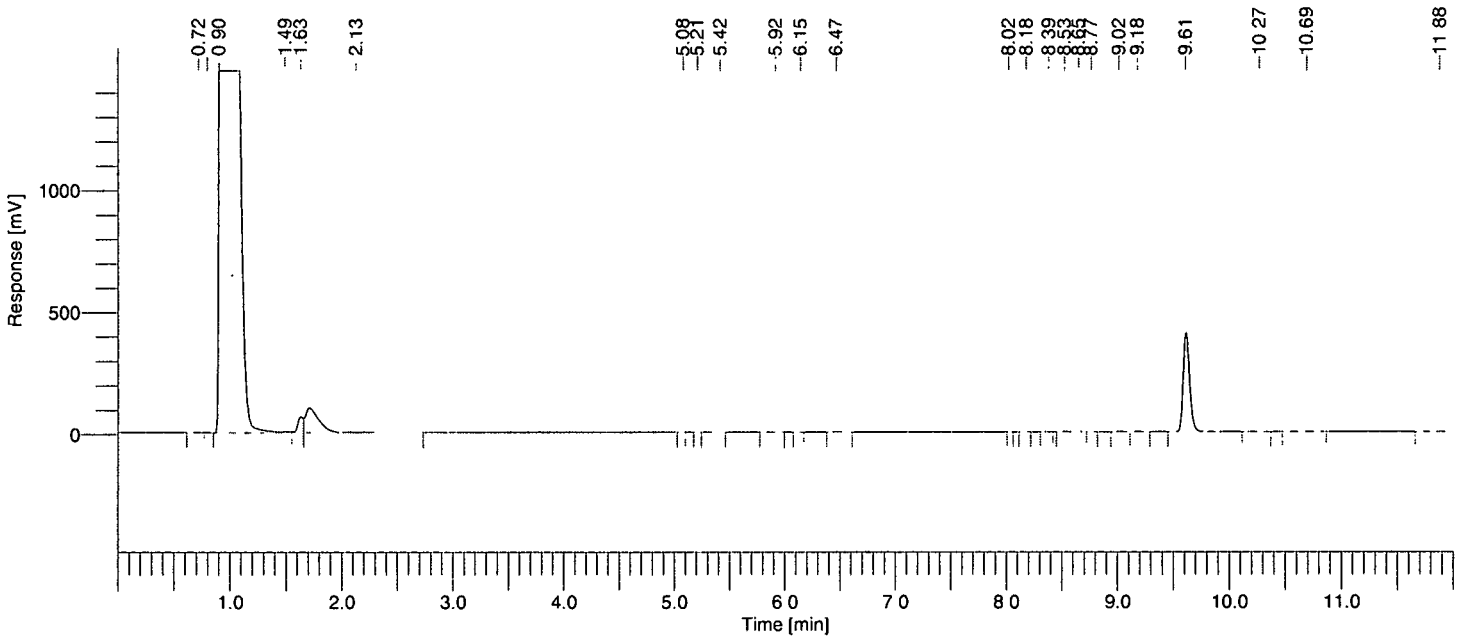
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
2		0.779	2.61	34344.94	6422.08	0.35
19		6.478	21.72	47672.91	10839.83	0.48
23		8.467	28.39	46755.53	8910.53	0.47
25		9.031	30.28	21012.42	5331.16	0.21
26		9.196	30.83	19651.36	4819.75	0.20
27		9.625	32.27	9649397.40	2.43e+06	97.95
31		10.872	36.45	32306.22	7131.34	0.33
				9851140.77	2.47e+06	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:16 PM
Reprocess Number	: ry80m-108-pelc: 499		
Sample Name	: stressed 3% H2O2 25C A	Data Acquisition Time	: 1/8/02 12:25:05 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/6	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 5		

Result File : D:\Projects\L383548\RawData\60659.129.006.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



Single Injection Report

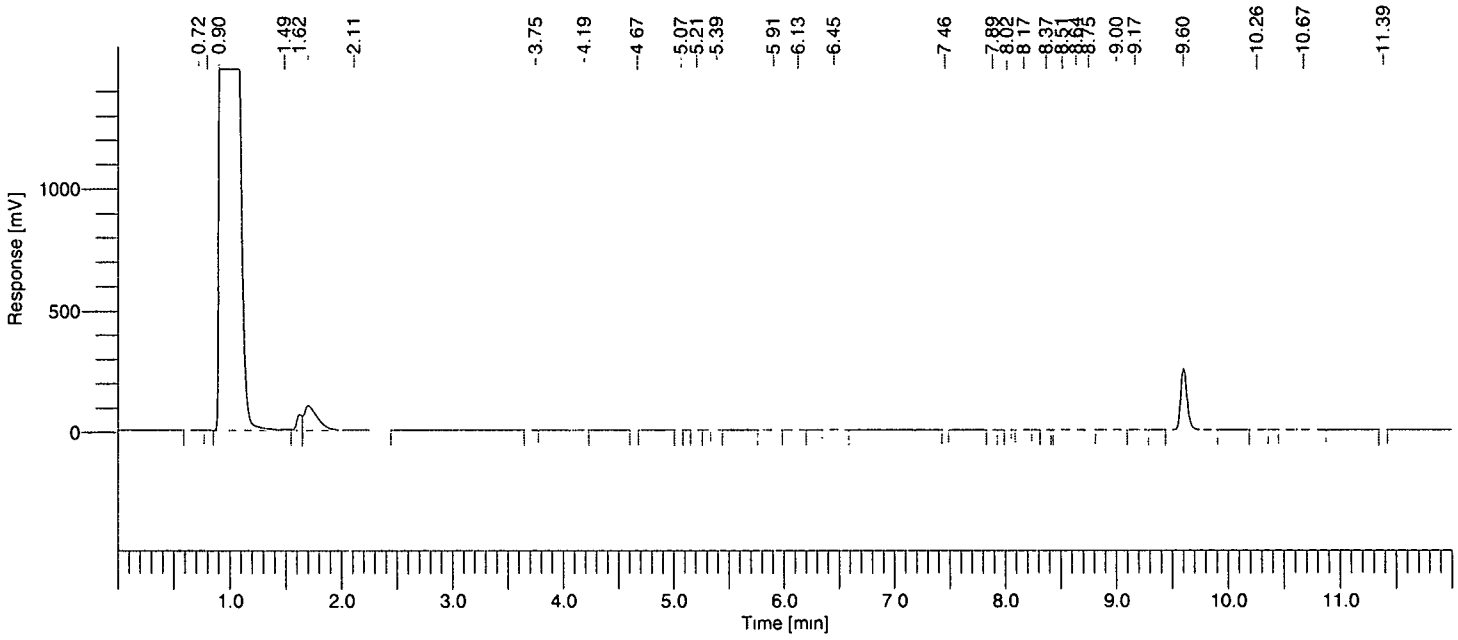
Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
3		0.903	1.25	38473909.00	2.89e+06	87.75
4		1.494	2.07	75621.55	8315.54	0.17
5		1.633	2.27	417040.55	126891.49	0.95
6		1.711	2.37	1660110.88	197481.10	3.79
7		2.129	2.95	56301.13	2543.07	0.13
13		6.468	8.97	16268.38	3695.33	0.04
20		9.017	12.51	10064.15	2563.47	0.02
21		9.182	12.74	10796.37	2671.71	0.02
22		9.613	13.34	3123458.18	791750.65	7.12
				43843570.20	4.02e+06	100.00

Warning -- Signal level out-of-range in peak

Software Version	: 6.2.0.0.0:B27	Date	: 1/8/02 1:15:17 PM
Reprocess Number	: ry80m-108-pelc: 500		
Sample Name	: stressed 3% H2O2 25C B	Data Acquisition Time	: 1/8/02 12:42:38 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/7	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 6		

Result File : D:\Projects\L383548\RawData\60659.129.007.rst
Sequence File : D:\Sequences\L383548\60659.129.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

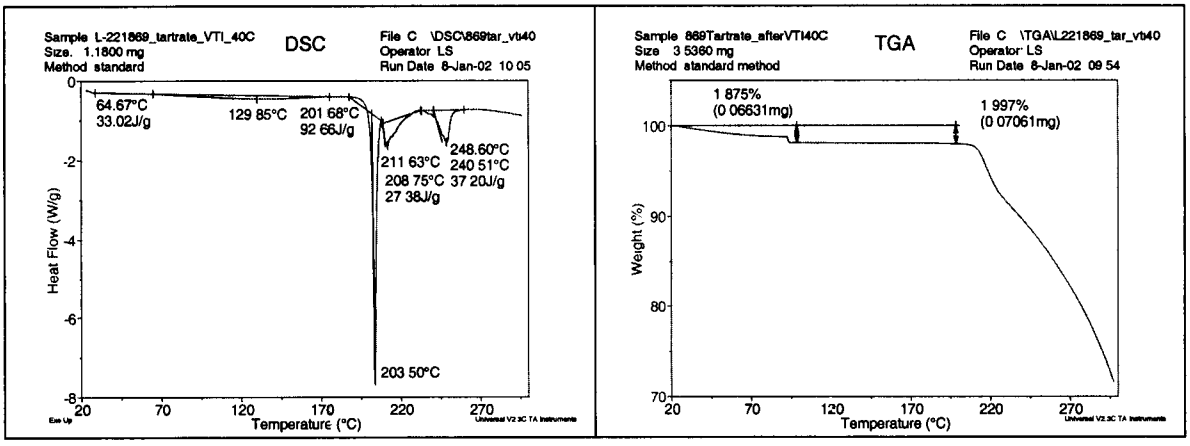
Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
3		0.903	1.25	38320051.04	2.89e+06	90.33
4		1.490	2.07	69073.41	7888.58	0.16
5		1.623	2.25	406400.85	126059.93	0.96
6		1.701	2.36	1649120.26	197420.39	3.89
7		2.112	2.93	28243.34	1855.09	0.07
16		6.454	8.95	11559.63	2616.73	0.03
27		9.600	13.31	1939479.55	492521.69	4.57
				42423928.08	3.71e+06	100.00

Warning -- Signal level out-of-range in peak

R. Leigh Shultz
08 Jan 2002

Subject: Thermal analysis of L-221869 tartrate salt (post-VTI)

The solid from the VTI run was analyzed by DSC and TGA.



The solid melts at 203.5 °C. As shown in the graph of the TGA data, it abruptly loses weight at 100 °C (1.875% loss). The theoretical weight loss for a hemihydrate is 1.64%.

Subject: Bulk photostability of L-224715-000T001

In order to assess the bulk photostability of the free base of L-224715, two samples were prepared. Sample L contained 1.53 mg of material; sample D contained 1.34 mg of material. Both samples were placed in test tubes and capped. The dark sample (D) was wrapped tightly in aluminum foil; both samples were placed in the Rayonet for 17 hours under fluorescent light. They will be dissolved and assayed by HPLC when the irradiation is complete.

Rebecca Leigh Shultz
08 Jan 2002

RLS 08 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
09 Jan 2002

Subject: pKa determination for L-221869 free base

Mass (mol) of L-221869: 39.22mg (1.007×10^{-4} mol)

Volume of water used: 50.0 mL

Mass of added KBr: 0

Concentration of HCl titrant: 0.10 N

Volume HCl added (total, mL)	pH of solution
0	9.32
0.100	8.85
0.200	8.59
0.300	8.38
0.400	8.20
0.500	8.04
0.600	7.86
0.700	7.65
0.800	7.38
0.900	6.90
1.000	4.32
1.100	3.60
—	—
—	—

Rebecca Leigh Shultz
09 Jan 2002

Scott Pfeiffer 21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
09 Jan 2002

Subject: Bulk photostability of L-224715-000T001

The bulk photostability samples were removed from the Rayonet and dissolved in 3.0 mL 0.1% phosphoric acid. These solutions were then diluted with 16.0 mL 0.1% phosphoric acid each and shaken gently to mix. An aliquot (1.0 mL) of each solution was placed in an HPLC vial for analysis. The samples were analyzed in the sample set 60659.132.seq using the method L221869_method1.

The dark sample has an area % parent of 96.32; the light sample has an area % parent of 96.80. No differences are observed between the two samples, and no degradation of the bulk is observed in either sample. L-224715-000R is photostable under fluorescent light for at least one week.

Subject: pKa determination for L-221869-000R

A sample of L-221869 free base (60659-127, 39.22 mg) was weighed into a 100-mL beaker and dissolved in 50.0 mL of deionized, boiled water. The solution was sonicated for 5 minutes to fully dissolve the solid and degas the solution. A small magnetic stir bar was then added to the solution, the beaker was placed on a magnetic stir plate and stirred, and a glass pH electrode was immersed in the solution. pH measurements were taken as 0.1 mL aliquots of 0.10 N HCl were added to the solution, producing the raw data shown on page 131 of this notebook. The data was analyzed using Microsoft Excel and the method discussed on page 22 of this notebook, producing a pKa value for L-221869 of 7.98 with a scatter of 0.13. The spreadsheet is printed on page 60659-133.

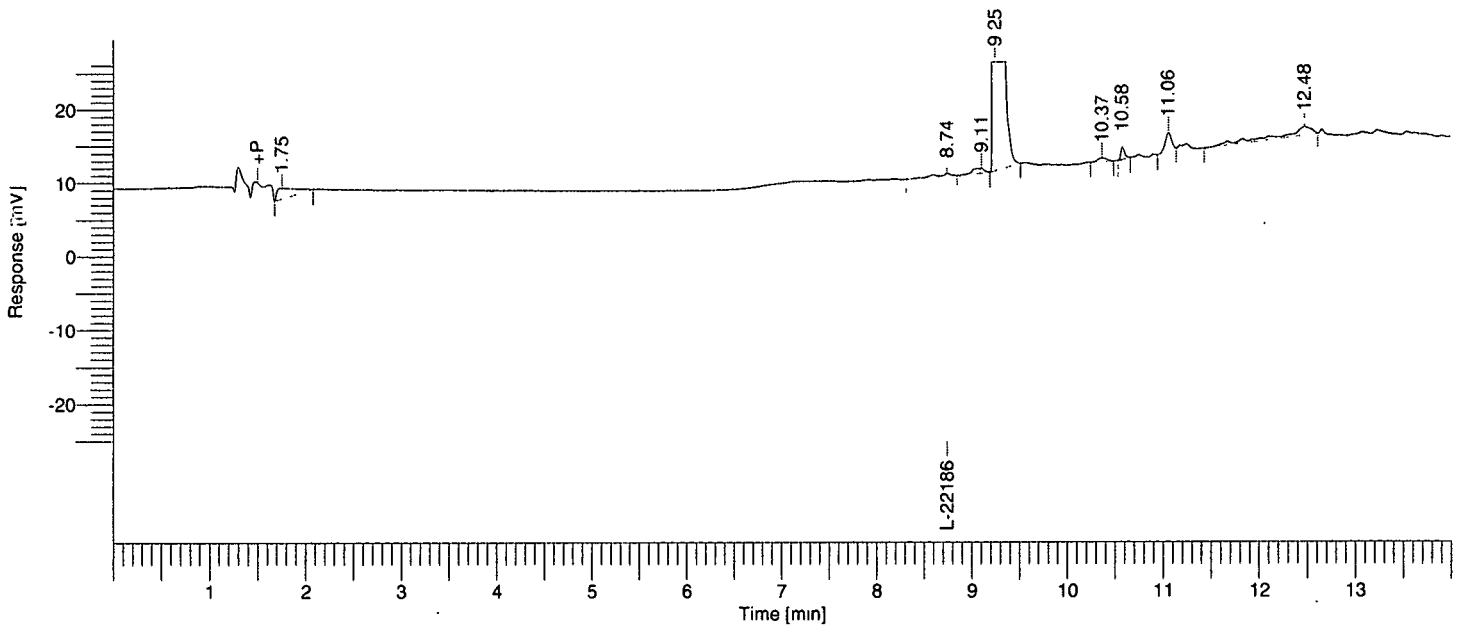
Rebecca Leigh Shultz
09 Jan 2002

RLS 09 Jan 2002

Cynthia Rhodes 21 Jan 2002
COUNTERSIGNATURE DATE

Software Version	: 6.2.0.0.0:B27	Date	: 1/9/02 12:54:22 PM
Reprocess Number	: ry80m-108-pelc: 501		
Sample Name	: L224715 bulk dark	Data Acquisition Time	: 1/9/02 11:20:55 AM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L224715\RawData\60659.132.003.rst
Sequence File : D:\Sequences\L224715\60659.132.seq
Sample Notes:
L-221869 method 1 (J. Remenar)
Metasil AQ column
12 minute run time
phosphoric acid/ACN



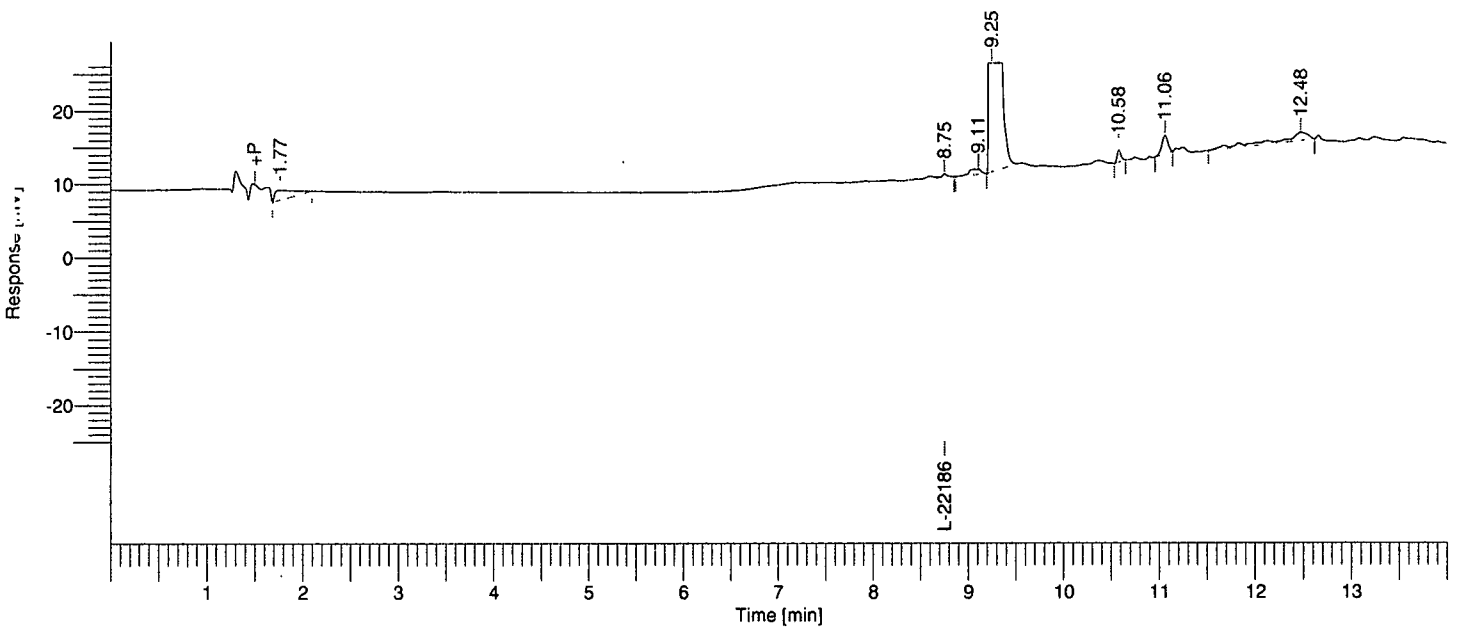
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.754	0.20	37066.87	2732.53	1.14
3		9.107	1.04	11176.47	1308.18	0.35
4		9.249	1.06	3120086.74	847484.47	96.32
7		11.060	1.26	21234.78	4790.94	0.66
8		12.477	1.43	49576.06	2208.70	1.53
				3239140.91	858524.83	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/9/02 12:54:23 PM
Reprocess Number	: ry80m-108-pelc: 502		
Sample Name	: L224715 bulk light	Data Acquisition Time	: 1/9/02 11:40:27 AM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

Result File : D:\Projects\L224715\RawData\60659.132.004.rst
Sequence File : D:\Sequences\L224715\60659.132.seq
Sample Notes:
L-221869 method 1 (J. Remenar)
Metasil AQ column
12 minute run time
phosphoric acid/ACN



Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.766	0.20	35277.75	2528.89	0.95
3		9.112	1.04	12452.61	1492.30	0.34
4		9.250	1.06	3592599.38	949195.94	96.80
6		11.062	1.26	21302.85	4806.88	0.57
7		12.478	1.43	49638.10	2158.01	1.34
				3711270.68	960182.02	100.00

pKa Determination							
Compound: L-221869-000R							
Solvent: water (100%)							
Titrant: 0.1 N HCl							
Titrant (mL)	**pH**	**C(0) minus col D**	**C(0) dim. by tenths**	**C/D**	**log(col E)**	**pKa (B + F)**	**Ka**
0.00	9.32	0.0E+00	1.0E-04	0.000			
0.10	8.85	1.0E-05	9.0E-05	0.111	-0.95	7.90	1.3E-08
0.20	8.59	2.0E-05	8.0E-05	0.250	-0.60	7.99	1.0E-08
0.30	8.38	3.0E-05	7.0E-05	0.429	-0.37	8.01	9.7E-09
0.40	8.20	4.0E-05	6.0E-05	0.667	-0.18	8.02	9.5E-09
0.50	8.04	5.0E-05	5.0E-05	1.000	0.00	8.04	9.1E-09
0.60	7.86	6.0E-05	4.0E-05	1.500	0.18	8.04	9.2E-09
0.70	7.65	7.0E-05	3.0E-05	2.333	0.37	8.02	9.6E-09
0.80	7.38	8.0E-05	2.0E-05	4.000	0.60	7.98	1.0E-08
0.90	6.90	9.0E-05	1.0E-05	9.000	0.95	7.85	1.4E-08
1.00	4.32	1.0E-04	0.0E+00				
						avg Ka	1.1E-08
						-pKa	-7.98
						pKa	7.98

R. Leigh Shultz
09 Jan 2002

Rebecca Leigh Shultz
09 Jan 2002

Cathy Sparks 21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Jan 2002

Subject: Bulk and solution stability of L-221869-000R (crystalline free base)

The bulk stability data for L-221869-000R (60659-93) are shown below. Data are reported in area % relative to samples kept for the same amount of time at –20 °C.

Bulk stability of L-221869-000R

Conditions	Rel Area % L-221869		
	1 wk	2 wk	4 wk
5 °C/amb RH	98.5	106.2	100.0
25 °C/60% RH	101.1	95.5	100.6
40 °C/amb RH	94.3	100.7	100.6
40 °C/75% RH	102.0	97.3	99.8
60 °C/amb RH	98.5	100.0	100.3
80 °C/amb RH	98.1	89.4	101.7

Significant loss of parent is observed after two weeks at 40 °C/75% RH and 25 °C/60% RH, but no degradates are seen in the LC traces. The four-week data does not show the same trend and may more accurately represent the stability of the compound.

The solution stability data for L-221869-000R (60659-94) are shown below. Data are reported in area % relative to samples stored at –20 °C.

Solution stability of L-221869-000R

	5 °C			25 °C			40 °C			80 °C		
Condition	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk
water	103.3	94.6	98.9	99.8	82.0	89.4	90.7	70.2	52.7	0.4	0.0	0.0
pH 2	106.2	94.4	99.4	105.3	98.9	99.4	99.2	103.8	99.1	102.6	104.2	95.4
pH 4	99.7	98.3	96.9	100.9	100.2	96.9	104.6	97.7	97.7	103.1	101.3	92.5
pH 6	89.2	103.9	99.2	103.1	100.2	99.1	94.3	108.5	98.4	35.8	11.9	0.4
pH 7.4 saline	102.8	101.3	99.2	93.3	98.7	97.1	99.7	88.4	82.1	0.8	0.0	0.0
pH 8	106.6	86.6	98.8	107.5	91.4	91.5	88.2	72.6	57.1	0.0	0.0	0.0
pH 10	107.5	95.2	99.3	94.9	85.4	81.5	80.2	56.6	34.1	0.0	0.0	0.0

Significant loss of parent is observed in water at 25 °C over the 4-week time period. The compound is most stable to hydrolysis at pH 2 and pH 4, though less de-amination is seen at pH 2. De-amination becomes more likely as the storage temperature rises. The compound is not stable for significant amounts of time (>2 weeks) above pH 6 due to rapid hydrolysis.

The solution photostability data (60659-110) for L-221869-000R are tabulated below and are reported in area % relative to samples wrapped in aluminum foil.

Solution photostability of L-221869-000R

Condition	Rel Area % L-221869
water	98.2
pH 2	99.7
pH 4	100.0
pH 6	100.1
pH 8	100.4
pH 10	100.0

Solutions of L-221869 are photostable to laboratory light for at least one week.

Rebecca Leigh Shultz
15 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Jan 2002

Subject: Bulk and solution stability for L-221869 p-toluenesulfonate salt

The bulk stability data (60659-101) for the p-toluenesulfonate salt of L-221869 are shown the table below. Data are reported in area % relative to samples stored at –20 °C.

Bulk thermal stability of L-221869 p-toluenesulfonate

Conditions	Rel Area % L-221869		
	1 wk	2 wk	4 wk
40 °C/75% RH	nd	98.5	100.1
80 °C/amb RH	nd	99.9	99.8

nd = no data due to LC error

An LC error resulted in no data being collected during analysis of the one-week samples. Nonetheless. the two- and four-week samples indicate that the p-TSA salt is stable toward hydrolysis and de-amination over 4 weeks.

The solution stability data (60659-101) for the p-toluenesulfonate salt of L-221869 are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Solution thermal stability of L-221869 p-toluenesulfonate

Conditions	Rel Area % L-221869, 40 °C			Rel Area % L-221869, 80 °C		
	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk
water	87.4	75.3	56.1	0.0	0.0	0.0
pH 2	100.1	100.3	100.4	99.5	97.3	94.7
pH 4	100.1	96.5	100.0	101.7	95.4	93.3
pH 6	102.3	96.8	98.6	35.9	7.8	0.5
pH 8	89.0	73.4	54.0	0.0	0.0	0.0
pH 10	76.0	53.4	28.4	0.0	0.0	0.0

The salt is most stable at pH 2 and pH 4, though some hydrolysis is observed at 80 °C under each of these conditions. The salt is unstable in water and in buffers above pH 6. The solution stability of the p-toluenesulfonate salt is very similar to that of the HCl, benzenesulfonate, and tartrate salts.

Rebecca Leigh Shultz
15 Jan 2002

15 Jan 2002
RLS

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Jan 2002

Subject: Bulk and solution stability of L-221869 HCl salt

The bulk stability data (60659-101) for the HCl salt of L-221869 are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Bulk thermal stability of L-221869 HCl salt

Conditions	Rel Area % L-221869		
	1 wk	2 wk	4 wk
40 °C/75% RH	nd	97.2	98.6
80 °C/amb RH	nd	99.5	100.8

nd = no data due to LC error

No data was collected during analysis of the 1-week samples due to an LC error. The data at 40 °C/75% RH suggest that some hydrolysis of the parent is resulting in loss of L-221869. This is expected based on the relatively high hygroscopicity of this salt. The HCl salt is deemed a poor candidate for development based on this hygroscopicity and high-humidity stability data.

The solution stability data (60659-101) for the HCl salt of L-221869 are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Solution thermal stability of L-221869 HCl salt

Conditions	Rel Area % L-221869, 40 °C			Rel Area % L-221869, 80 °C		
	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk
water	88.0	82.5	64.9	nd	0.0	0.3
pH 2	100.4	99.7	100.0	nd	97.6	96.0
pH 4	99.7	100.1	99.8	nd	97.0	93.7
pH 6	103.2	100.1	99.0	nd	8.2	0.6
pH 8	92.3	82.4	59.5	nd	0.0	0.0
pH 10	80.1	60.0	33.5	nd	0.0	0.0

The HCl salt is most stable in solution at pH 2, though some hydrolysis is still observed at 80 °C at this pH. The salt is very unstable above pH 6. This solution stability data is not very different from data obtained from other salts of L-221869; however, the bulk stability data argues against development of this salt.

Rebecca Leigh Shultz
15 Jan 2002

RLS 15 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Jan 2002

Subject: Bulk and solution stability of L-221869 benzenesulfonate salt

The bulk stability data for the benzenesulfonate salt of L-221869 (60659-106) are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Bulk thermal stability of L-221869 benzenesulfonate salt

Conditions	Rel Area % L-221869		
	1 wk	2 wk	4 wk
40 °C/75% RH	100.5	100.1	97.3
80 °C/amb RH	100.0	98.5	99.6

Some loss of parent is observed over 4 weeks at 40 °C/75% RH, but no degradates appear in the HPLC trace. The bulk appears to be stable toward de-amination over 4 weeks at 80 °C.

The solution stability data for the benzenesulfonate salt of L-221869 (60659-106) are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Solution thermal stability of L-221869 benzenesulfonate salt

Conditions	Rel Area % L-221869, 40 °C			Rel Area % L-221869, 80 °C		
	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk
water	87.5	73.0	52.1	0.0	0.0	0.0
pH 2	100.2	97.0	99.2	98.4	94.7	92.8
pH 4	99.9	97.9	99.6	98.5	95.5	94.6
pH 6	100.1	96.5	97.0	33.6	9.3	0.6
pH 8	88.0	74.0	55.2	0.0	0.0	0.0
pH 10	73.2	54.0	29.5	0.0	0.0	0.4

The benzenesulfonate salt is unstable in unbuffered water at both 40 and 80 °C. This is most likely a pH effect, as hydrolysis is faster above pH 6. The salt is most stable toward hydrolysis between pH 2 and 4. No de-amination is observed at pH 2. Based on chemical stability data, the benzenesulfonate salt of L-221869 is a possibility for development, although the pace of the degradation in plain water could be a problem for such a soluble drug.

Rebecca Leigh Shultz
15 Jan 2002

15 Jan 2002
RLS

21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Jan 2002

Subject: Bulk and solution stability of L-221869 L-tartaric acid salt

The bulk stability data for the L-tartaric acid salt of L-221869 (60659-106) are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Bulk thermal stability of L-221869 tartrate salt

Conditions	Rel Area % L-221869		
	1 wk	2 wk	4 wk
40 °C/75% RH	102.7	99.6	99.5
80 °C/amb RH	101.9	99.0	99.4

Minimal loss of parent is observed over 4 weeks; the loss is probably not statistically significant, and no degradates are observed by HPLC.

The solution stability data for the tartrate salt of L-221869 (60659-106) are shown in the table below. Data are reported in area % relative to samples stored at –20 °C.

Solution thermal stability of L-221869 tartrate salt

Conditions	Rel Area % L-221869, 40 °C			Rel Area % L-221869, 80 °C		
	1 wk	2 wk	4 wk	1 wk	2 wk	4 wk
water	99.5	92.5	87.5	1.6	0.0	0.0
pH 2	99.7	99.0	99.6	98.9	97.6	94.8
pH 4	95.9	94.4	100.0	95.0	92.6	90.8
pH 6	99.8	98.7	98.9	37.1	11.6	1.5
pH 8	92.2	80.3	63.8	0.0	0.0	0.0
pH 10	83.9	60.1	34.7	0.0	0.0	0.5

The tartrate salt is most stable between pH 2 and 4, similar to other salt of L-221869. Although the stability of the salt in water at 80 °C is poor, the stability of the salt at 40 °C in water shows a marked improvement over the other salts of this compound. This is likely a pH effect; the tartaric acid in the salt buffers the water solutions and affords a pH between 3 and 4, where the drug is most stable. Based on this data, the tartaric acid salt has a distinct advantage over the benzenesulfonate salt for development. The table below compares the degradation (both hydrolysis and de-amination) of L-221869 in the benzenesulfonate and tartrate salt forms at 0.1 mg/mL salt in water at 40 °C.

Comparison of degradation in salts of L-221869

Salt of L-221869	Rel Area % Hydrolysis			Rel Area % De-amination		
	1wk	2wk	4wk	1wk	2wk	4wk
Benzenesulfonate	8.9	19.3	46.6	3.3	6.4	22.3
L-Tartrate Hemihydrate	1.9	5.0	9.5	1.0	2.6	4.1

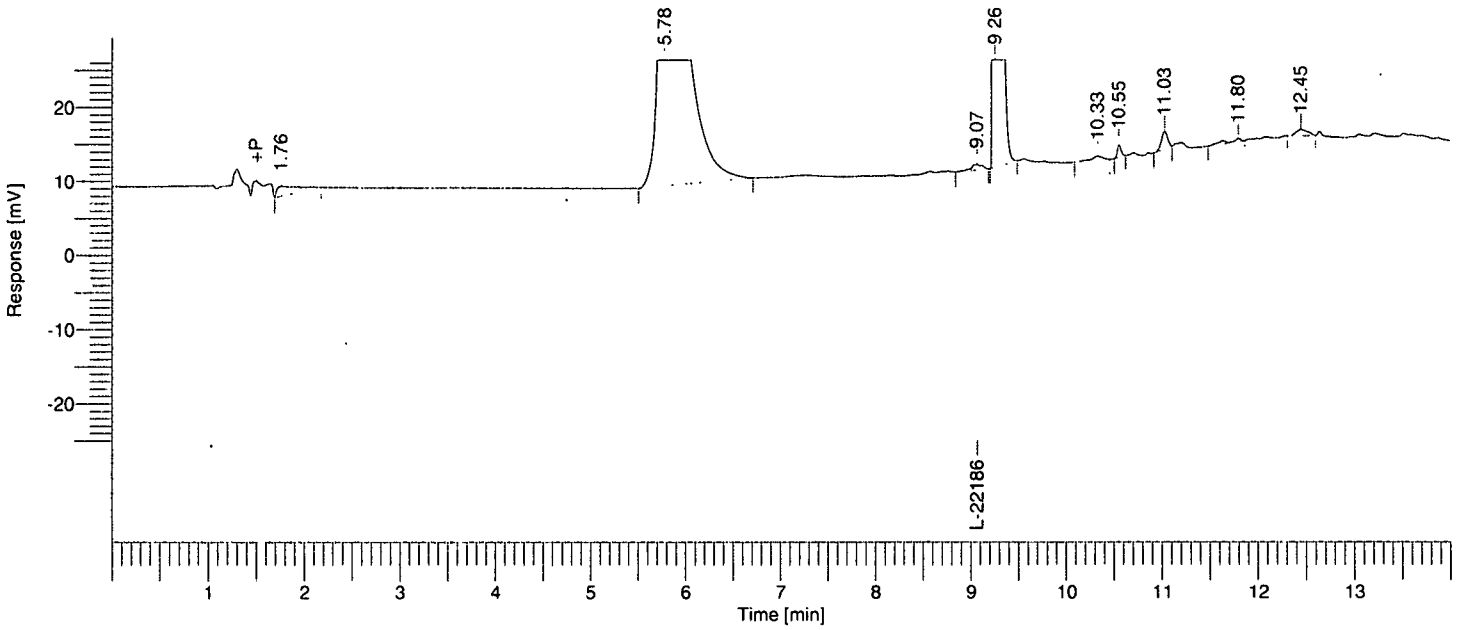
The data clearly indicate that the tartrate salt is much more stable in unbuffered water. This is an advantage for the tartrate salt should some of the salt dissolve in a formulation.

Rebecca Leigh Shultz
15 Jan 2002

21 Jan 2002
COUNTERSIGNATURE DATE

Software Version	: 6.2.0.0.0:B27	Date	: 1/21/02 8:10:37 AM
Reprocess Number	: ry80m-108-pelc: 505		
Sample Name	: L224715 besylate in water 2	Data Acquisition Time	: 1/18/02 12:59:27 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

Result File : D:\Projects\L224715\RawData\60659.142\003.rst
Sequence File : D:\Sequences\L224715\60659.142-20020118-121253.idx



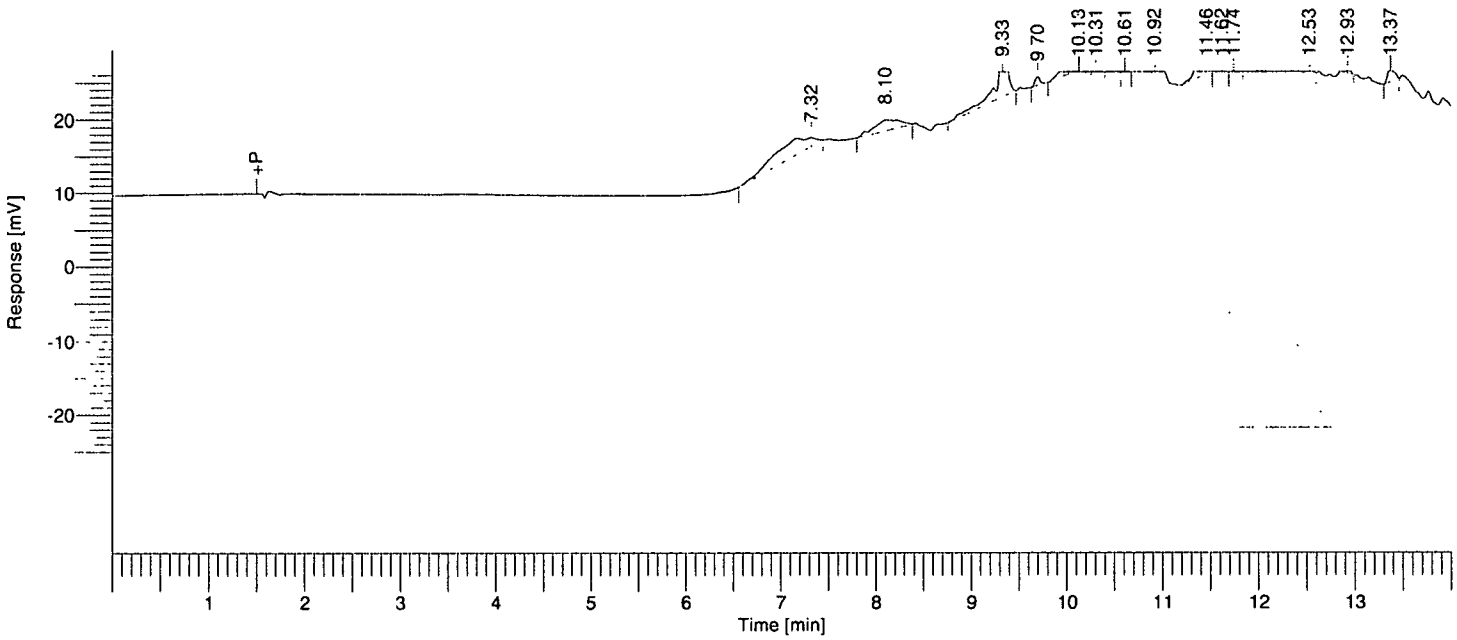
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.761	0.19	39007.93	2463.55	0.74
2		5.781	0.64	2131102.37	136599.36	40.16
3	L-221869	9.068	1.00	11799.17	1482.35	0.22
4		9.256	1.02	3090814.57	809674.04	58.24
7		11.029	1.22	20076.40	4572.39	0.38
9		12.446	1.37	13875.23	1666.27	0.26
				5306675.67	956457.95	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/21/02 8:10:35 AM
Reprocess Number	: ry80m-108-pelc: 503		
Sample Name	: water	Data Acquisition Time	: 1/18/02 12:20:25 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/1	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L224715\RawData\60659.142.001.rst
Sequence File : D:\Sequences\L224715\60659.142-20020118-121253.idx



Single Injection Report

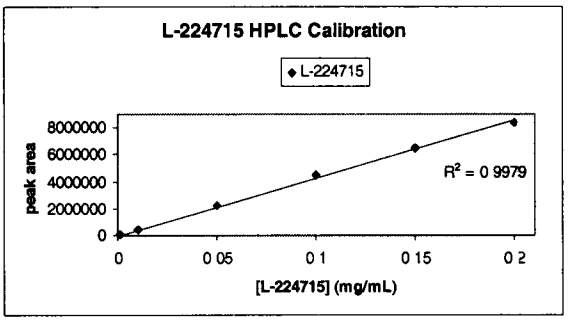
Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		7.321	0.83	120440.89	2283.70	19.91
2		8.099	0.92	53336.97	2815.95	8.82
3		9.331	1.06	173010.11	36466.26	28.60
5		10.129	1.15	34428.42	1825.03	5.69
6		10.307	1.17	71110.41	26089.82	11.76
7		10.609	1.20	10220.59	4017.41	1.69
8		10.923	1.24	23974.03	5426.06	3.96
9		11.459	1.30	46131.36	4619.46	7.63
10		11.621	1.32	22972.50	2986.54	3.80
12		12.530	1.42	21503.33	8010.13	3.56
13		12.927	1.47	10317.10	3786.45	1.71
14		13.372	1.52	17411.53	3572.35	2.88
				604857.25	101899.15	100.00

R. Leigh Shultz
15 Jan 2002

Subject: Solubility of L-224715-000T

The solubility of L-224715-000T was investigated in water, saline, 0.01 N HCl, and buffers ranging in pH from 4-10 (60659-119). An LC calibration was performed using standard solutions of L-224715-000T001, and the calibration plot obtained is shown below.



The solubility data obtained were quantified using the calibration plot above and are tabulated below.

Solubility of L-224715-000T

Solvent	Sol L-224715 (mg/mL)	$pH_{initial}$	pH_{final}	Area % Hydrolysis
water	5.92	6.47	9.37	0.35
saline	5.74	5.80	9.31	0.35
0.01 N HCl	9.63	2.01	8.27	0.23
20 mM sodium acetate	11.78	4.02	8.10	0.19
20 mM sodium phosphate	11.89	6.05	8.13	0.23
20 mM sodium phosphate	6.39	7.92	8.97	0.37
20 mM sodium carbonate	6.47	10.02	10.07	0.46

The solubilities of L-224715 in water and saline are equilibrium values. However, equilibrium solubilities were not obtained across the pH range because the drug neutralized the 20 mM buffers and raised the pH of the solutions. This experiment will have to be repeated with more concentrated buffers to obtain actual solubility data as a function of pH.

Rebecca Leigh Shultz

15 Jan 2002

RLS 15 Jan 2002

COUNTERSIGNATURE 21 Jan 2002 DATE

R. Leigh Shultz
18 Jan 2002

Subject: Initiation of bulk and solution stability (abbreviated) for L-224715 benzenesulfonate salt

A sample of the benzenesulfonate salt of L-224715 was received from K. Hansen (70130-347). A stock solution of the salt was made by dissolving 10.54 mg salt in ~~10.00~~ mL of water in a scintillation vial. This stock solution was used to make two samples for immediate HPLC analysis: an aliquot (0.10 mL) of the stock solution was diluted to 1.0 mL with water in each of two HPLC vials. These samples were analyzed immediately in the sample set 60659.142.seq using the method L221869_method1.mth.

10.54mL typo RLS 21Jan 2002

The stock solution of the benzenesulfonate salt prepared above were used to make stability samples for the salt (54 samples were prepared). One set of vials was made for each of three time points (1, 2, and 4 weeks). Within each time point, a set of vials was placed in each of 3 stability stations: –20 °C (samples R), 40 °C (samples C), and 80 °C (samples F). In each station at each time point, samples were prepared at pH 2, 4, 6, 8, 10, and in water. (Buffers used were made on 17 Jan 2002: pH 2, commercial 0.01 N HCl; pH 4, 20 mM sodium acetate; pH 6 and 8, 20 mM sodium phosphate; and pH 10, 20 mM sodium carbonate.) The vials were crimped with Teflon-lined caps, labeled as 60659-142, and placed in the stability stations at 10:00 am. Bulk stability was also initiated the salt. Nine vials were prepared according to the table below. The vials were placed in the appropriate stability stations at 10:00 am.

Sample	Conditions	Mass salt (mg)
60659-142R1	–20 °C 1 wk	1.17
60659-142 R2	–20 °C 2 wk	1.48
60659-142 R4	–20 °C 4 wk	1.30
60659-142 D1	40/75 1wk	2.05
60659-1421 D2	40/75 2 wk	1.69
60659-142 D4	40/75 4 wk	1.38
60659-142 F1	80 °C 1 wk	1.81
60659-142 F2	80 °C 2 wk	2.62
60659-142 F4	80 °C 4 wk	1.95

Rebecca Leigh Shultz
18 Jan 2002

RLS 18 Jan 2002

Kathy Rhodes 21 Jan 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
21 Jan 2002

Subject: Initiation of bulk and solution stability (abbreviated) for L-224715 tartrate salt

A sample of the tartrate salt of L-224715 was received from K. Hansen (70130-359). A stock solution of the salt was made by dissolving 7.32 mg salt in 7.32 mL of water in a scintillation vial. This stock solution was used to make three samples for immediate HPLC analysis: an aliquot (0.10 mL) of the stock solution was diluted to 1.0 mL with water in each of two HPLC vials, while a third aliquot (0.050 mL) was diluted to 1.0 mL with water in a third HPLC vial. These samples were analyzed immediately in the sample set 60659.143.seq using the method L221869_method1.mth along with six standard solutions of L-224715 free base (see NB 60659-119).

The stock solution of the tartrate salt prepared above was used to make stability samples for the salt (54 samples were prepared). One set of vials was made for each of three time points (1, 2, and 4 weeks). Within each time point, a set of vials was placed in each of 3 stability stations: –20 °C (samples R), 40 °C (samples C), and 80 °C (samples F). In each station at each time point, samples were prepared at pH 2, 4, 6, 8, 10, and in water. (Buffers used were made on 17 Jan 2002: pH 2, commercial 0.01 N HCl; pH 4, 20 mM sodium acetate; pH 6 and 8, 20 mM sodium phosphate; and pH 10, 20 mM sodium carbonate.) The vials were crimped with Teflon-lined caps, labeled as 60659-143, and placed in the stability stations at 2:00 pm. Bulk stability was also initiated the salt. Nine vials were prepared according to the table below. The vials were placed in the appropriate stability stations at 2:00 pm.

Sample	Conditions	Mass salt (mg)
60659-143 R1	–20 °C 1 wk	1.14
60659-143 R2	–20 °C 2 wk	1.26
60659-143 R4	–20 °C 4 wk	1.08
60659-143 D1	40/75 1wk	1.11
60659-143 D2	40/75 2 wk	1.01
60659-143 D4	40/75 4 wk	1.21
60659-143 F1	80 °C 1 wk	1.34
60659-143 F2	80 °C 2 wk	1.38
60659-143 F4	80 °C 4 wk	1.12

Subject: Native pH of L-224715 besylate and tartrate salts (at 1.0 mg/mL)

The pH of the stock solution of the tartrate salt of L-224715 made above was measured using the pH meter and a glass electrode. The pH of the 1.0 mg/mL solution was 3.98, indicating that the tartrate is a 1:1 salt and forms a buffer in water. The pH of the stock solution of the besylate salt of L-224715 (60659-142) was also measured in the same manner; the pH of this 1.0 mg/mL solution was 6.66.

Subject: Initial HPLC analysis of L-224715 benzenesulfonate salt

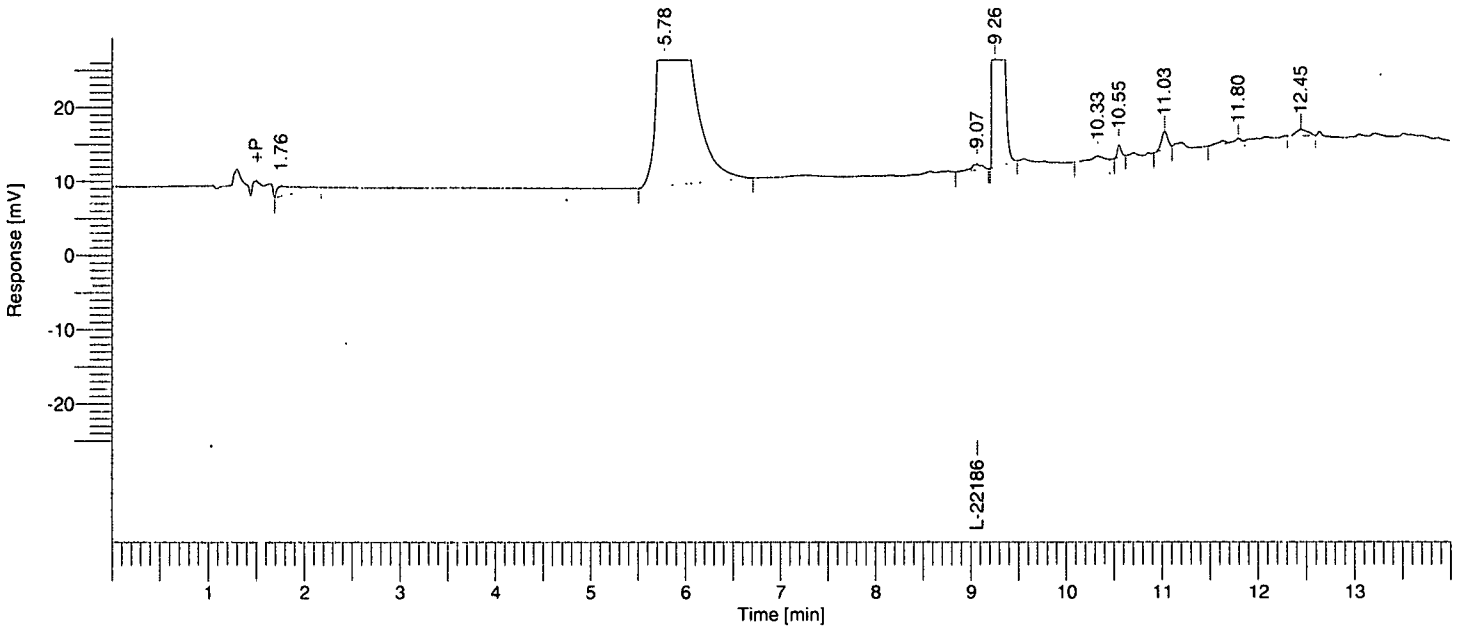
The HPLC analysis of the benzenesulfonate salt of L-224715 in water (fresh) shows an area % of 58.24% L-224715 (benzenesulfonic acid = 40.16 area %). The parent elutes at 9.26 minutes, while the benzenesulfonic acid elutes at 5.78 minutes (RRT 0.62).

Rebecca Leigh Shultz
21 Jan 2002

25 Mar 2002
COUNTERSIGNATURE DATE

Software Version	: 6.2.0.0.0:B27	Date	: 1/21/02 8:10:37 AM
Reprocess Number	: ry80m-108-pelc: 505		
Sample Name	: L224715 besylate in water 2	Data Acquisition Time	: 1/18/02 12:59:27 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

Result File : D:\Projects\L224715\RawData\60659.142\003.rst
Sequence File : D:\Sequences\L224715\60659.142-20020118-121253.idx



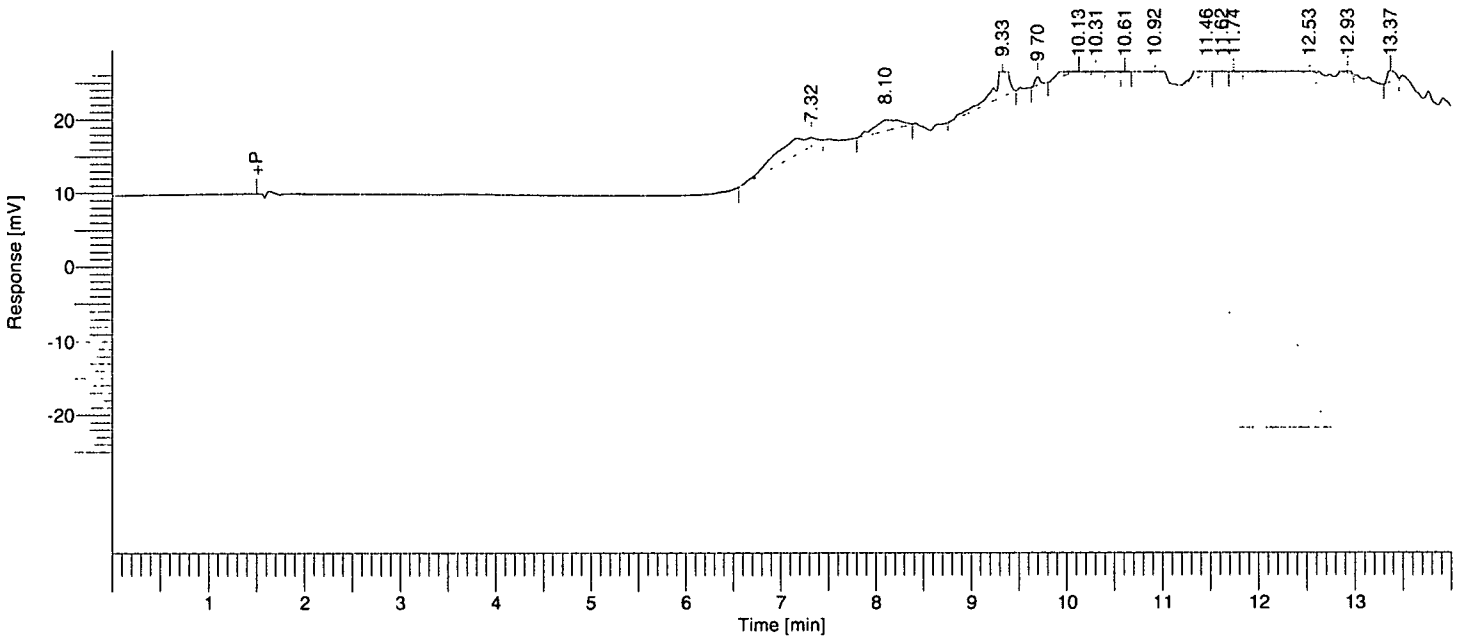
Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.761	0.19	39007.93	2463.55	0.74
2		5.781	0.64	2131102.37	136599.36	40.16
3	L-221869	9.068	1.00	11799.17	1482.35	0.22
4		9.256	1.02	3090814.57	809674.04	58.24
7		11.029	1.22	20076.40	4572.39	0.38
9		12.446	1.37	13875.23	1666.27	0.26
				5306675.67	956457.95	100.00

Software Version	: 6.2.0.0.0:B27	Date	: 1/21/02 8:10:35 AM
Reprocess Number	: ry80m-108-pelc: 503		
Sample Name	: water	Data Acquisition Time	: 1/18/02 12:20:25 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/1	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File : D:\Projects\L224715\RawData\60659.142.001.rst
Sequence File : D:\Sequences\L224715\60659.142-20020118-121253.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		7.321	0.83	120440.89	2283.70	19.91
2		8.099	0.92	53336.97	2815.95	8.82
3		9.331	1.06	173010.11	36466.26	28.60
5		10.129	1.15	34428.42	1825.03	5.69
6		10.307	1.17	71110.41	26089.82	11.76
7		10.609	1.20	10220.59	4017.41	1.69
8		10.923	1.24	23974.03	5426.06	3.96
9		11.459	1.30	46131.36	4619.46	7.63
10		11.621	1.32	22972.50	2986.54	3.80
12		12.530	1.42	21503.33	8010.13	3.56
13		12.927	1.47	10317.10	3786.45	1.71
14		13.372	1.52	17411.53	3572.35	2.88
				604857.25	101899.15	100.00

R. Leigh Shultz
22 Jan 2002

Subject: Bulk stability of binary mixtures of L-224715 benzenesulfonate and gelatin/HPMC

In order to investigate the stability of L-224715 benzenesulfonate salt (70130-347) in a dry-filled capsule formulation, binary mixtures of the salt were prepared with gelatin (lab grade, type B, Fisher lot no. 987519) and with HPMC (USP, 23537, 6 cps). Samples of the salt were weighed out (see table below), as were samples of gelatin and HPMC. The salt sample was ground gently with a mortar and pestle with ca. 85 mg of the excipient; the rest of the excipient was then added, and the mixture was ground until uniform.

Mass L-224715 salt (mg)	Mass L-224715 parent (mg) FB = 0.72(Bs salt)	Excipient	Total mass (exc + salt) (mg)	% w/w drug loading
22.2	16.0	gelatin	320.3	5.0
22.9	16.5	HPMC	319.6	5.2

Samples of the mixtures were then weighed into vials (10 vials for each mixture) for stability studies according to the following table.

Sample (gelatin)	Mass mixture (mg)	Sample (HPMC)	Mass mixture (mg)
60659-144 A1/gel	21.44	60659-144 A1/HPMC	20.01
60659-144 A2/gel	19.49	60659-144 A2/HPMC	20.33
60659-144 A4/gel	20.68	60659-144 A4/HPMC	20.91
60659-144 C1/gel	20.41	60659-144 C1/HPMC	19.95
60659-144 C2/gel	21.11	60659-144 C2/HPMC	20.75
60659-144 C4/gel	19.56	60659-144 C4/HPMC	19.70
60659-144 D1/gel	20.76	60659-144 D1/HPMC	20.97
60659-144 D2/gel	21.00	60659-144 D2/HPMC	20.02
60659-144 D4/gel	20.50	60659-144 D4/HPMC	21.19
60659-144 phys/gel	128.99	60659-144 phys/HPMC	126.70

Samples A, C, and D were placed in the stability stations at 3:30 pm. Samples D had open caps; the rest of the samples had closed caps. Samples 60659-144 phys/gel and phys/HPMC were reserved for NMR studies.

Subject: Bulk stability of binary mixtures of L-221869 tartrate and gelatin/HPMC

The above procedure was repeated with the tartrate salt of L-221869 (72061-54).

Mass L-221869 salt (mg)	Mass L-221869 parent (mg) FB = 0.71(Tar salt)	Excipient	Total mass (exc + salt) (mg)	% w/w drug loading
22.6	16.1	gelatin	324.0	5.0
22.9	16.3	HPMC	320.9	5.1

Samples of the mixtures were then weighed into vials (10 vials for each mixture) for stability studies according to the following table (continued on next page, 60659-145).

Rebecca Leigh Shultz
22 Jan 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
22 Jan 2002

Subject: Bulk stability of binary mixtures of L-221869 tartrate and gelatin/HPMC (continued from pg144)

Sample (gelatin)	Mass mixture (mg)	Sample (HPMC)	Mass mixture (mg)
60659-144a A1/gel	21.26	60659-144a A1/HPMC	20.65
60659-144a A2/gel	21.22	60659-144a A2/HPMC	19.91
60659-144a A4/gel	21.16	60659-144a A4/HPMC	19.59
60659-144a C1/gel	21.25	60659-144a C1/HPMC	20.42
60659-144a C2/gel	21.75	60659-144a C2/HPMC	20.43
60659-144a C4/gel	20.08	60659-144a C4/HPMC	20.11
60659-144a D1/gel	20.74	60659-144a D1/HPMC	20.36
60659-144a D2/gel	21.37	60659-144a D2/HPMC	20.91
60659-144a D4/gel	20.64	60659-144a D4/HPMC	19.88
60659-144a phys/gel	124.37	60659-144a phys/HPMC	126.45

Samples A, C, and D were placed in the stability stations at 5:00 pm. Samples D had open caps; the rest of the samples had closed caps. Samples 60659-144a phys/gel and phys/HPMC were reserved for NMR studies.

Rebecca Leigh Shultz
22 Jan 2002

R. Leigh Shultz
23 Jan 2002

Subject: SSNMR analysis of L-221869 and L-224715 samples

A sample of L-221869 L-tartrate hemihydrate (ca. 105 mg) was weighed into a vial and taken to Bob Wenslow for solid-state NMR analysis. Both ^{13}C and ^{19}F experiments will be done with closed and open sample containers to determine the reversibility of the hydration of the tartrate salt.

Small samples were removed from samples 60659-144 phys/gel (21.49 mg) and phys/HPMC (19.05 mg) and from samples 60659-144a phys/gel (22.74 mg) and phys/HPMC (20.54 mg). These samples were reserved for HPLC method development. The remaining samples were taken to Bob Wenslow for SSNMR analysis. These measurements will be the time zero measurements for physical stability of the salts in these binary mixtures. The samples will be placed on stability after the NMR experiments are completed.

Rebecca Leigh Shultz
23 Jan 2002

[illegible] 25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
23 Jan 2002

Subject: Solubility of L-221869 tartrate salt

In order to determine the solubility of the crystalline L-221869 tartrate salt (hemi-hydrate) in several solvents, 8 samples of the salt (72061-54) were weighed into tared test tubes according to the table below. An aliquot of solvent was added to each vial, and the vial was shaken to see if the solid dissolved. If the solid did not dissolve immediately, the vial was placed on the rotator to equilibrate overnight.

Solvent	Mass salt (mg)	Volume added (mL)	$pH_{initial}$	Observations
water	11.66	0.5	6.13	solid sinks, then dissolves
0.9% NaCl	6.73	0.5	5.57	solid floats, sinks, dissolves
0.01N HCl	10.03	0.5	2.08	slow to dissolve
20 mM sodium acetate	4.70	0.5	3.99	soluble
20 mM sodium phosphate	5.45	0.5	6.01	soluble
MeOH	6.14	0.4	n/a	milky at first, then soluble
EtOH	9.17	0.5	n/a	milky – equilibrate
2-PrOH	6.57	0.5	n/a	milky - equilibrate

All of the samples were soluble in the aliquots added except the ethanol and 2-propanol samples. These samples were placed on the rotator at 6:00 pm.

Subject: Solubility of L-224715 free base, besylate salt, and tartrate salt

In order to determine the solubility of the crystalline free base (-000T001), crystalline L-224715 besylate salt (anhydrous used, 70130-347) and the crystalline L-224715 tartrate salt (hemi-hydrate, 70130-359) in several solvents, a procedure similar to the one above was followed. The details appear in the table below.

Salt of L-224715	Solvent	Mass salt (mg)	Volume added (mL)	$pH_{initial}$	Observations
free base	MeOH	4.96	0.2	n/a	soluble
free base	EtOH	4.35	0.2	n/a	soluble
free base	2-PrOH	4.44	0.4	n/a	soluble (v. slow)
free base	PEG 400	7.24	0.4	n/a	soluble (v. slow)
free base	glycerol	6.43	1.0	n/a	soluble upon heating
besylate	water	4.99	0.5	6.13	solid sinks, then dissolves
besylate	0.01N HCl	4.37	0.5	2.08	soluble (fast)
besylate	MeOH	4.38	0.2	n/a	soluble (fast)
besylate	EtOH	4.16	0.2	n/a	soluble (fast when shaken)
besylate	2-PrOH	4.24	0.5	n/a	milky – equilibrate
L-tartrate	water	5.75	0.5	6.13	floats, then dissolves (fast)
L-tartrate	0.01N HCl	5.47	0.5	2.08	soluble (fast)
L-tartrate	MeOH	5.46	0.2	n/a	soluble (slow)
L-tartrate	EtOH	5.86	0.5	n/a	milky – equilibrate
L-tartrate	2-PrOH	5.44	0.5	n/a	milky - equilibrate

The three alcoholic samples which did not dissolve readily were placed on the rotator at 6:00 pm.

Rebecca Leigh Shultz
31 Jan 2002

COUNTERSIGNATURE 25 Mar 2002 DATE

R. Leigh Shultz
24 Jan 2002

Subject: Continuation of solubility experiments for L-221869 and L-224715 (from 60659-146)

The solubility samples were removed from the rotator at 9:00 am. They were transferred to centrifuge filters and centrifuged for 10 minutes at 10000 rpm to separate the supernatant from the remaining solids. An aliquot of the supernatant was then removed via pipet (the volume of the aliquot was calculated by estimating the approximate solubility) and diluted to 15.00 mL with 0.1% phosphoric acid according to the following table.

Salt	Solvent	Volume aliquot (mL)
L-221869 tartrate	EtOH	0.075
L-221869 tartrate	2-PrOH	0.100
L-224715 free base	PEG 400	0.050
L-224715 free base	glycerol	0.050
L-224715 besylate	2-PrOH	0.125
L-224715 tartrate	EtOH	0.100
L-224715 tartrate	2-PrOH	0.125

An aliquot of each 15-mL solution was then transferred to an HPLC vial and analyzed using the method L221869_method1. Standard solutions of L-221869 (tosylate salt) and L-224715 (free base), made and used previously for calibration, were run in the same sample set (60659.146.seq) to calibrate the samples.

Subject: Bulk stability of binary mixtures of L-224715 tartrate and gelatin/HPMC

In order to investigate the stability of L-224715 tartrate salt (70130-359) in a dry-filled capsule formulation, binary mixtures of the salt were prepared with gelatin (lab grade, type B, Fisher lot no. 987519) and with HPMC (USP, 23537, 6 cps). Samples of the salt were weighed out (see table below), as were samples of gelatin and HPMC. The salt sample was ground gently with a mortar and pestle with ca. 85 mg of the excipient; the rest of the excipient was then added, and the mixture was ground until uniform.

Mass L-224715 salt (mg)	Mass L-224715 parent (mg) FB = 0.719(Tar salt)	Excipient	Total mass (exc + salt) (mg)	% w/w drug loading
22.4	16.1	gelatin	327.7	4.9
22.9	16.5	HPMC	328.1	5.0

Samples of the mixtures were then weighed into vials (10 vials for each mixture) for stability studies according to the following table.

Sample (gelatin)	Mass mixture (mg)	Sample (HPMC)	Mass mixture (mg)
60659-147 A1/gel	20.23	60659-147 A1/HPMC	21.45
60659-147 A2/gel	23.09	60659-147 A2/HPMC	21.29
60659-147 A4/gel	21.12	60659-147 A4/HPMC	20.33
60659-147 C1/gel	21.66	60659-147 C1/HPMC	21.58
60659-147 C2/gel	22.58	60659-147 C2/HPMC	21.07
60659-147 C4/gel	19.96	60659-147 C4/HPMC	22.29
60659-147 D1/gel	21.20	60659-147 D1/HPMC	20.09
60659-147 D2/gel	19.32	60659-147 D2/HPMC	20.36
60659-147 D4/gel	20.58	60659-147 D4/HPMC	21.16
60659-147 phys/gel	125.72	60659-147 phys/HPMC	114.58

Samples A, C, and D were placed in the stability stations at 5:30 pm. Samples D had open caps; the rest of the samples had closed caps. Samples 60659-147 phys/gel and phys/HPMC were taken to Bob Wenslow for NMR studies (t = 0 samples).

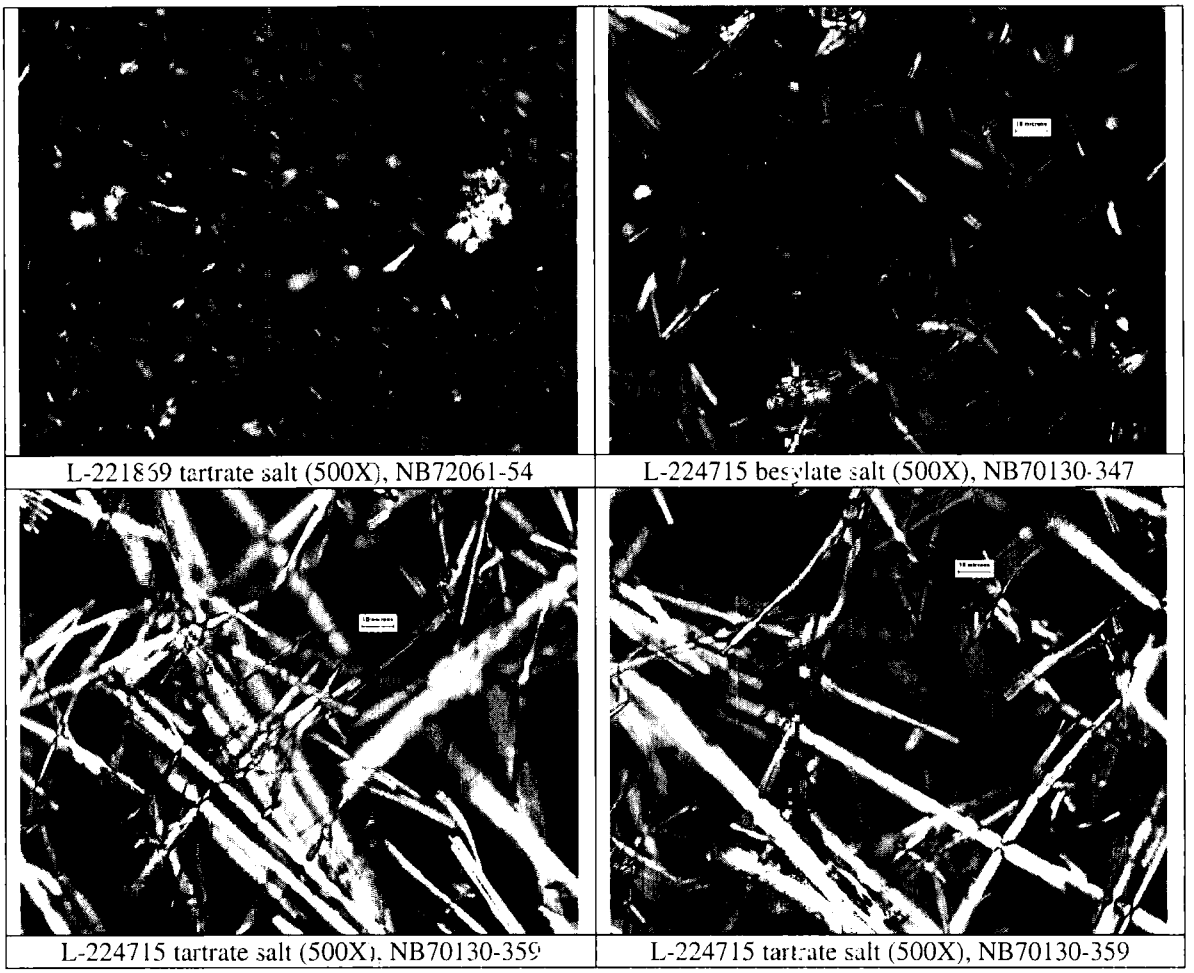
COUNTERSIGNATURE DATE

Rebecca Leigh Shultz 01 Feb 2002 Tuff Phodes 25 Mar 2002

R. Leigh Shultz
24 Jan 2002

Subject: Microscope images of L-221869 tartrate salt and L-224715 besylate and tartrate salts

Optical microscopic images of the tartrate salt of L-221869 and the tartrate and besylate salts of L-224715 were obtained and are shown below.



L-221869 tartrate salt (500X), NB72061-54

L-224715 besylate salt (500X), NB70130-347

L-224715 tartrate salt (500X), NB70130-359

L-224715 tartrate salt (500X), NB70130-359

The tartrate salt of L-221869 has a needle-like morphology; the individual needles are very fine and average around 5 µm in length. Some agglomeration is observed in the sample. The besylate salt of L-224715 also exhibits a needle-like morphology. The average needle length is ca. 10 µm with an aspect of 5. The tartrate salt of L-224715 consists of very long, thin needles (average length 100 µm, aspect ca. 50) which seem to agglomerate lengthwise into clusters of 5-10 needles.

Rebecca Leigh Shultz
01 Feb 2002

COUNTERSIGNATURE DATE
25 Mar 2002

R. Leigh Shultz
25 Jan 2002

Subject: L-224715 besylate salt bulk and solution stability samples

The 1-week bulk (3) and solution (18) stability samples for the besylate salt of L-224715 (60659-142) were removed from the stability stations at 7:00 am. They were placed in the freezer until they could be analyzed. No physical changes were observed in any of the bulk samples.

Rebecca Leigh Shultz 01 Feb 2002

28 Jan 2002

Subject: L-224715 tartrate salt bulk and solution stability samples

The 1-week bulk (3) and solution (18) stability samples for the tartrate salt of L-224715 (60659-143) were removed from the stability stations at 3:00 pm. They were placed in the freezer until they could be analyzed. No physical changes were observed in any of the bulk samples.

Rebecca Leigh Shultz 01 Feb 2002

29 Jan 2002

Subject: Bulk stability of binary mixtures of L-224715 besylate and L-221869 tartrate with gelatin/HPMC

The 1-week bulk chemical stability samples for L-224715 besylate (60659-144) and L-221869 tartrate (60659-144a) in binary mixtures with gelatin and HPMC were removed from the stability stations at 8:00 am. They were placed in the freezer until method development is completed.

Subject: HPLC analysis of L-224715 besylate and tartrate 1-week samples

The 1-week stability samples of L-224715 besylate (60659-142) and tartrate (60659-143) were removed from the freezer at 8:30 am and allowed to come to ambient temperature. The solid samples were each dissolved in 19.0 mL of 0.1% phosphoric acid, and 1-mL aliquots of these solutions were placed in HPLC vials for analysis. Both the bulk and solution samples were assayed in the sample set 60659.149.seq using the method L221869_method1.

Rebecca Leigh Shultz 01 Feb 2002

31 Jan 2002

Subject: Bulk stability of binary mixtures of L-224715 tartrate with gelatin/HPMC

The 1-week bulk chemical stability samples for L-224715 tartrate (60659-147) in binary mixtures with gelatin and HPMC were removed from the stability stations at 8:00 am. They were placed in the freezer until method development is completed.

Rebecca Leigh Shultz
01 Feb 2002

Cynthia Phoebe 25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
01 Feb 2002

Subject: L-224715 besylate salt bulk and solution stability samples

The 2-week bulk (3) and solution (18) stability samples for the besylate salt of L-224715 (60659-142) were removed from the stability stations at 8:30 am. No physical changes were observed in any of the bulk samples. They were allowed to come to ambient temperature, and the solid samples were each dissolved in 19.0 mL of 0.1% phosphoric acid. Aliquots (1 mL) of these solutions were placed in HPLC vials for analysis. Both the bulk and solution samples were assayed in the sample set 60659.150.seq using the method L221869_method1.

Subject: Stoichiometry of L-224715 tartrate salt – data analysis

The HPLC data from two different concentrations of the tartarte salt were analyzed against known standards of the free base (60659-119). The data are shown below. The theoretical [715] for the 1:1 salt is calculated as a hemi-hydrate.

Sample	[salt] (known) (mg/mL)	[715] (from LC) (mg/mL)	Theo [715], 1:1 salt (mg/mL)	Theo [715], 2:1 salt (mg/mL)
A	0.1	0.095	0.072	0.084
B	0.1	0.092	0.072	0.084
C	0.05	0.052	0.036	0.042

The data seem to indicate that L-224715 tartrate is actually a hemitartrate salt. However, ^{1}H NMR spectroscopy (K. Hansen, Process Research) indicates 1:1 stoichiometry. In addition, the pH of a solution of the salt in water is between 3 and 4, indicating a free carboxylic acid group (1:1 salt). All three of the sample solutions were made by diluting a 1 mg/mL stock solution of the tartrate salt. The stock solution was made (60659-143) by dissolving 7.32 mg of salt in 7.32 mL of water. It is possible that there was a large error in the concentration of the stock solution, producing abnormally high concentrations of L-224715.

Subject: Solubility of L-224715-000T001 in alcohols and oily vehicles

Solubility data for the crystalline free base of L-224715 was calculated from the data shown on 60659-146. The solubility data are shown in the table below.

Solvent	Sol. Parent (mg/mL)
PEG 400	>18.1
glycerol	~6.4*
methanol	>24.8
ethanol	>21.8
2-propanol	>11.1

* free base goes into glycerol at this conc. when heated.

None of the data are equilibrium values, since the solid completely dissolved in the solvent in each case. However, the glycerol had to be heated to dissolve the drug, so the solubility in glycerol is not likely to be much higher than noted above. The free base of L-224715 is very soluble in methanol, ethanol, and 2-propanol.

Rebecca Leigh Shultz
01 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
01 Feb 2002

Subject: Solubility data for L-221869 tartrate hemihydrate

The solubilities of L-221869 tartrate hemihydrate (72061-54) in a variety of aqueous solutions and alcohols was determined by analyzing the HPLC data for the samples against a calibration curve for L-221869 (60659-146, 147). The results are shown in the table below.

Solvent	Sol. Salt (mg/mL)	Sol. Parent (mg/mL)	$pH_{initial}$	pH_{final}
water	>23.3	>16.5	6.13	3.54
0.9% NaCl	>13.5	>9.6	5.57	3.43
0.01 N HCl	>20.1	>14.3	2.08	3.23
20 mM sodium acetate	>9.4	>6.7	3.99	3.69
20 mM sodium phosphate	>10.9	>7.7	6.01	3.72
methanol	>15.4	>10.9	n/a	n/a
ethanol	0.82	0.59	n/a	n/a
2-propanol	0.17	0.12	n/a	n/a

The salt dissolved completely in all of the solvents except ethanol and 2-propanol. The solids remaining from these two solvents were examined under the microscope. No difference in morphology or birefringence was noted from those of the original solid.

Subject: Solubility data for L-224715 besylate and tartrate salts

The solubilities of L-224715 besylate (70130-347) and tartrate hemihydrate (70130-359) in a variety of aqueous solutions and alcohols was determined by analyzing the HPLC data for the samples against a calibration curve for L-224715 (60659-146, 147). The results are shown in the tables below.

A. Solubility data for L-224715 besylate salt (anhydrous salt used)

Solvent	Sol. Salt (mg/mL)	Sol. Parent (mg/mL)	$pH_{initial}$	pH_{final}
water	>10.0	>7.2	6.13	5.75
0.01 N HCl	>8.7	>6.3	2.08	2.16
methanol	>21.9	>15.8	n/a	n/a
ethanol	>20.8	>15.0	n/a	n/a
2-propanol	5.47	3.94	n/a	n/a

B. Solubility data for L-224715 tartrate hemihydrate

Solvent	Sol. Salt (mg/mL)	Sol. Parent (mg/mL)	$pH_{initial}$	pH_{final}
water	>11.5	>8.3	6.13	3.57
0.01 N HCl	>10.9	>7.8	2.08	2.98
methanol	>27.3	>19.6	n/a	n/a
ethanol	1.14	0.82	n/a	n/a
2-propanol	0.093	0.067	n/a	n/a

Both salts are very soluble in water, 0.01N HCl, and methanol. The tartrate salt is much less soluble in ethanol than the besylate salt, and the besylate is 60 times as soluble in 2-propanol as the tartarte salt. The lower solubility of the tartrate salt in 2-propanol would make it a better candidate for wet granulation with that solvent. The samples which did not completely dissolve were analyzed by microscopy; no form changes were evident. Although the anhydrous besylate salt was used for the experiment, these solubility number most likely represent the solubility of the hemihydrate form of the salt which should form in water or in wet organic solvents.

COUNTERSIGNATURE DATE

Rebecca Leigh Shultz 01 Feb 2002 Tuff Rhodes 25 Mar 2002

R. Leigh Shultz
04 Feb 2002

Subject: Bulk and solution stability of L-224715 tartrate salt

The two-week stability samples for the tartrate salt of L-224715 were removed from the stability stations at 9:00 am. The R2 pH=W sample vial had broken and began to leak as it thawed; the liquid was collected and transferred to a new vial with a glass pipet. The 3 bulk stability samples were dissolved in 19.0 mL 0.1% phosphoric acid each, and an aliquot from each solution was transferred to an HPLC vial for analysis. The solution samples and the three bulk stability samples were analyzed in the sample set 60659.152.seq using the method L221869_method1.

Subject: Physical stability of L-221869 tartrate and L-224715 besylate with gelatin and HPMC

The samples 60659-144 phys/gel, 60659-144 phys/HPMC, 60659-144a phys/gel, and 60659-144a phys/HPMC were returned by Bob Wenslow. Each of the samples was divided into two vials, and one vial was placed in the 40 °C/ambient RH oven. The other vial for each sample was placed in the 40 °C/75% RH oven. Details are shown in the table below.

Original sample ID	New sample ID	Compound	Conditions	Mass of mixture (mg)
60659-144 phys/gel	60659-144 phys1/gel	L-224715 besylate	40 °C/amb RH	42.50
60659-144 phys/gel	60659-144 phys2/gel	L-224715 besylate	40 °C/75% RH	52.53
60659-144 phys/HPMC	60659-144 phys1/HPMC	L-224715 besylate	40 °C/amb RH	40.03
60659-144 phys/HPMC	60659-144 phys2/HPMC	L-224715 besylate	40 °C/75% RH	55.60
60659-144a phys/gel	60659-144a phys1/gel	L-221869 tartrate	40 °C/amb RH	40.55
60659-144a phys/gel	60659-144a phys2/gel	L-221869 tartrate	40 °C/75% RH	50.37
60659-144a phys/HPMC	60659-144a phys1/HPMC	L-221869 tartrate	40 °C/amb RH	42.71
60659-144a phys/HPMC	60659-144a phys2/HPMC	L-221869 tartrate	40 °C/75% RH	45.43

The vials were placed in the stability chambers at 2:00 pm. They will be monitored by SSNMR to ascertain the physical stability of the mixtures.

Subject: Fluorescence of L-221869 and L-224715

The 0.01mg/mL stock solutions of L-221869 tosylate salt and L-224715 free base were analyzed quickly in the fluorimeter to determine if they fluoresce. Both compounds emit weakly at 285 nm when excited between 250-260 nm.

Rebecca Leigh Shultz
04 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
05 Feb 2002

Subject: Bulk stability of L-224715 besylate and L-221869 tartrate with gelatin/HPMC

The two-week samples of L-224715 besylate with gelatin and HPMC (60659-144) were removed from the stability stations at 9:30 am. The two-week samples of L-221869 tartrate with gelatin and HPMC (60659-145) were also removed at that time. The two sets of samples were placed in the freezer (40 °C/75% RH samples were capped) until analysis could be done.

Subject: Bulk and solution stability of L-224715 phosphate salt

A sample of the phosphate salt of L-224715 was received from Process Research (K. Hansen, NB 70316-25). A stock solution of the salt (1.0 mg/mL salt) was made by dissolving 9.16 mg of the salt in 9.16 mL of water. The pH of the stock solution was measured to be 5.61 at 25 °C. The stock solution of the phosphate salt was used to make stability samples for the salt (54 samples were prepared). One set of vials was made for each of three time points (1, 2, and 4 weeks). Within each time point, a set of vials was placed in each of 3 stability stations: –20 °C (samples R), 40 °C (samples C), and 80 °C (samples F). In each station at each time point, samples were prepared at pH 2, 4, 6, 8, 10, and in water. (Buffers used were made on 17 Jan 2002: pH 2, commercial 0.01 N HCl; pH 4, 20 mM sodium acetate; pH 6 and 8, 20 mM sodium phosphate; and pH 10, 20 mM sodium carbonate.) The vials were crimped with Teflon-lined caps, labeled as 60659-153, and placed in the stability stations at 4:00 pm. Bulk stability was also initiated the salt. Nine vials were prepared according to the table below. The vials were placed in the appropriate stability stations at 4:00 pm.

Sample	Conditions	Mass salt (mg)
60659-153 R1	–20 °C 1 wk	1.57
60659-153 R2	–20 °C 2 wk	1.74
60659-153 R4	–20 °C 4 wk	2.03
60659-153 D1	40/75 1wk	1.89
60659-153 D2	40/75 2 wk	2.21
60659-153 D4	40/75 4 wk	1.78
60659-153 F1	80 °C 1 wk	1.16
60659-153 F2	80 °C 2 wk	2.31
60659-153 F4	80 °C 4 wk	2.21

Rebecca Leigh Shultz
05 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
05 Feb 2002

Subject: Solution stability of L-224715 besylate and tartrate salts (summary of 2-week data)

The two-week stability results for L-224715 besylate and L-221869 tartrate were compared; the table below shows relative amounts (in terms of area percent) of hydrolysis and de-amination at 40 °C in water for each salt.

Salt of L-224715	Rel Area % Hydrolysis			Rel Area % De-amination		
	1wk	2wk	4wk	1wk	2wk	4wk
Benzenesulfonate	8.3	18.2		3.6	7.4	
L-Tartrate Hemihydrate	6.3	13.7		4.2	6.1	

The results, very different from those with the tartrate and besylate salts of L-221869 (see 60659-138), indicate that the pH of the tartrate stability samples is close to that of the besylate samples. Several HPLC samples were then analyzed to determine their pH (pH meter with glass electrode):

Sample	Salt	pH
60659-142 C1 pH=W	L-224715 besylate (40 °C, 1 wk)	7.7
60659-142 C2 pH=W	L-224715 besylate (40 °C, 2 wks)	7.7
60659-142 R2 pH=W	L-224715 besylate (–20 °C, 2 wks)	7.6
60659-143 C1 pH=W	L-224715 tartrate (40 °C, 1 wk)	7.5
60659-143 C2 pH=W	L-224715 tartrate (40 °C, 2 wks)	7.6
60659-143 R2 pH=W	L-224715 tartrate (–20 °C, 2 wks)	6.8

The pH of a saturated solution of the tartrate salt of L-224715 in water (60659-151) is 3.6, so the reason for the pH increase over time is not clear. Two new solutions were made: a solution of the besylate salt at 0.1 mg/mL (from stock 60659-142) and a solution of the tartrate salt at 0.1 mg/mL (from stock 60659-143). The pH's of these solutions were 7.1 (besylate) and 5.1 (tartrate). The 1.0-mg/mL stock solution of the tartrate salt had a pH of 4.1, closer to that of the saturated solution. However, the pH of the stability samples has still increased 2.5 pH units over 2 weeks, which is the cause of the lack of stability of the tartrate. The 0.1 mg/mL solutions of the besylate and tartrate were placed in the 40 °C/75% RH oven at 1:30 pm (closed) and will be monitored over time for changes in pH.

Rebecca Leigh Shultz
05 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
07 Feb 2002

Subject: Bulk stability of binary mixtures of L-224715 phosphate and gelatin/HPMC

In order to investigate the stability of L-224715 phosphate salt (70316-25) in a dry-filled capsule formulation, binary mixtures of the salt were prepared with gelatin (lab grade, type B, Fisher lot no. 987519) and with HPMC (USP, 23537, 6 cps). Samples of the salt were weighed out (see table below), as were samples of gelatin and HPMC. The salt sample was ground gently with a mortar and pestle with ca. 85 mg of the excipient; the rest of the excipient was then added, and the mixture was ground until uniform.

Mass L-224715 salt (mg)	Mass L-224715 parent (mg) FB = 0.806(PO_4 salt)	Excipient	Total mass (exc + salt) (mg)	% w/w drug loading
21.06	16.97	gelatin	323.49	5.2
21.01	16.93	HPMC	321.06	5.3

Samples of the mixtures were then weighed into vials (10 vials for each mixture) for stability studies according to the following table.

Sample (gelatin)	Mass mixture (mg)	Sample (HPMC)	Mass mixture (mg)
60659-155 A1/gel	20.32	60659-155 A1/HPMC	20.14
60659-155 A2/gel	20.72	60659-155 A2/HPMC	19.95
60659-155 A4/gel	20.24	60659-155 A4/HPMC	20.56
60659-155 C1/gel	19.76	60659-155 C1/HPMC	20.52
60659-155 C2/gel	19.56	60659-155 C2/HPMC	21.79
60659-155 C4/gel	19.68	60659-155 C4/HPMC	21.34
60659-155 D1/gel	19.48	60659-155 D1/HPMC	20.47
60659-155 D2/gel	20.25	60659-155 D2/HPMC	20.84
60659-155 D4/gel	20.00	60659-155 D4/HPMC	20.23
60659-155 phys/gel	121.96	60659-155 phys/HPMC	113.53

Samples A, C, and D were placed in the stability stations at 2:30 pm. Samples D had open caps; the rest of the samples had closed caps. Samples 60659-155 phys/gel and phys/HPMC were taken to Bob Wenslow for solid-state ^{19}F NMR studies.

Subject: Solution stability of L-224715 tartrate and besylate salts

The 0.1 mg/mL solutions of the besylate and tartrate salts in water (60659-154) were removed from the oven at 10:00 am. The pH of the besylate solution was 8.22 (an increase of 1.1 pH units), and the pH of the tartrate solution was 7.54 (and increase of almost 2.5 pH units). An aliquot (0.75 mL) of each solution was transferred to an HPLC vial for degradate analysis, and the rest of the solutions were placed back in the 40 °C/75% RH oven. The two samples were assayed in the sample set 60659.155.seq using the method L221869_method1.

The LC runs for both of the salts follow this page. Both salts show some degradation, but not to the extent seen in the one- and two-week samples. This indicates that the degradation is a result of the rise in pH rather than the other way around. The cause of the rise in pH remains unknown.

Rebecca Leigh Shultz
07 Feb 2002

25 Mar 2002
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Software Version	: 6.2.0.0.0:B27	Date	: 2/7/02 4:18:26 PM
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nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
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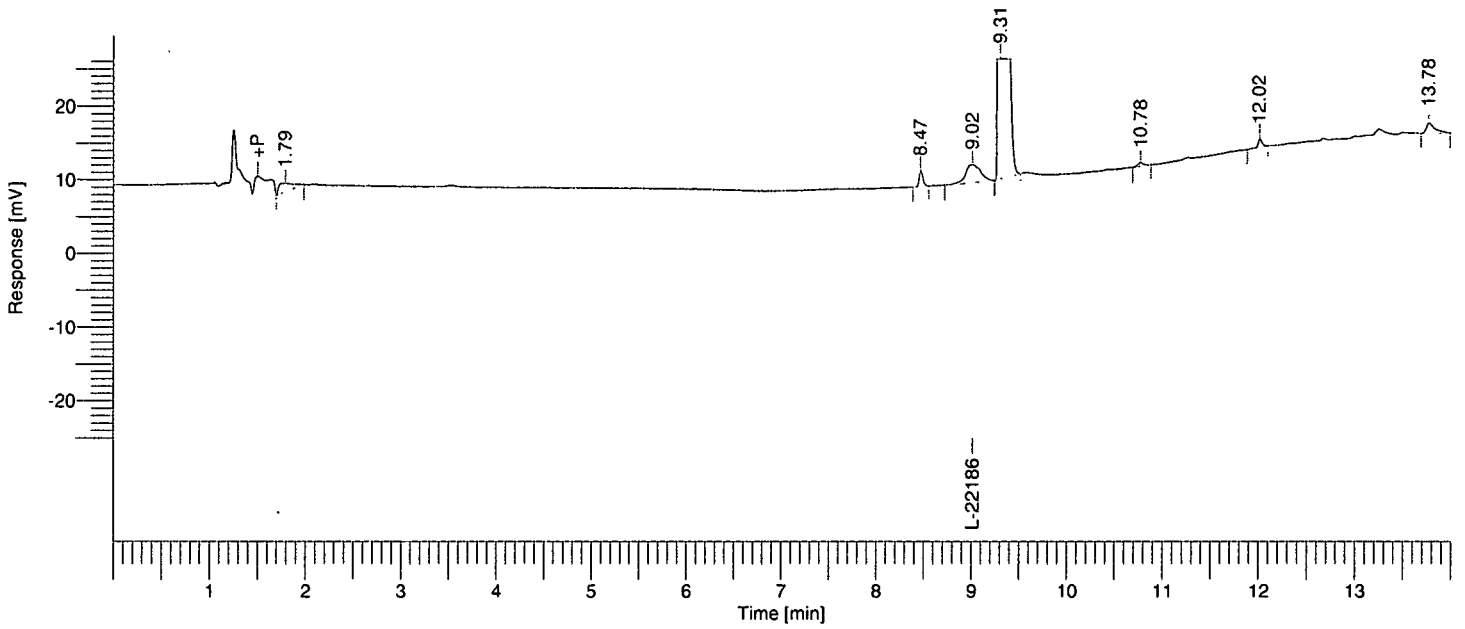

Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]	
1		1.789	0.20	23939.57	2217.44	0.80	
2		8.469	0.94	11519.99	4080.29	0.38	0.4%
3	L-221869	9.016	1.00	51214.67	4684.01	1.71	
4		9.309	1.03	2890340.07	773802.60	96.52	
7		13.780	1.53	17510.91	2790.43	0.58	0.6%
				2994525.22	787574.77	100.00	

Software Version	: 6.2.0.0.0:B27	Date	: 2/7/02 4:18:25 PM
Reprocess Number	: ry80m-108-pelc: 632		
Sample Name	: 7-15 Bs pHW 40C 2 days	Data Acquisition Time	: 2/7/02 3:41:55 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

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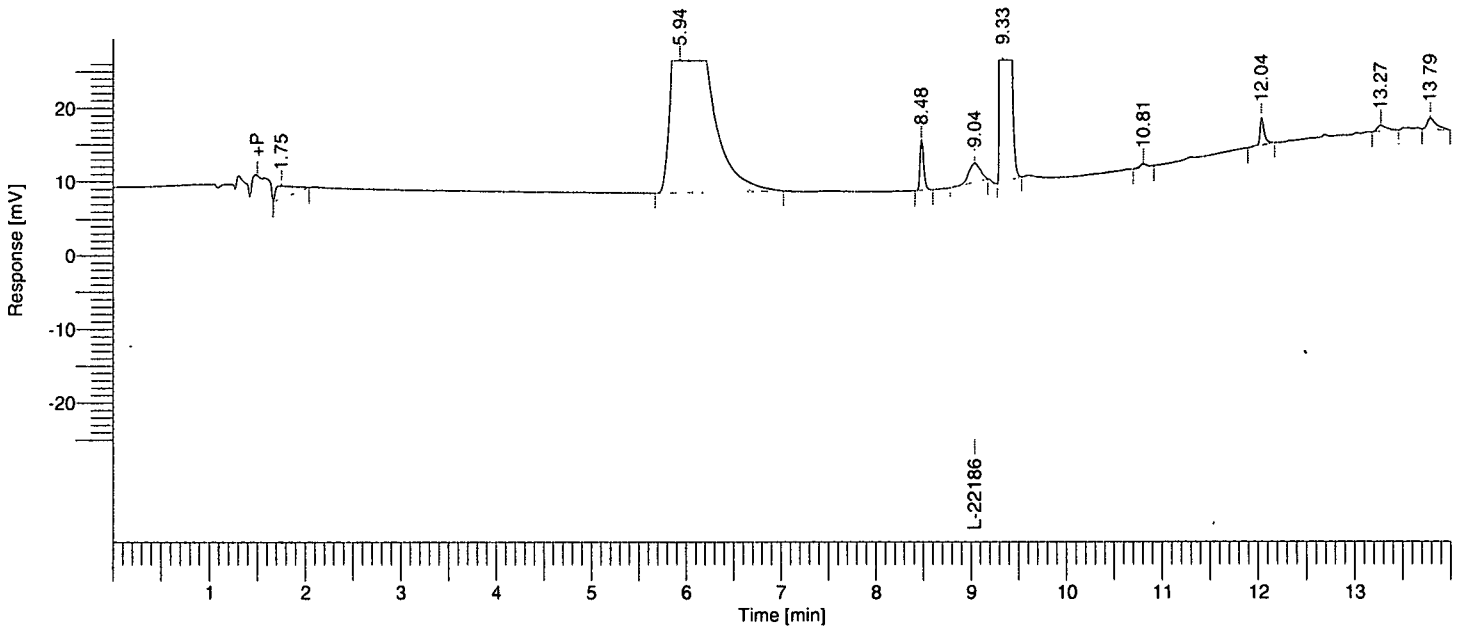

Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.750	0.19	41050.09	3110.28	0.77
2		5.936	0.66	2250861.96	131600.45	42.28
3		8.478	0.94	37416.13	13171.37	0.70
4	L-221869	9.039	1.00	41233.63	4891.41	0.77
5		9.332	1.03	2900716.34	770340.27	54.49
7		12.037	1.33	22596.51	7208.44	0.42
8		13.272	1.47	10450.08	1581.13	0.20
9		13.790	1.53	19179.11	2971.89	0.36
				5323503.85	934875.24	100.00

R. Leigh Shultz
11 Feb 2002

Subject: Syringe filter retention test for L-221869, L-224715; Test of MetaSil guard column

A Metasil MetaGuard cartridge was inserted into the Perkin Elmer LC system just before the column in preparation for running HPMC and gelatin samples. In order to test the guard column's performance, samples of L-224715 and L-221869 (standard solutions, concentrations 0.15 and 0.1 mg/mL, respectively) were run using the method L221869_method1. In addition, each one of the above samples was syringe-filtered at 0.2 microns before analysis; the first 0.75 mL to come through the filter was labeled "initial filtrate," and the remaining solution was labeled "bulk filtrate." The area counts of the initial and bulk samples will be compared to determine if L-221869 and L-224715 are retained on the syringe filters. The four samples were run in the sample set 60659.156A.seq.

Analysis of the chromatograms from sequence 60659.156A indicated that perhaps the guard column was causing a problem: all of the peaks in each chromatogram were split into two peaks. The guard column was removed from the system, and the samples were re-run to see if that solved the problem (same sequence). Removing the guard column eliminated the problem, so the gelatin and HPMC samples will have to be run without a guard column. Additionally, the chromatograms showed less than 1% loss of compound (for both L-221869 and L-224715) after syringe filtration. The gelatin and HPMC samples will therefore be syringe-filtered to removed particulates before analysis.

Subject: Test of assay for L-221869/L-224715 gelatin and HPMC binary mixtures

The samples of L-221869 tartrate with gelatin and HPMC and L-224715 with gelatin and HPMC which had been retained for HPLC method development (see 60659-145) were each extracted with 9.0 mL 0.1& phosphoric acid (3 x 3.0 mL). The gelatin samples appeared to completely dissolve, while the HPMC samples still had pariculates in them. Portions of each of the samples were syringe-filtered at 0.2 microns into HPLC vials. These filtered samples were analyzed in the sequence 60659.156B.seq using the method L221869_method1. No guard column was used for the assay.

Rebecca Leigh Shultz
11 Feb 2002

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25 Mar 2002
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R. Leigh Shultz
12 Feb 2002

Subject: HPLC assay for binary mixtures of L-224715/L-221869 with gelatin and HPMC

The HPLC test run data with gelatin and HPMC (60659-156) showed that HPMC will not be a problem for analysis using the current LC method. Chromatograms produced from HPMC mixtures look the same as those from neat drug, likely due to low solubility of HPMC in 0.1% phosphoric acid. Gelatin samples, however, looked vastly different; peaks from gelatin elute at 1.3 and 1.7 minutes (sharp) and a large, broad band elutes from ca. 7.4 minutes to ca. 9.6 minutes. The origin of these peaks was confirmed by analysis of a sample of gelatin (18.6 mg) dissolved in 9.0 mL 0.1% phosphoric acid; the solution was filtered at 0.2 microns prior to LC analysis (60659.157.seq). Chromatograms follow this page a supplementary material.

The tail of the large gelatin peak prevents baseline resolution of L-224715 or L-221869, and the carboxylate hydrolysis degradate is completely covered by it. However, it is unlikely that changes in the LC method will result in resolution of these issues; gelatin is proteinaceous, and both L-221869 and L-224715 are β-amino acid derivatives. The carboxylate hydrolysis degradate is also either a β-amino acid or a derivative as well. The LC method will be used as is for both gelatin and HPMC samples. Analysis of the water sample run after the gelatin mixtures indicated that gelatin was being retained on the column after the runs. The column was washed by injecting water 3 times using the method L-221869_method1. Detectable levels of gelatin remained after the washes, but they were much lower than before the wash. The column used to run these samples will therefore be used to run the gelatin binary mixtures; a separate column will be used to run pure drug samples and HPMC mixtures.

Subject: Bulk and solution stability of L-224715 phosphate salt (1-week data)

The 1-week bulk and solution stability samples for L-224715 phosphate (60659-153) were removed from the stability chambers at 11:00 am. The samples were placed in the freezer until they could be analyzed.

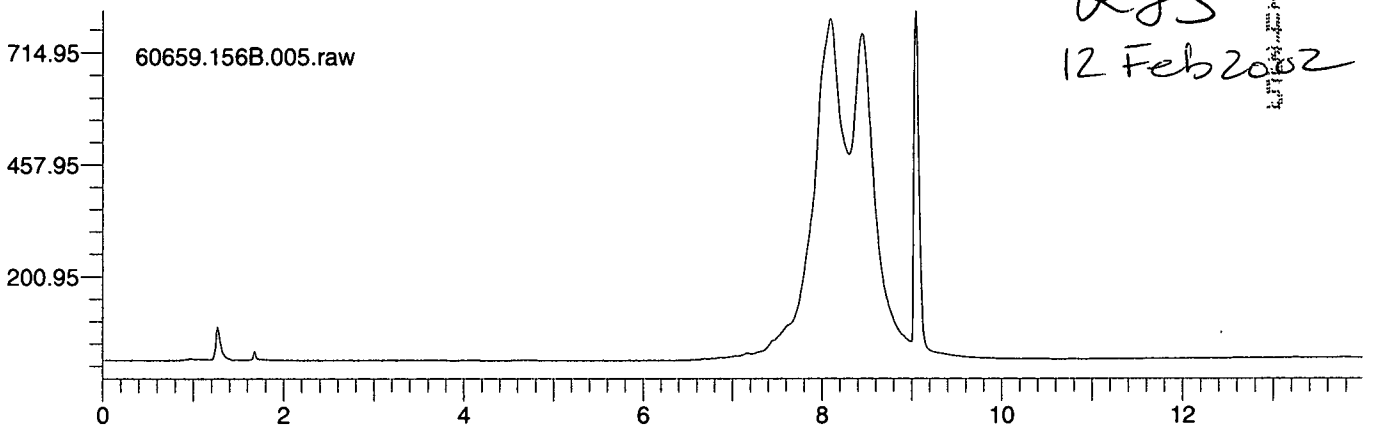
Subject: Analysis of gelatin mixtures (1- and 2-week) for L-224715 besylate, tartrate/L-221869 tartrate

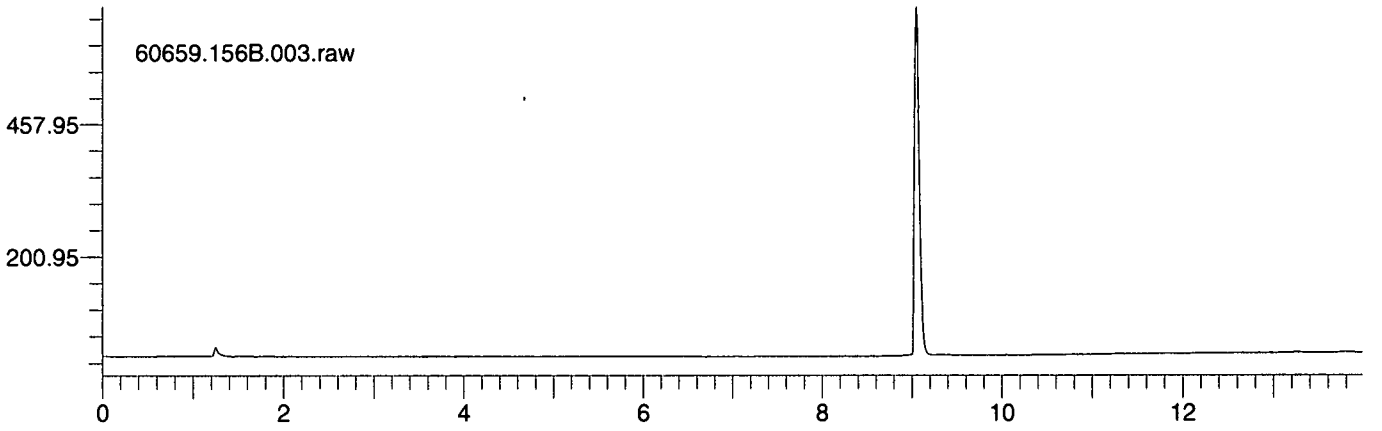
The 1- and 2-week stability samples with gelatin for L-224715 besylate (60659-144), L-221869 tartrate (60659-144a), and L-224715 tartrate (60659-147) were removed from the freezer at 1:00 pm. No changes were noted in the samples kept at 5 or 40 °C; however, samples stored at 40 °C/75% RH were noticeably clumped and stuck to the vial. Each sample was extracted with 0.1% phosphoric acid (3 x 3.0 mL), and a 1-mL aliquot of the resulting solution was syringe-filtered at 0.2 microns into an HPLC vial for analysis. The samples were analyzed in the sample set 60659.157B.seq using the method L-221869_method1.

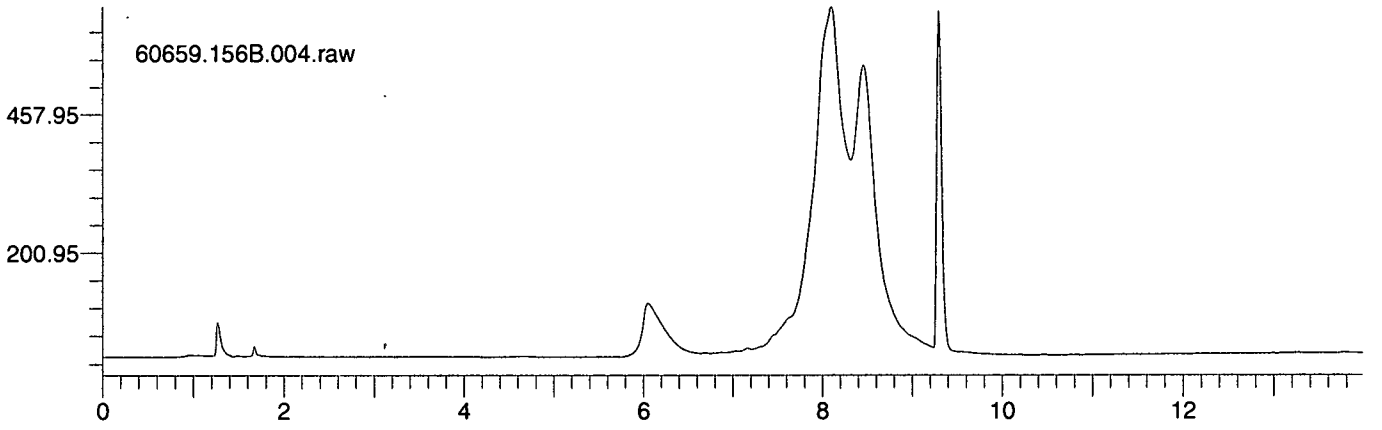
Rebecca Leigh Shultz
12 Feb 2002

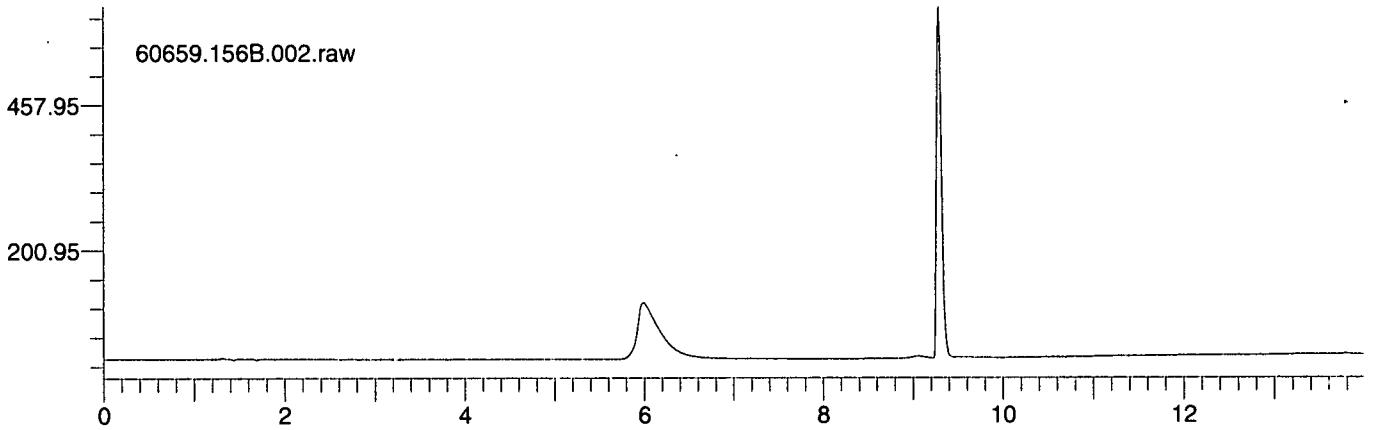
25 Mar 2002
COUNTERSIGNATURE DATE

60659-157#1
RSS
12 Feb 2002









66659-157-2
RJS
12Feb2002

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Sample Name	: water	Data Acquisition Time	: 2/11/02 4:47:34 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/1	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

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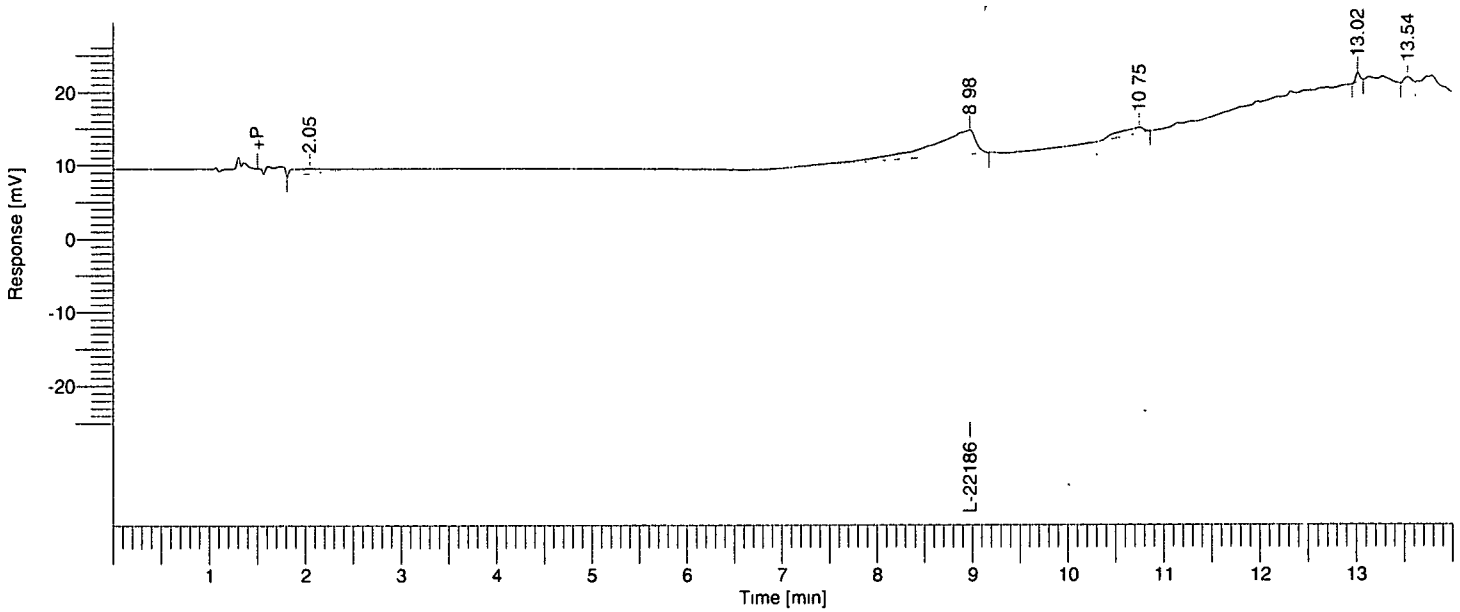

Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		2.046	0.23	35513.27	1345.82	12.31
2	L-221869	8.978	1.00	219827.09	6397.98	76.17
3		10.751	1.20	33262.87	1516.39	11.53
				288603.23	9260.19	100.00

60659-157-3
RSS
12 Feb 2002

Software Version	: 6.2.0.0.0:B27	Date	: 2/12/02 8:24:46 AM
Reprocess Number	: ry80m-108-pelc: 650		
Sample Name	: 715 Bs HPMC t0	Data Acquisition Time	: 2/11/02 5:07:05 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/2	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

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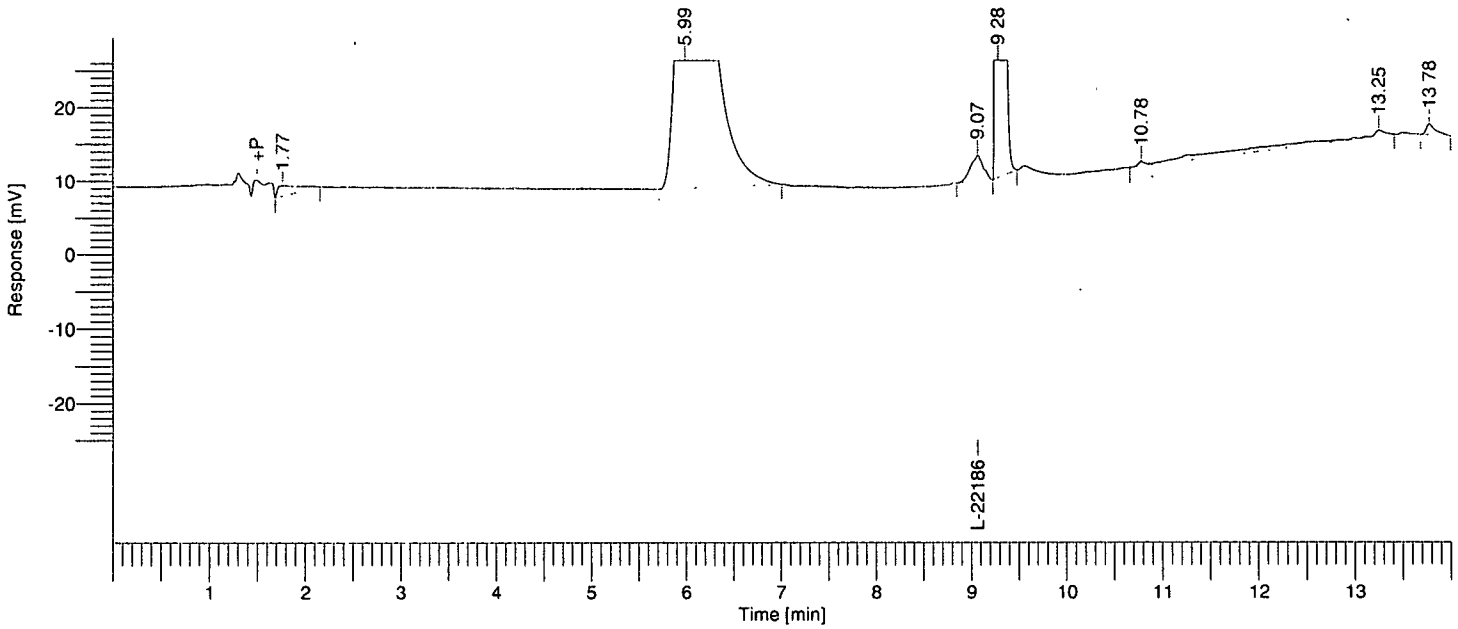

Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.770	0.20	41533.20	2672.22	0.49
2		5.989	0.66	3467379.37	193812.80	41.22
3	L-221869	9.069	1.00	63627.64	6967.90	0.76
4		9.283	1.02	4710683.59	1.21e+06	56.00
6		13.251	1.46	109932.15	1513.00	1.31
7		13.775	1.52	18860.87	2848.40	0.22
				8412016.82	1.42e+06	100.00

60659-157-4
RJS
12 Feb2002

Software Version	: 6.2.0.0.0:B27	Date	: 2/12/02 8:24:47 AM
Reprocess Number	: ry80m-108-pelc: 651		
Sample Name	: 869 tar HPMC t0	Data Acquisition Time	: 2/11/02 5:26:37 PM
Instrument Name	: PELC	Channel	: A
Rack/Vial	: 1/3	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

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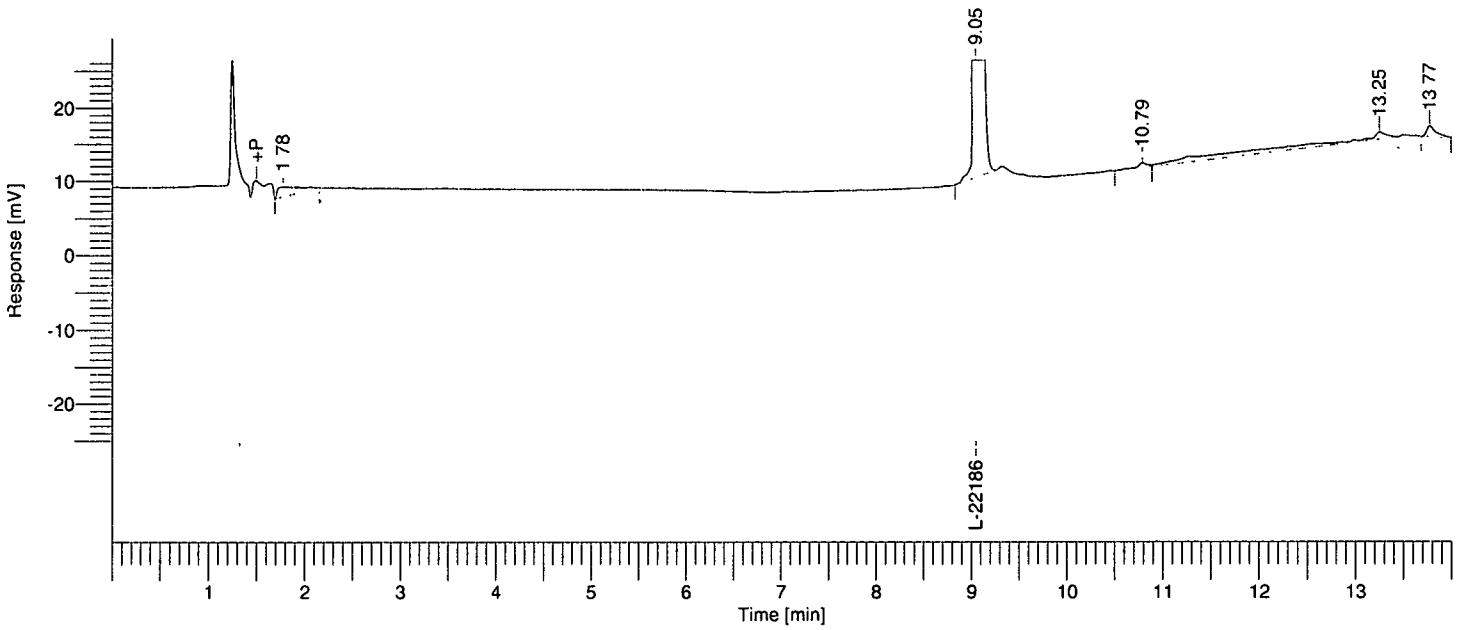
Sequence File : D:\Sequences\L224715\60659.156B.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.777	0.20	39990.23	2589.70	0.78
2	L-221869	9.049	1.00	4923185.32	1.32e+06	96.49
4		13.252	1.46	120344.00	1847.42	2.36
5		13.774	1.52	18853.40	2887.47	0.37
				5102372.95	1.32e+06	100.00

60659-157-5
RSS
12 Feb 2002

Software Version	: 6.2.0.0.0:B27	Date	: 2/12/02 8:24:48 AM
Reprocess Number	: ry80m-108-pelc: 652		
Sample Name	: 715 bs gelatin t0	Data Acquisition Time	: 2/11/02 5:46:09 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/4	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 4		

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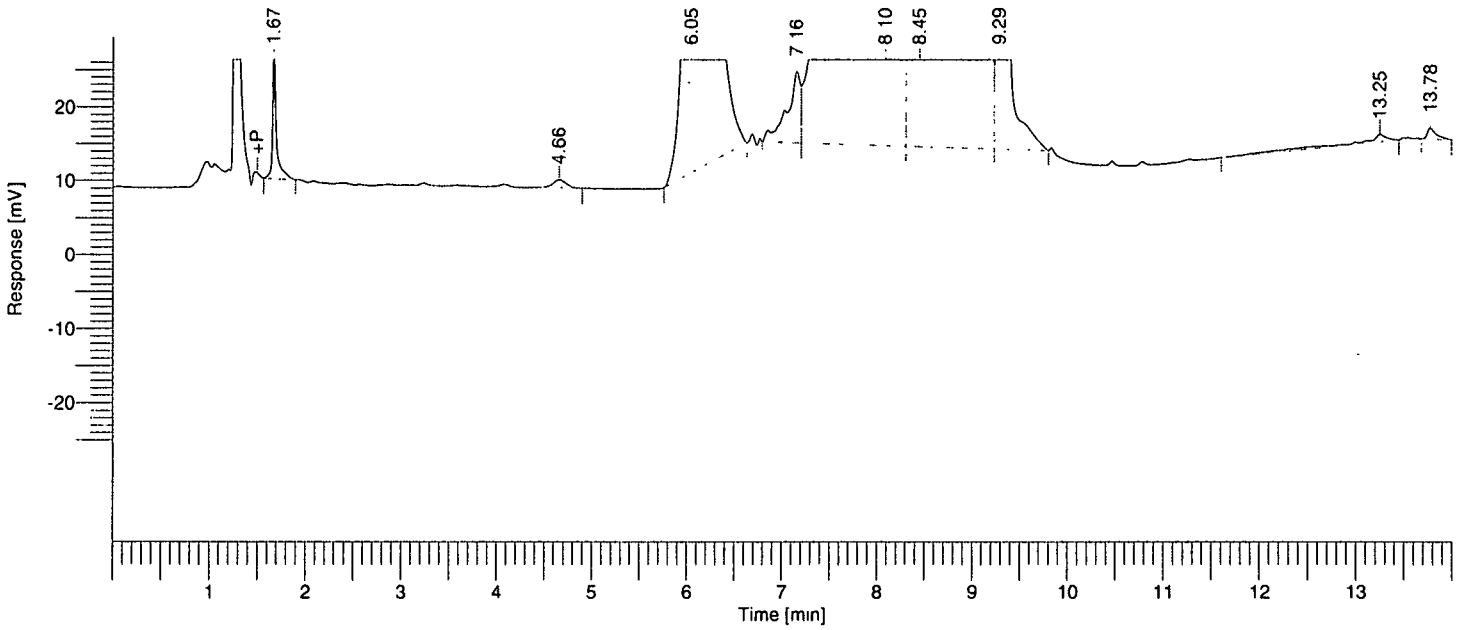


Single Injection Report

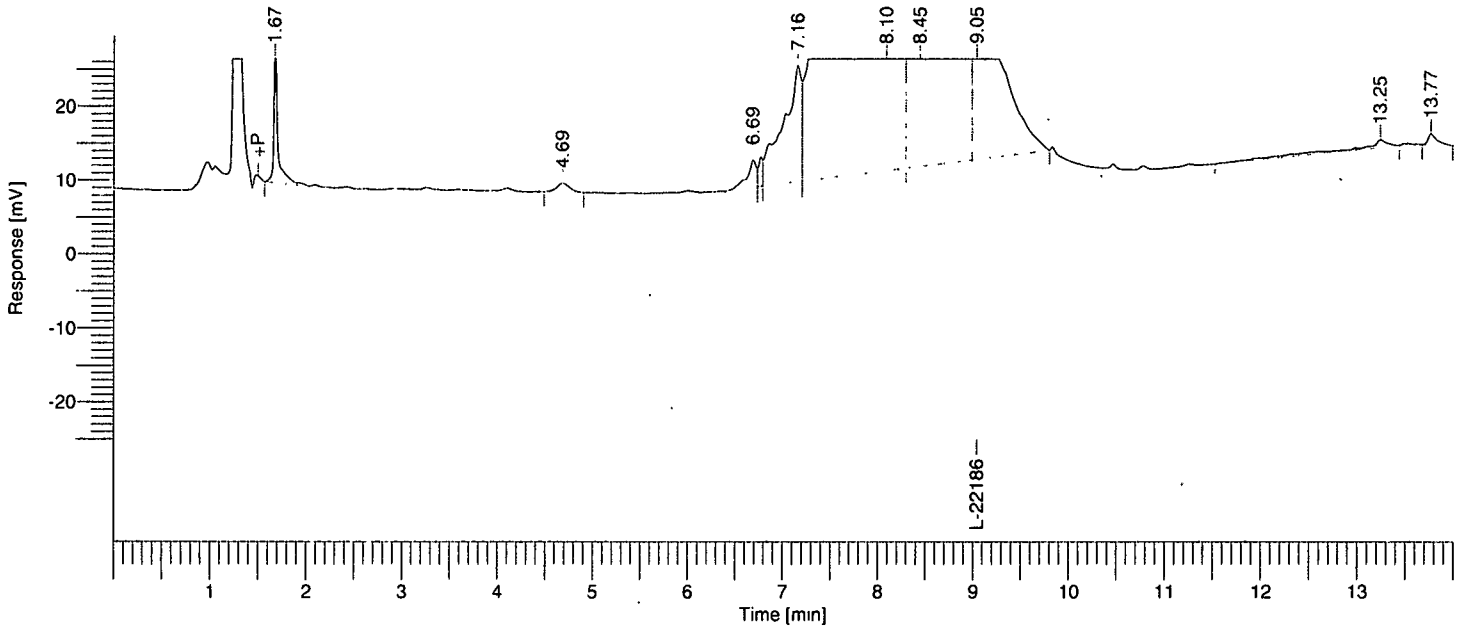
Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.672	0.19	94730.01	36247.24	0.16
2		4.661	0.53	18703.47	2061.71	0.03
3		6.052	0.69	3156375.80	187402.33	5.44
4		7.163	0.81	186770.23	18855.03	0.32
5		8.097	0.92	30171324.27	1.25e+06	52.04
6		8.455	0.96	19239371.54	1.04e+06	33.18
7		9.291	1.05	5043966.64	1.24e+06	8.70
8		13.251	1.50	46402.80	1914.03	0.08
9		13.775	1.56	18843.06	2908.60	0.03
				57976487.82	3.79e+06	100.00

60659-157-6
RJS
12 Feb 2002

Software Version	: 6.2.0.0.0:B27	Date	: 2/12/02 8:24:49 AM
Reprocess Number	: ry80m-108-pelc: 653		
Sample Name	: 869 tar gelatin t0	Data Acquisition Time	: 2/11/02 6:05:41 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/5	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 5		

Result File : D:\Projects\L224715\RawData\60659.156B.005.rst
Sequence File : D:\Sequences\L224715\60659.156B.idx



Single Injection Report

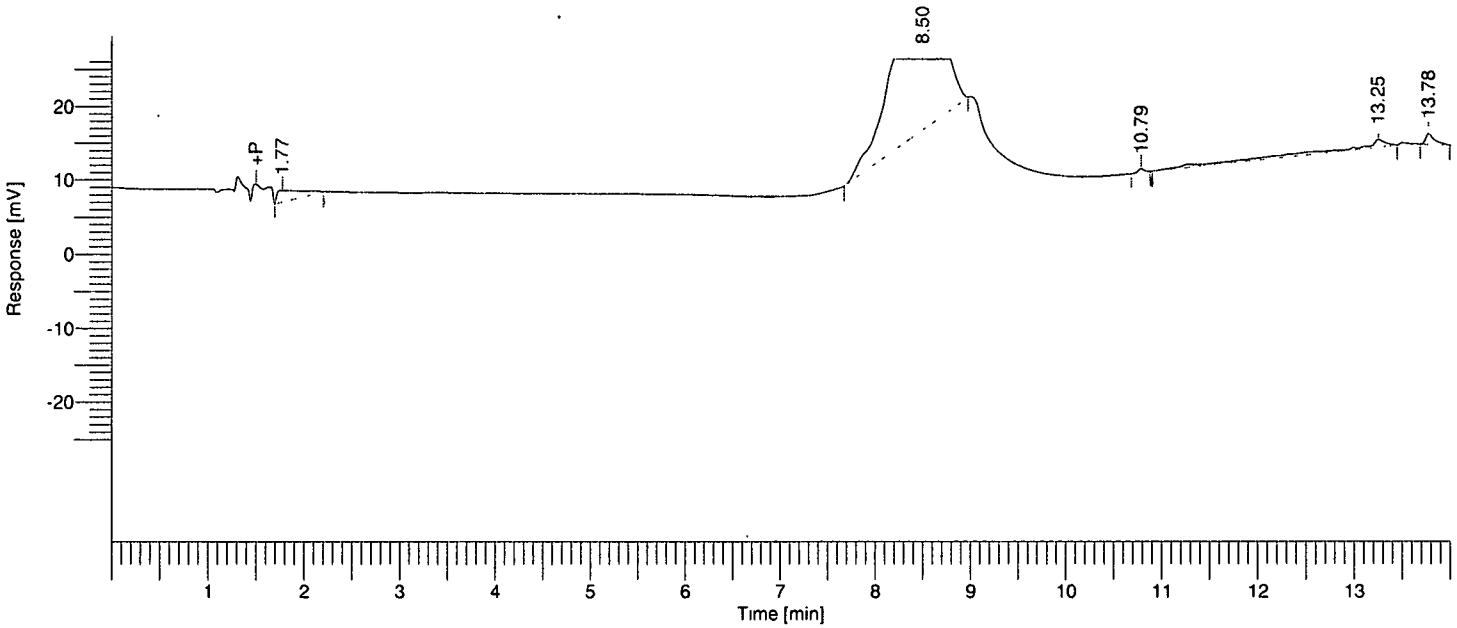
Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.675	0.19	107951.80	39543.48	0.16
2		4.685	0.52	20093.64	2195.89	0.03
3		6.691	0.74	49511.69	7233.23	0.07
4		7.164	0.79	419503.36	30498.70	0.61
5		8.097	0.90	35833023.46	1.52e+06	51.86
6		8.449	0.93	25720112.90	1.45e+06	37.22
7	L-221869	9.046	1.00	6880075.37	1.55e+06	9.96
8		13.246	1.46	47328.81	1951.01	0.07
9		13.772	1.52	18589.15	2865.07	0.03
				69096190.19	4.61e+06	100.00

60659-157-7
RJS
12 Feb 2002

Software Version	: 6.2.0.0.0:B27	Date	: 2/12/02 8:24:50 AM
Reprocess Number	: ry80m-108-pelc: 654		
Sample Name	: water	Data Acquisition Time	: 2/11/02 6:25:12 PM
nstrument Name	: PELC	Channel	: A
Rack/Vial	: 1/1	Operator	: lhshultz
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 6		

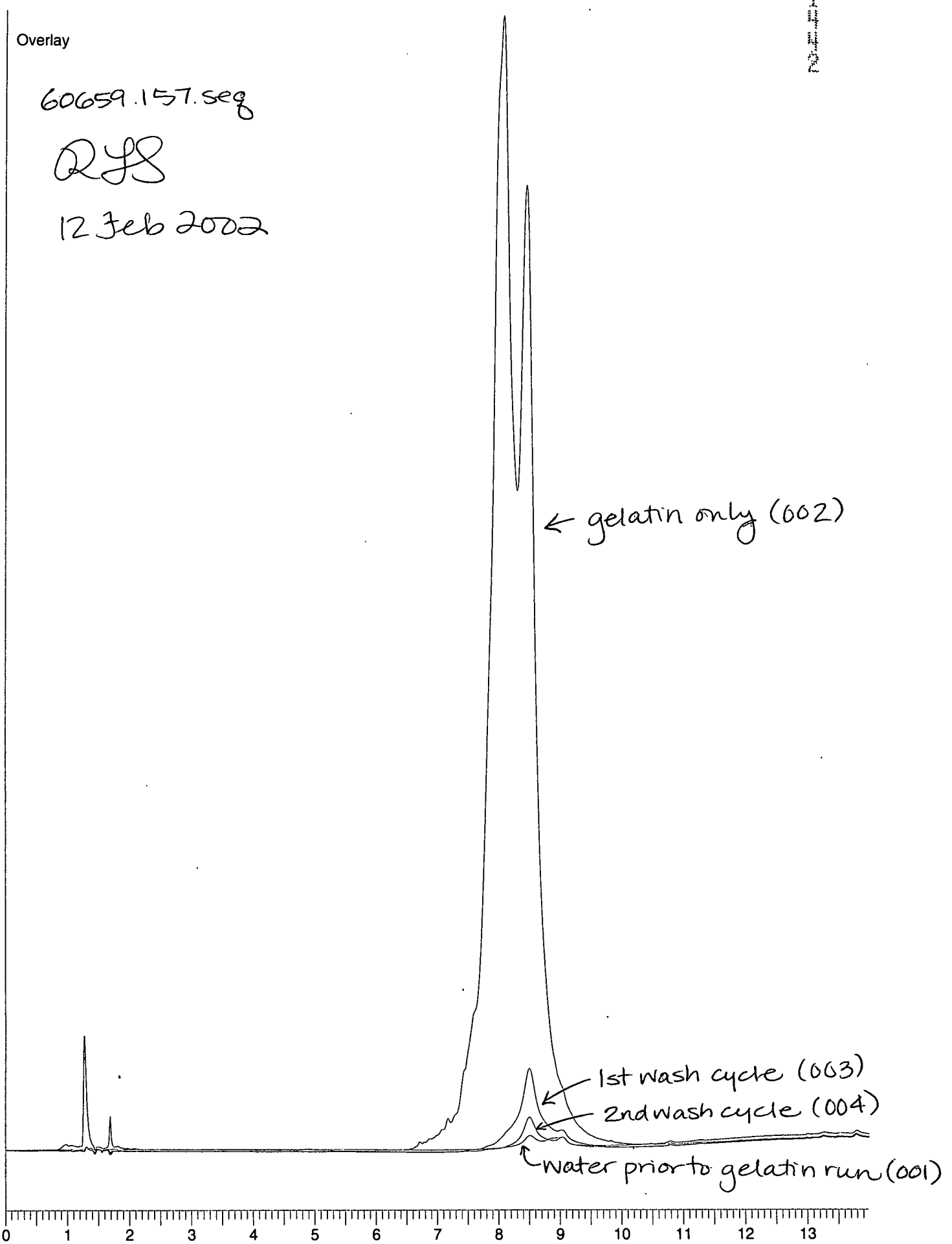
Result File : D:\Projects\L224715\RawData\60659.156B.006.rst
Sequence File : D:\Sequences\L224715\60659.156B.idx



Single Injection Report

Rebecca Leigh Shultz
NB 60659

Peak #	Component Name	Time [min]	RRT	Area [uV*sec]	Height [uV]	Area [%]
1		1.774	0.20	49901.77	2944.55	2.08
2		8.503	0.96	2258920.04	114300.10	94.16
4		13.253	1.50	71503.33	1935.73	2.98
5		13.777	1.56	18605.24	2879.38	0.78
				2398930.38	122059.77	100.00


Overlay
60659.157.seq
RJS
12 Feb 2002
← gelatin only (002)
1st wash cycle (003)
2nd wash cycle (004)
water prior to gelatin run (001)
0
1
2
3
4
5
6
7
8
9
10
11
12
13

R. Leigh Shultz
12 Feb 2002

Subject: Solubility of L-224715 phosphate and L-224715 besylate

In order to determine the solubility of L-224715 phosphate in a variety of solvents, samples of the salt (NB70316-25) were weighed into tared test tubes according to the table below. Solvent was added to each tube in 0.10 mL (or 0.05 mL) increments. The water sample dissolved after addition of 0.150 mL; the saline and pH 2 samples dissolved after addition of 0.100 mL. The methanol, ethanol, and 2-propanol samples did not dissolve completely and were left to equilibrate on the rotator overnight (started at 10:30 am). In addition, a sample of the besylate salt of L-224715 was weighed out and water was added to more accurately determine the solubility in water. The besylate salt did not dissolve after addition of 0.3 mL of water, but addition of an additional 0.05 mL of water caused complete dissolution of the solid. The details of the experiment appear in the table below.

Salt of L-224715	Solvent	Mass salt (mg)	Volume added (mL)	pH_{intial}	Observations
phosphate	water	15.02	0.10 + 0.05	6.13	very soluble
phosphate	0.9% NaCl	7.19	0.10	5.57	dissolved immediately
phosphate	0.01N HCl	7.51	0.10	2.08	dissolved immediately
phosphate	methanol	7.76	5 x 0.10	n/a	wettable
phosphate	ethanol	8.54	5 x 0.10	n/a	wettable
phosphate	2-propanol	6.13	5 x 0.10	n/a	wettable
besylate	water	18.64	(3 x 0.10) + 0.05	6.13	soluble (slowly)

The data indicate that the solubility of the phosphate salt in water is >100.1 and <150.2 mg/mL salt (equivalent to >80.7 and <121.1 mg/mL L-224715). The solubility in saline is >71.9 mg/mL salt (>58.0 mg/mL parent) and in 0.01N HCl is >75.1 mg/mL salt (>60.5 mg/mL parent). The solubility of the besylate is >53.3 mg/mL salt (38.4 mg/mL L-224715). The alcoholic solubility samples for the phophate salt will be assayed by HPLC after equilibration.

Subject: Analysis of HPMC mixtures (1- and 2-week) for L-224715 besylate, tartrate/L-221869 tartrate

The 1- and 2-week stability samples with HPMC for L-224715 besylate (60659-144), L-221869 tartrate (60659-144a), and L-224715 tartrate (60659-147) were removed from the freezer at 4:00 pm. No changes were noted in the samples kept at 5 or 40 °C; however, samples stored at 40 °C/75% RH were slightly yellowed. Each sample was extracted with 0.1% phosphoric acid (3 x 3.0 mL), transferred to a 20-mL scintillation vial, and the vials were placed in the refrigerator (5 °C) overnight for analysis on 13 Feb 2002.

Rebecca Leigh Shultz
12 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
13 Feb 2002

Subject: Solubility of L-224715 phosphate salt

The methanol, ethanol, and 2-propanol solubility samples were removed from the rotator at 9:00 am. The tubes were centrifuged to settle the remaining solids, and aliquots of the supernatants were removed (pipet), diluted with 0.1% phosphoric acid, and assayed by HPLC (sequence 60659.159) to determine the concentration. The specific dilutions are outlined in the table below, along with the solubility results, obtained from the calibration found on page 60659-143.

Solvent	Volume aliquot (mL)	Volume total solution (mL)	Solubility (mg/mL salt)	Solubility (mg/mL L-224715)
methanol	0.15	3.00	0.41	0.33
ethanol	0.30	3.00	0.045	0.036
2-propanol	0.30	0.60	0.098	0.079

The salt is highly soluble in water but much less soluble in alcohols. The higher solubility in 2-propanol with respect to ethanol is unusual and may be a result of evaporation of solvent from a very small volume of solution (this result should be repeated for confirmation).

Subject: Bulk and solution stability of L-224715 phosphate salt (1-week samples)

The 1-week samples for the phosphate salt were removed from the freezer (60659-157) and were allowed to come to ambient temperature. The solid samples were dissolved in 3.0 mL of 0.1% phosphoric acid each, and each solution was then diluted to 19.0 mL. Aliquots of these three solutions were placed in HPLC vials for analysis along with the solution samples. The samples were analyzed in the sequence 60659.159.seq using the method L-221869_method1.

Subject: Analysis of HPMC mixtures (1-and 2-week) for L-224715 besylate. tartrate/L-221869 tartrate

The HPMC solutions (60659-158) were removed from the refrigerator and allowed to come to ambient temperature. A 1-mL aliquot of each solution was syringe-filtered at 0.2 microns into an HPLC vial for analysis. The samples were analyzed in the sample set 60659.159.seq using the method L221869_method1.

Rebecca Leigh Shultz
13 Feb 2002

RLS 13 Feb 2002

Kathy Pfeifer 25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
14 Feb 2002

Subject: Physical stability of L-224715 besylate (anhydrate form A and hemi-hydrate) and phosphate salts

In order to assess the physical stability of the salts of L-224715, samples of besylate anhydrate form A (70316-9), besylate hemi-hydrate (70316-29), phosphate (70316-35) were weighed into vials and placed in the stability chambers at 8:30 am. A desiccator containing a saturated solution of LiCl was used to create 10% RH; it was stored in the dark on the bench. The table below details the samples prepared.

Salt	Form	Condition	Mass (mg salt)
besylate	anhydrate form A	25 °C/60% RH	22.83
besylate	anhydrate form A	40 °C/75% RH	22.40
besylate	anhydrate form A	40 °C/amb RH	23.90
besylate	hemi-hydrate	25 °C/10% RH	24.88
besylate	hemi-hydrate	40 °C/75% RH	22.84
besylate	hemi-hydrate	40 °C/amb RH	22.20
phosphate	anhydrate	25 °C/60% RH	21.94
phosphate	anhydrate	40 °C/75% RH	21.10
phosphate	anhydrate	40 °C/amb RH	21.79

Samples will be removed from the vials at 1, 2, and 4 weeks to determine physical stability of each form (by DSC/TGA).

The stability ovens were drained and moved to make room for the X-ray, causing the samples to have to be removed. They were not under the correct conditions for ca. 6 hours. This applies to all samples currently on stability.

Subject: Stability of L-224715 phosphate salt in binary mixtures with gelatin/HPMC

The 1-week samples of phosphate salt with gelatin and HPMC (60659-155) were removed from the stability stations at 10:00 am and placed in the freezer until they could be analyzed.

Rebecca Leigh Shultz
15 Feb 2002

15 Feb 2002

Subject: Stability of L-224715 besylate salt (chemical, 4-week samples)

The four-week samples of the besylate salt of L-224715 were removed from the stability stations at 9:00 am. They were placed in the freezer until they could be analyzed.

Rebecca Leigh Shultz
15 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
15 Feb 2002

Subject: Physical stability of L-224715 tartrate and phosphate salts with gelatin/HPMC

The gelatin and HPMC mixtures of L-224715 tartrate and phosphate (60659-147 and –155, respectively) were received back from Bob Wenslow. Each sample was then separated into two vials according to the table below. Vials phys1 were placed in the 40 °C/amb RH oven at 5:00 pm; vials phys2 were placed in the 40 °C/75% RH oven (open cap) at 5:00 pm.

Sample ID	Salt of L-224715	Mass (mg)
60659-147 phys1/gel	L-tartrate hemihydrate	47.98
60659-147 phys2/gel	L-tartrate hemihydrate	55.10
60659-147 phys1/HPMC	L-tartrate hemihydrate	47.46
60659-147 phys2/HPMC	L-tartrate hemihydrate	49.99
60659-155 phys1/gel	phosphate	51.71
60659-155 phys2/gel	phosphate	47.31
60659-155 phys1/HPMC	phosphate	50.48
60659-155 phys2/HPMC	phosphate	46.30

The vials will be checked for form changes periodically by SSNMR.

Rebecca Leigh Shultz
19 Feb 2002

19 Feb 2002

25 Mar 2002
COUNTERSIGNATURE DATE

R. Leigh Shultz
19 Feb 2002

Subject: Bulk and solution stability of L-224715 tartrate salt (4-week samples)

The 4-week samples of L-224715 tartrate salt (bulk and solution, 60659-143) were removed from the stability stations at 1:00 pm. They were placed in the freezer for storage and will be assayed if needed. These samples represent the *4 week plus 1 day* timepoint.

Subject: Stability of L-224715 besylate and L-221869 tartrate with gelatin and HPMC (4-wk samples)

The binary mixtures of L-224715 besylate and L-221869 tartrate salt (4-week samples, 60659-144, 145) were removed from the stability stations at 1:00 pm. They were placed in the freezer until they could be assayed by HPLC.

Subject: Bulk and solution stability of L-224715 phosphate (2-wk samples) and besylate (4-wk samples)

The 2-week stability samples (bulk and solution) for the phosphate salt of L-224715 (60659-153) were removed from the stability stations at 1:00 pm. There were allowed to come to ambient temperature, and the bulk samples were each dissolved in 19.0 mL of 0.1% phosphoric acid. Aliquots of these solutions were transferred to HPLC vials, and these bulk solutions were assayed in the sequence 60659.162.seq (method L221869_method1) along with the solution stability samples.

The 4-week stability samples (bulk and solution) for the besylate salt of L-224715 (60659-160) were removed from the freezer at 1:00 pm. They were allowed to warm to ambient temperature, and the bulk samples were each dissolved in 19.0 mL of 0.1% phosphoric acid. An aliquot of each bulk sample solution was transferred to an HPLC vial, and these bulk and solution samples were analyzed with the phosphate salt samples above in the same sample set.

Subject: Physical stability of L-224715 phosphate solubility samples in alcohols

The solids remaining in the methanol, ethanol,and 2-propanol solubility samples for the phosphate salt of L-224715 were examined under 200X magnification (optical microscope). No amorphous material was noted in any of the samples, and the crystal morphology did not appear different from a fresh sample of the salt in oil.

Rebecca Leigh Shultz
19 Feb 2002

RLS 19 Feb 2002

COUNTERSIGNATURE 25 Mar 2002 DATE

R. Leigh Shultz
20 Feb 2002

Subject: Detection of degradates in drug samples containing gelatin

In order to determine if changing the wavelength of detection in the LC method L221869_method1 would allow detection of small amounts of the hydrolysis degradate which coelutes with gelatin, a method (L221869_method1_260) was created from the original method with a new detection wavelength (260 nm). L-221869, L-224715, and their carboxylate hydrolysis degradates all have absorption maxima near that wavelength. A sample of gelatin dissolved in 0.1% phosphoric acid and syringe-filtered was run with the method, as was the sample 715 Bs D2g (L-224715 besylate salt, 5% drug load in gelatin, stored for 2 weeks at 40 °C/75% RH). The gelatin peak was much smaller with respect to the parent peak at this wavelength, but no hydrolysis degradate was detected. A sample containing a small amount of this degradate needs to be run to see if the degradate is visible.

Subject: pH changes in aqueous samples of L-224715 tartrate salt

NMR experiments (P. Dormer) performed on the tartrate salt of L-224715 in D2O at 29.6 mg/mL salt indicate that the salt does not disproportionate over 84 hours at 40 °C. The pH of the sample at the end of the NMR run was ca. 4, consistent with this result. However, the stability samples of the tartrate salt in water (0.1 mg/mL) experience a pH change of 2.5 units (5.1 to 7.6) over 48 hours at 40 °C. Therefore, a solution of concentration 29.6 mg/mL salt was made by dissolving 16.1 mg tartrate salt in 0.54 mL of water. The pH of this solution was 3.29. The tube containing the solution was placed at 40 °C at 2:30 pm. The pH will be monitored over time to see if it rises.

Subject: Stability of L-224715 and L-221869 salts with gelatin

The 1-week stability samples of L-224715 phosphate plus gelatin (1 week), L-224715 besylate plus gelatin (4 week), and L-221869 tartrate plus gelatin (4 week) were removed from the freezer at 2:00 pm. No physical changes were observed in the samples except that the 40 °C/75% RH samples had clumped and no longed flowed freely in their vials. Each sample was extracted with 0.1% phosphoric acid (3x3.0 mL), and a 1-mL aliquot of the resulting mixture was syringe-filtered at 0.2 microns into an HPLC vial for analysis. Each sample was analyzed twice, once at 210 nm (method L221869_method1) and once at 260 nm (L221869_method1_260), in the sample set 60659.163A.seq. The 1- and 2-week samples for the besylate of L-224715 and the tartrate of L-221869 were also added to this sequence and re-analyzed at 260 nm.

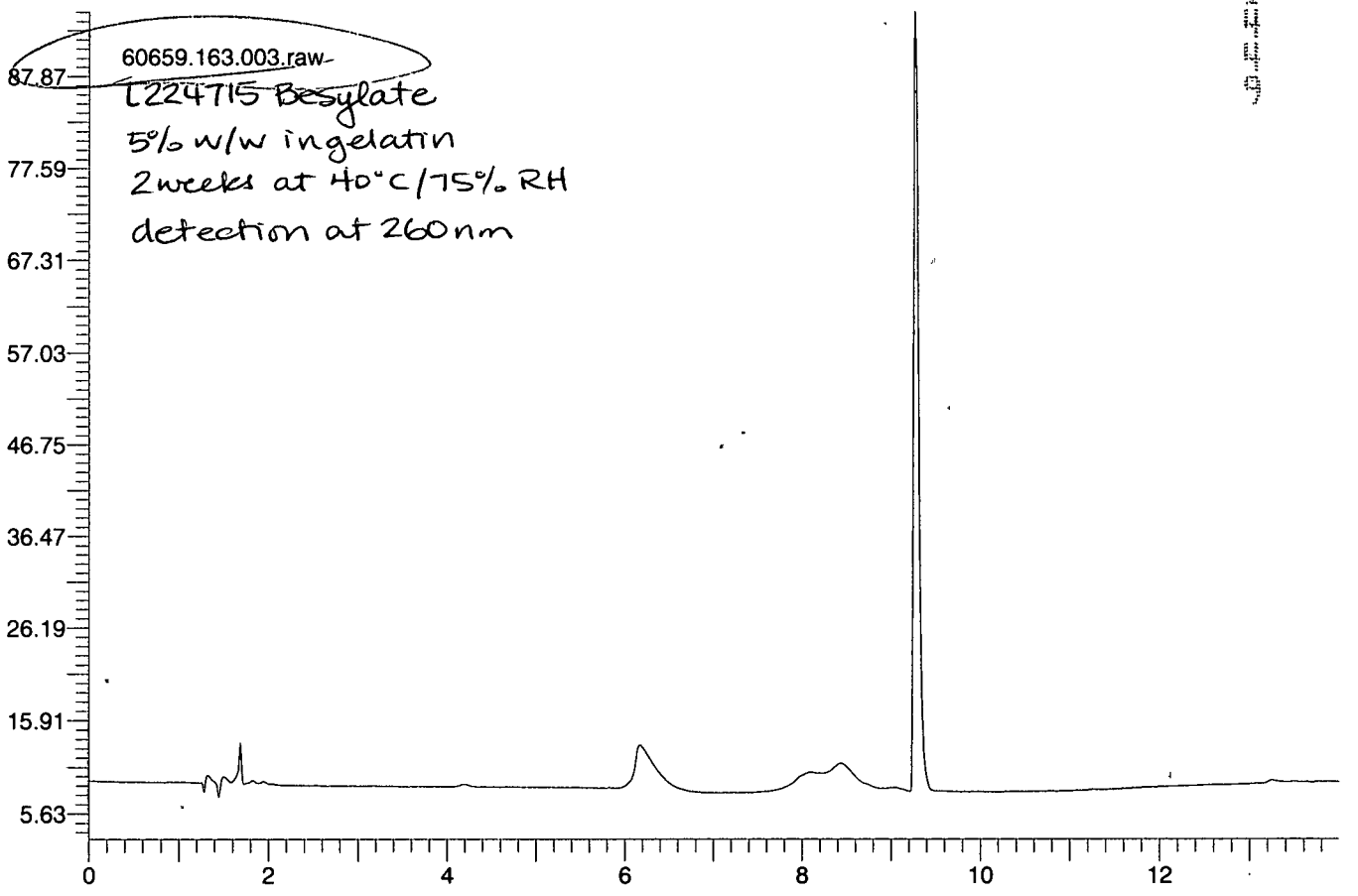
Subject: Thermal analysis of L-224715 phosphate salt (NB 70316-35)

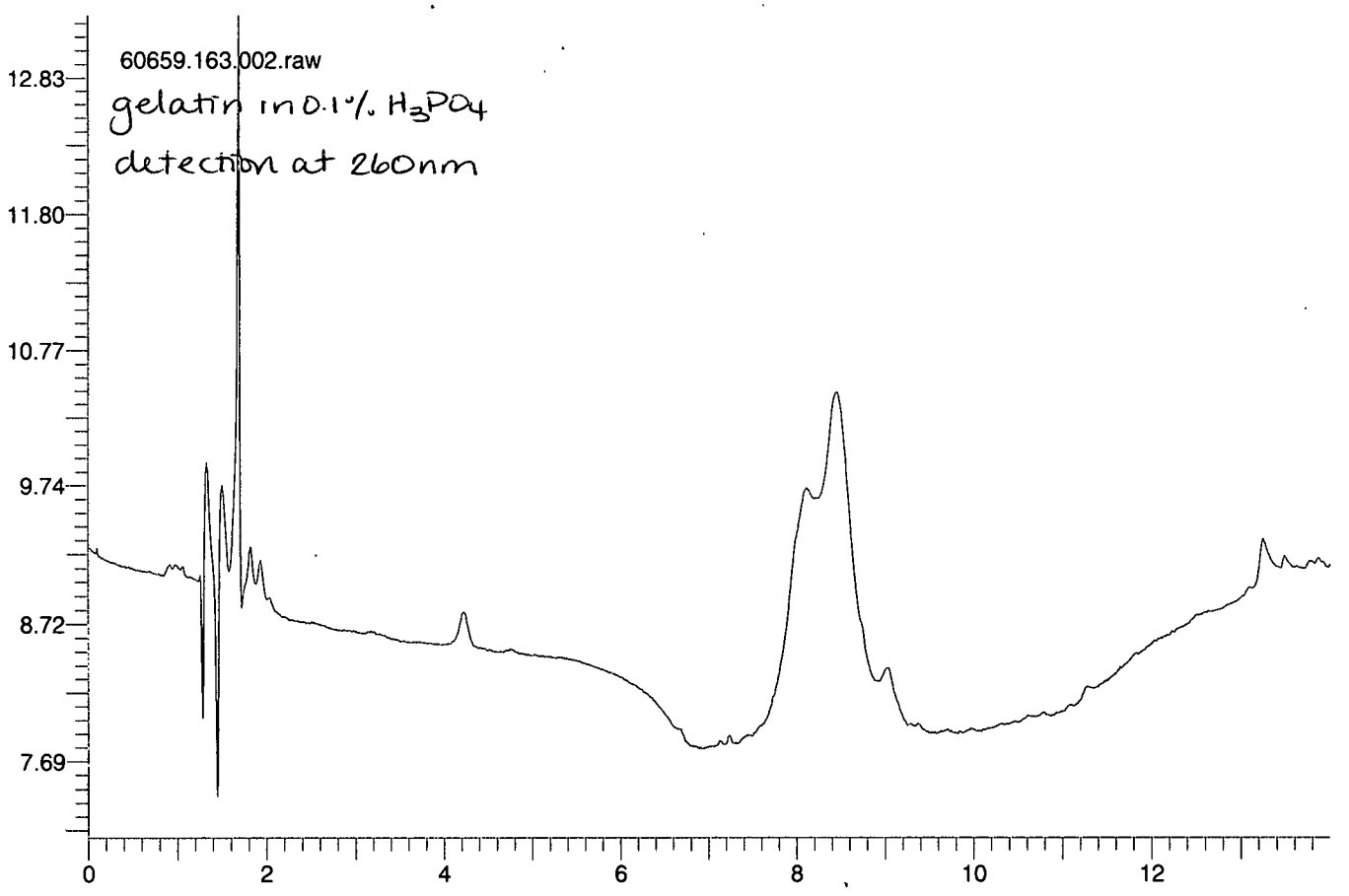
In order to have a point of reference from which to examine the physical stability of the phosphate salt of L-224715, DSC and TGA traces were obtained for the salt. Data and analysis can be found on the ~~next~~ notebook page ~~(164)~~. 60659-165

Correction
RLS
13Jun2002

Rebecca Leigh Shultz
20 Feb 2002

Witness 25 Mar 2002
COUNTERSIGNATURE DATE





Rebecca Leigh Shields
21 Feb 2002

R. Leigh Shultz
21 Feb 2002

Subject: Stability of L-224715 and L-221869 salts with HPMC

The 4-week samples of L-224715 tartrate with gelatin and HPMC were removed from the stability stations at 9:00 am. They were placed in the freezer in case analysis becomes necessary. The 2-week samples of L-224715 phosphate with gelatin and HPMC were also removed from the stability stations at 9:00 am. The gelatin samples were placed in the freezer, and the HPMC samples were prepared for analysis. The stability samples of L-224715 phosphate plus HPMC (1 week), L-224715 besylate plus HPMC (4 week), and L-221869 tartrate plus HPMC (4 week) were removed from the freezer at 9:00 am. No physical changes were observed in the samples except that the 40 °C/75% RH samples had darkened slightly. Each sample was extracted with 0.1% phosphoric acid (3x3.0 mL), and a 1-mL aliquot of the resulting mixture was syringe-filtered at 0.2 microns into an HPLC vial for analysis. Each sample was analyzed once at 210 nm (method L221869_method1) in the sample set 60659.164A.seq.

Subject: pH changes in aqueous samples of L-224715 tartrate salt

The 29.6 mg/mL salt solution of the tartrate was removed from the 40 °C oven and allowed to cool to room temperature. The pH was measured using a pH meter; it was 3.33. The sample was capped and placed back at 40 °C for further aging.

Subject: Stability of L-224715 phosphate salt with 2 grades of mannitol

In order to investigate the stability of L-224715 phosphate salt (70316-35) with mannitol, binary mixtures of the salt were prepared with Pearlitol 200SD (lot 5053) and with SPI mannitol (lot 24340). Samples of the salt were weighed out (see table below), as were samples of the mannitols. The salt sample was ground gently with a mortar and pestle with ca. 85 mg of the excipient; the rest of the excipient was then added, and the mixture was ground until uniform.

Mass L-224715 salt (mg)	Mass L-224715 parent (mg) FB = 0.806(PO_4 salt)	Excipient	Total mass (exc + salt) (mg)	% w/w drug loading
40.02	32.26	Pearlitol	314.99	10.2
39.75	32.04	SPI	312.54	10.3

Samples of the mixtures were then weighed into vials (10 vials for each mixture) for stability studies according to the following table.

Sample (Pearlitol)	Mass mixture (mg)	Sample (SPI)	Mass mixture (mg)
60659-164 A1/Pearl	22.22	60659-164 A1/SPI	22.56
60659-164 A2/Pearl	20.36	60659-164 A2/SPI	21.43
60659-164 A4/Pearl	20.43	60659-164 A4/SPI	21.48
60659-164 C1/Pearl	20.08	60659-164 C1/SPI	21.65
60659-164 C2/Pearl	21.23	60659-164 C2/SPI	24.50
60659-164 C4/Pearl	21.09	60659-164 C4/SPI	20.67
60659-164 D1/Pearl	20.41	60659-164 D1/SPI	20.17
60659-164 D2/Pearl	22.68	60659-164 D2/SPI	20.89
60659-164 D4/Pearl	21.05	60659-164 D4/SPI	23.13
60659-164 phys/Pearl	115.71	60659-164 phys/SPI	103.06

Samples A, C, and D were placed in the stability stations at 5:30 pm. Samples D had open caps; the rest of the samples had closed caps. Samples 60659-164 phys/Pearl and phys/SPI were taken to Bob Wenslow for solid-state ^{19}F NMR studies.

Rebecca Leigh Shultz
25 Feb 2002

COUNTERSIGNATURE DATE
Cathy Rhodes 25 Mar 2002

R. Leigh Shultz
21 Feb 2002

Subject: Thermal analysis of L-224715 salts

DSC and TGA analyses were perfomed on the phosphate salt of L-224715 (see 60659-163), form A of the anhydrous besylate salt, and the hemi-hydrate of the besylate salt. The data follow this page as supplemental information. The findings from the experiments are detailed in the table below.

Salt of L-224715	NB Reference	mp onset (°C)	heat of melting (J/g)	% volatiles
phosphate	70316-35	207.51	166.4	0.053
besylate anhydrate form A	70316-9	172.88	56.76	0.043
besylate hemi-hydrate	70316-29 and 32139-133	172.40	56.90	2.008

The phosphate salt shows a melt and then immediate decomposition, so the integration of the endotherm is not quantitative and cannot be used for determination of crystallinity. The material is a single polymorph, however, and it is not hydrated.

The anhydrous besylate salt is also a single polymorph for now (form A) and has a sharp melting endotherm that is quantifiable. There is a slight weight loss in the TGA upon melting. The material remains anhydrous when stored at ambient conditions.

The hemi-hydrate of the besylate salt begins to lose water immediately upon heating, and all water is lost by 80 °C under a stream of nitrogen. Loss of water occurs in the DSC (closed pan) at 114 °C, causing formation of anhydrate form B (known from studies done by C. Lindemann, Physical Measurements). Form B undergoes a solid-solid transition to form A at 131 °C. The resulting form A has the same melting point as the anhydrate form A sample examined above (onset 172 °C).

Rebecca Leigh Shultz
25 Feb 2002

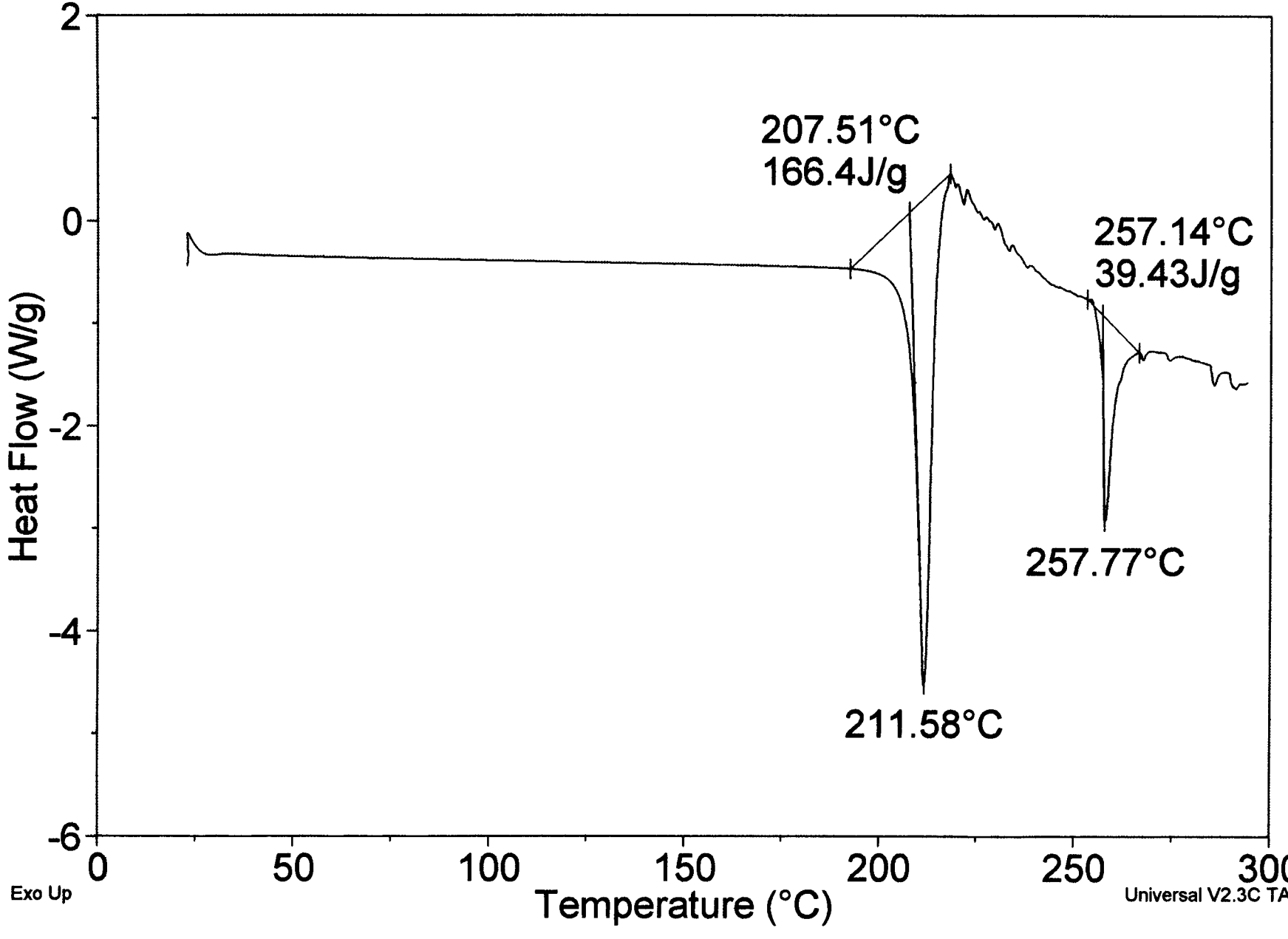
RLS 25 Feb 2002

COUNTERSIGNATURE 25 Mar 2002 DATE

Sample: L-224715 phosphate 70316-35
Size: 2.7600 mg
Method: standard

DSC

File: C:...\715_phos_35_t0
Operator: LS
Run Date: 20-Feb-02 16:45



60659-165-1
RJS
25 Feb 2002

Sample: L-224715 phosphate 70316-035

Size: 6.1670 mg

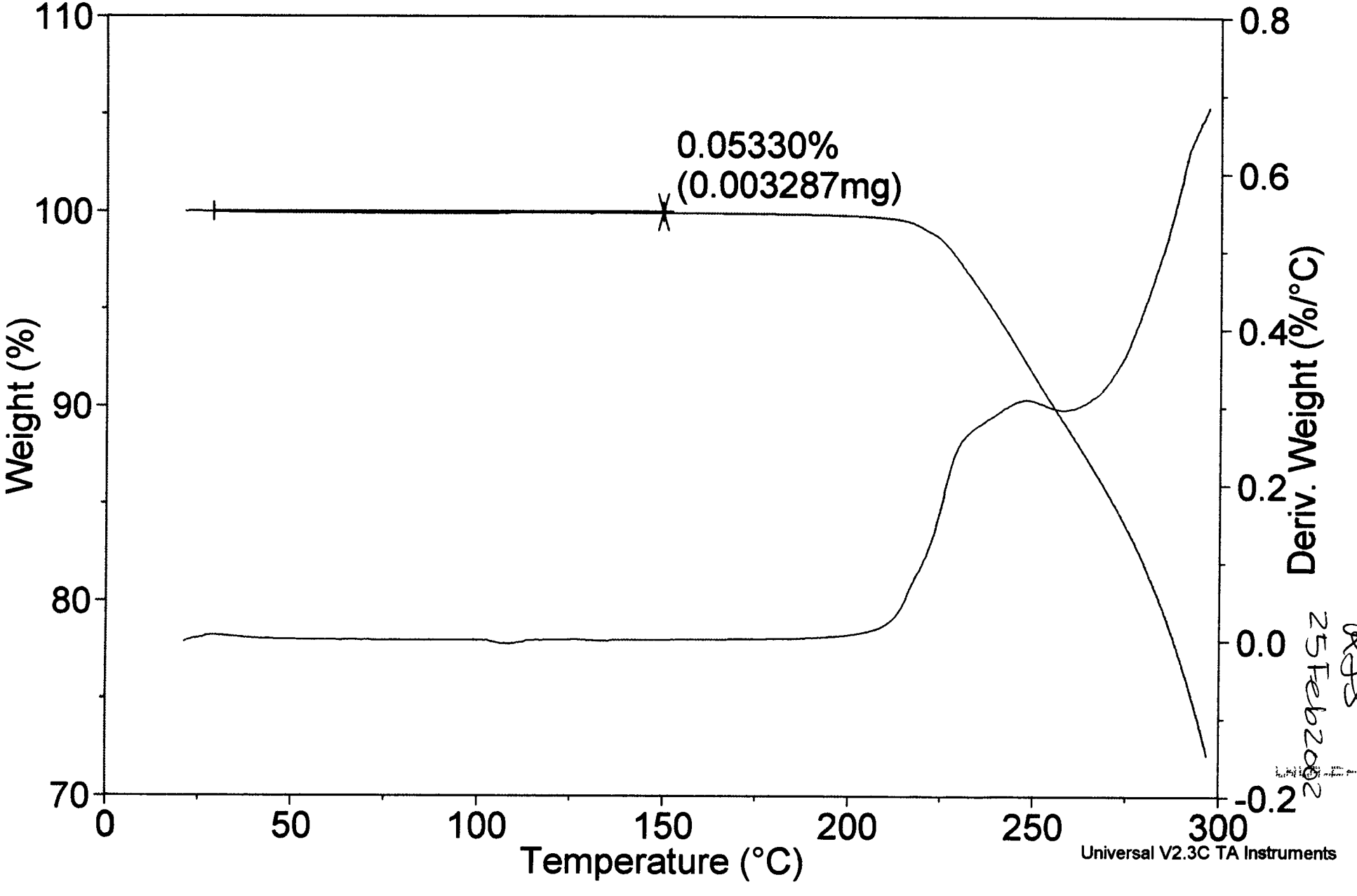
Method: standard method

TGA

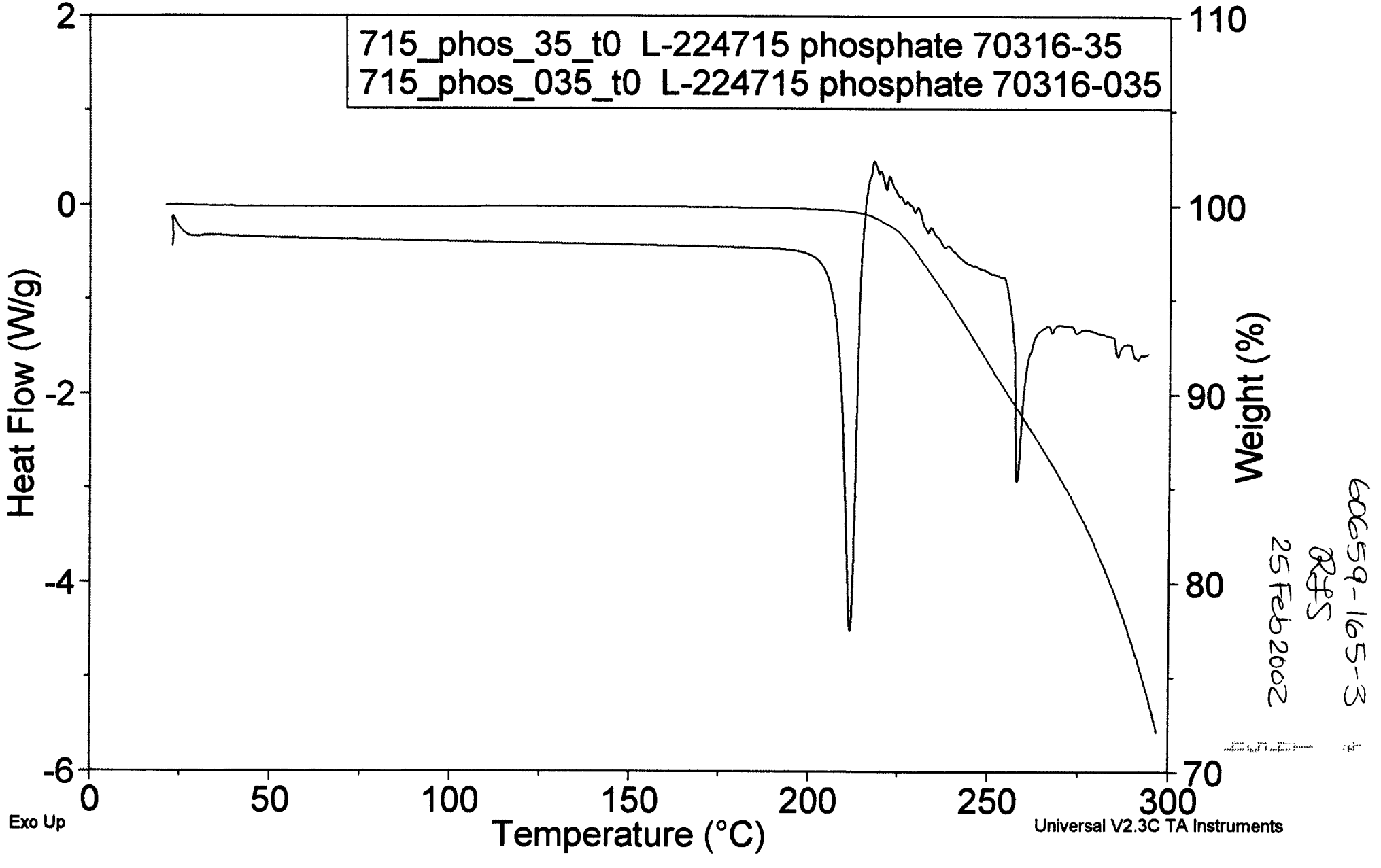
File: C:...\715_phos_035_t0

Operator: LS

Run Date: 20-Feb-02 16:46



60659-166 RFS 165-2
RFS
25 Feb 2002



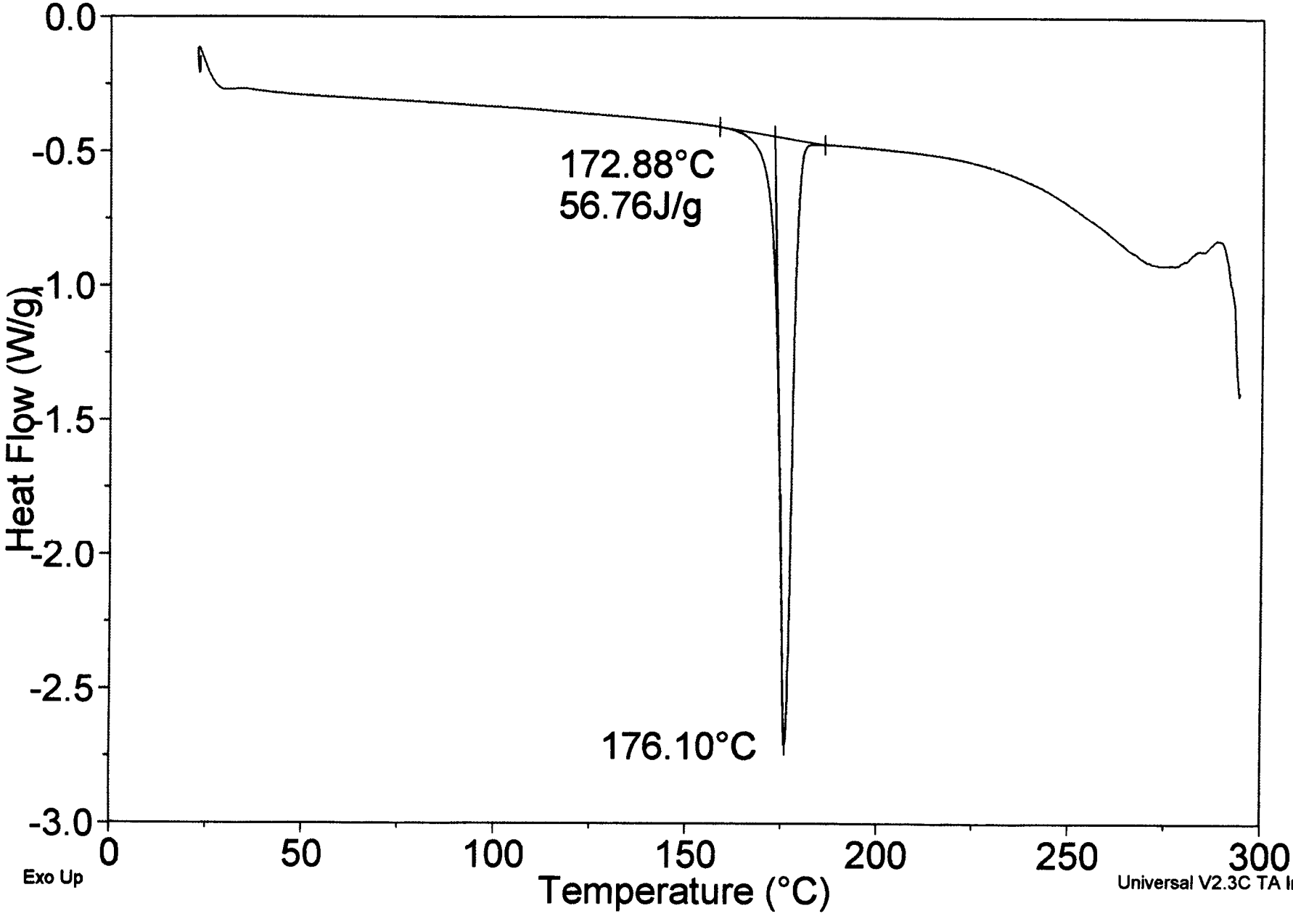
60659-165-3
RFS
25 Feb 2002

NB70316-009

Sample: L-224715 besylate anhy form A
Size: 1.9400 mg
Method: standard

DSC

File: C:...\715_Bes_anhy_A_009_t
Operator: LS
Run Date: 21-Feb-02 14:41

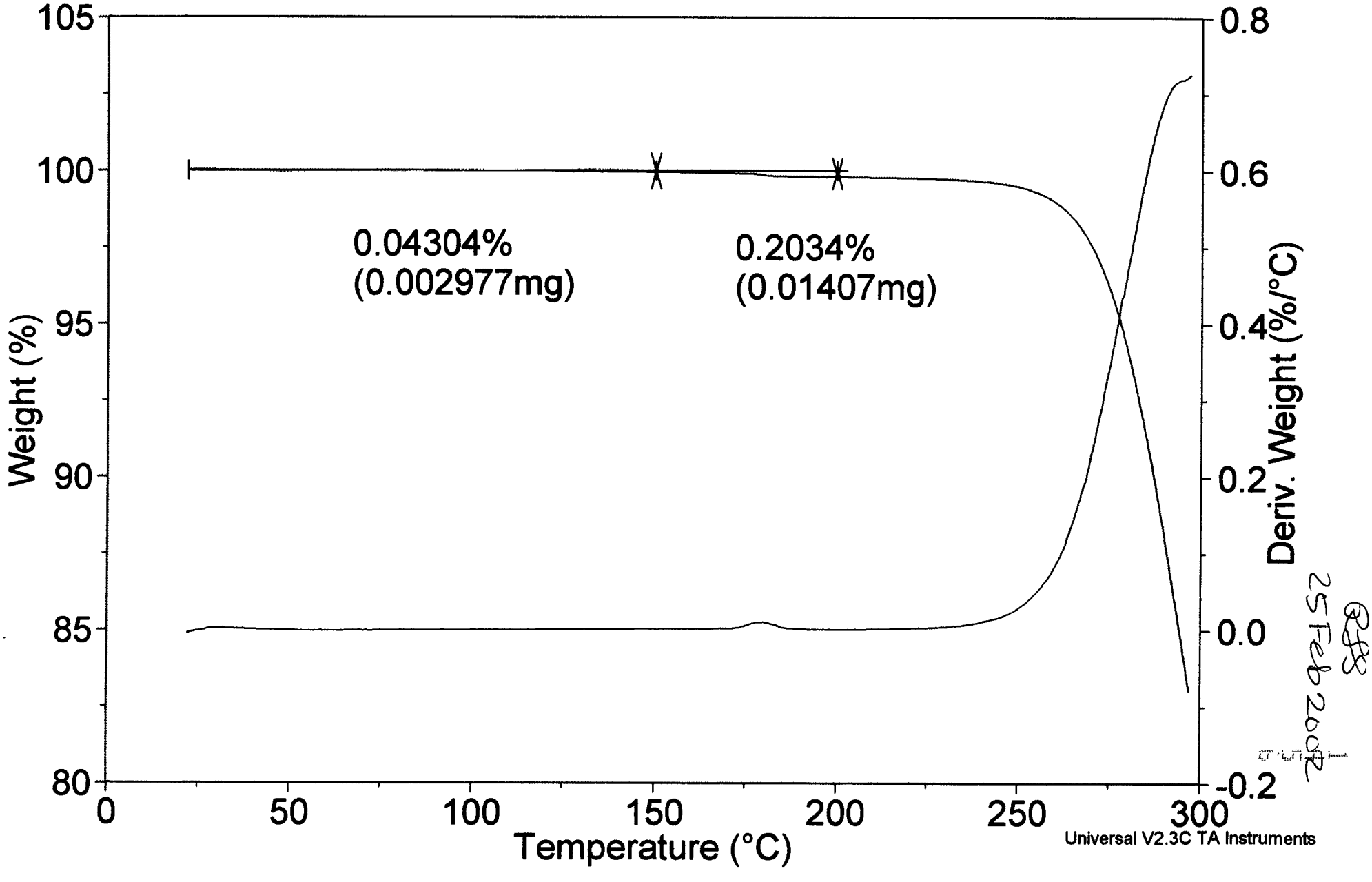


60659-165-4
RSS
25 Feb 2002

Sample: L-224715 besylate 70316-009
Size: 6.9180 mg
Method: standard method

TGA

File: C:...\715_bes_009_t0.000
Operator: LS
Run Date: 21-Feb-02 14:20



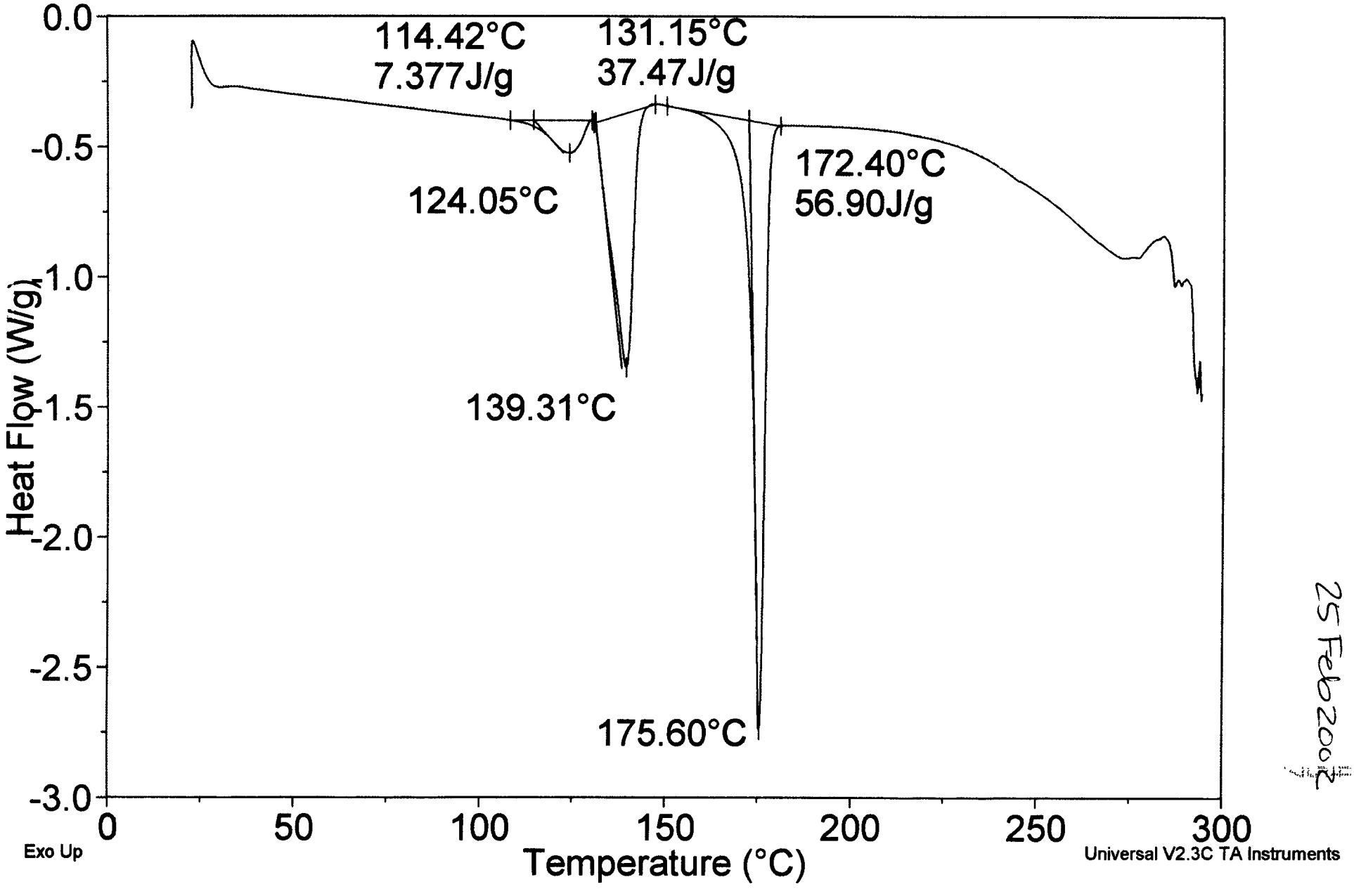
60659-165-5
RFS
25 Feb 2002

NB70316-029 / NB 32139-133

Sample: L-224715 besylate hemihydrate
Size: 2.1600 mg
Method: standard

DSC

File: C:...\715_Bes_hemihy_t0.00C
Operator: LS
Run Date: 21-Feb-02 15:22

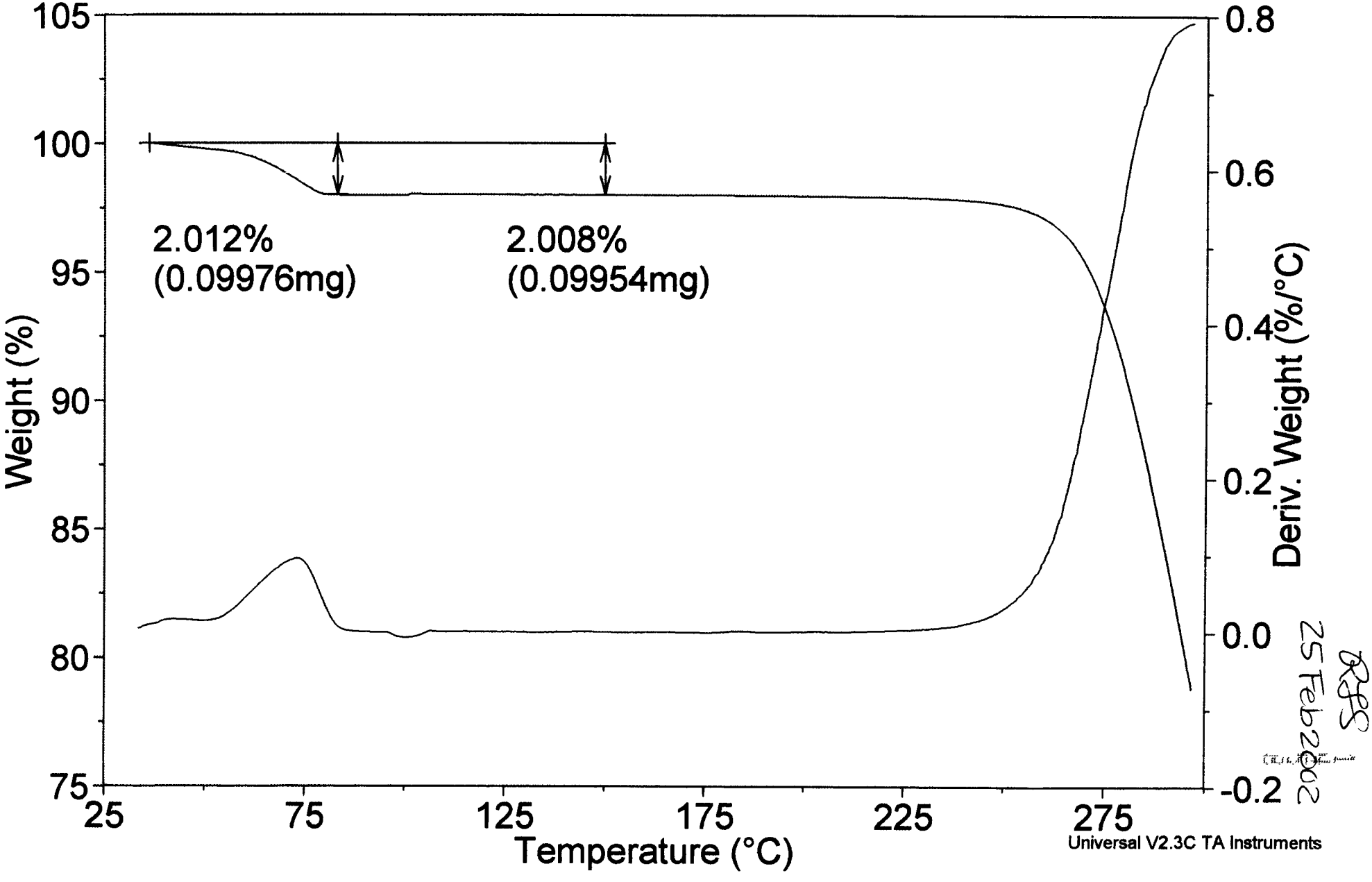


60659-165-6
RJS
25 Feb 2002

Sample: L-224715 besylate hemihydrate
Size: 4.9580 mg
Method: standard method

TGA

File: ...\715_bes_hyd_029_t0.001
Operator: LS
Run Date: 21-Feb-02 15:09



60659-1605-7
RFS
25 Feb 2002

R. Leigh Shultz
25 Feb 2002

Subject: Stability of L-224715 phosphate salt with gelatin (2-week)

The 2-week bulk stability samples of L-224715 with gelatin (60659-155) were removed from the freezer at 9:30 am and were allowed to come to room temperature. Each sample was extracted with 0.1% phosphoric acid (3x3.0 mL), and an aliquot of the resulting solution was filtered at 0.2 microns into an HPLC vial for analysis. Each of the three samples was assayed twice in the sequence 60659.166.seq using the methods L221869_method1 (210 nm detection) and L221869_method1_270 (270 nm detection).

Subject: Physical stability of L-224715 phosphate salt

The phosphate salt physical stability samples (60659-160) were removed from the stability stations, and samples were removed from each for TGA and DSC analysis. The vials were then placed back in the stability stations for further aging. The samples removed represent 11 days on stability. The DSC and TGA data for the samples are tabulated below and traces follow this page as supplemental material.

Conditions	mp onset (°C)	heat of melting (J/g)	% volatiles	Comments
unstressed	207.51	166.4	0.053	original material
25 °C/60% RH	207.55	175.7	0.092	no physical change
40 °C/amb RH	208.37	151.6	0.092	no physical change
40 °C/75% RH	208.69	205.5	0.020	TG started at 35 °C; no physical change

The phosphate salt decomposes immediately upon melting, so integration of the melt endotherm is unreliable and widely variable. However, overlaying the DSC and TGA data of the stressed samples with those of the unstressed material shows no physical changes over 11 days under the conditions studied.

Rebecca Leigh Shultz
25 Feb 2002

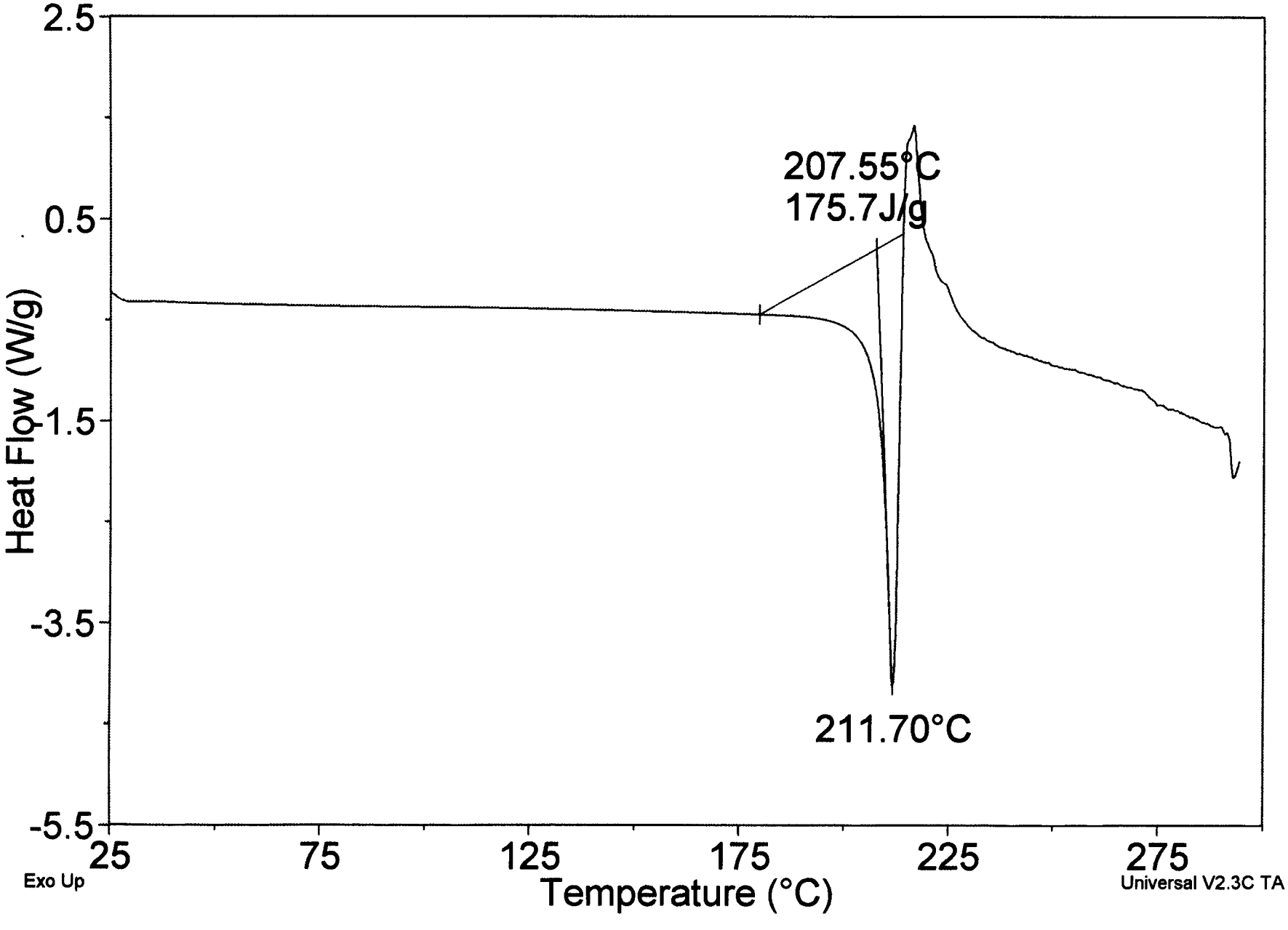
25 Feb 2002
RLS

25 Mar 2002
COUNTERSIGNATURE DATE

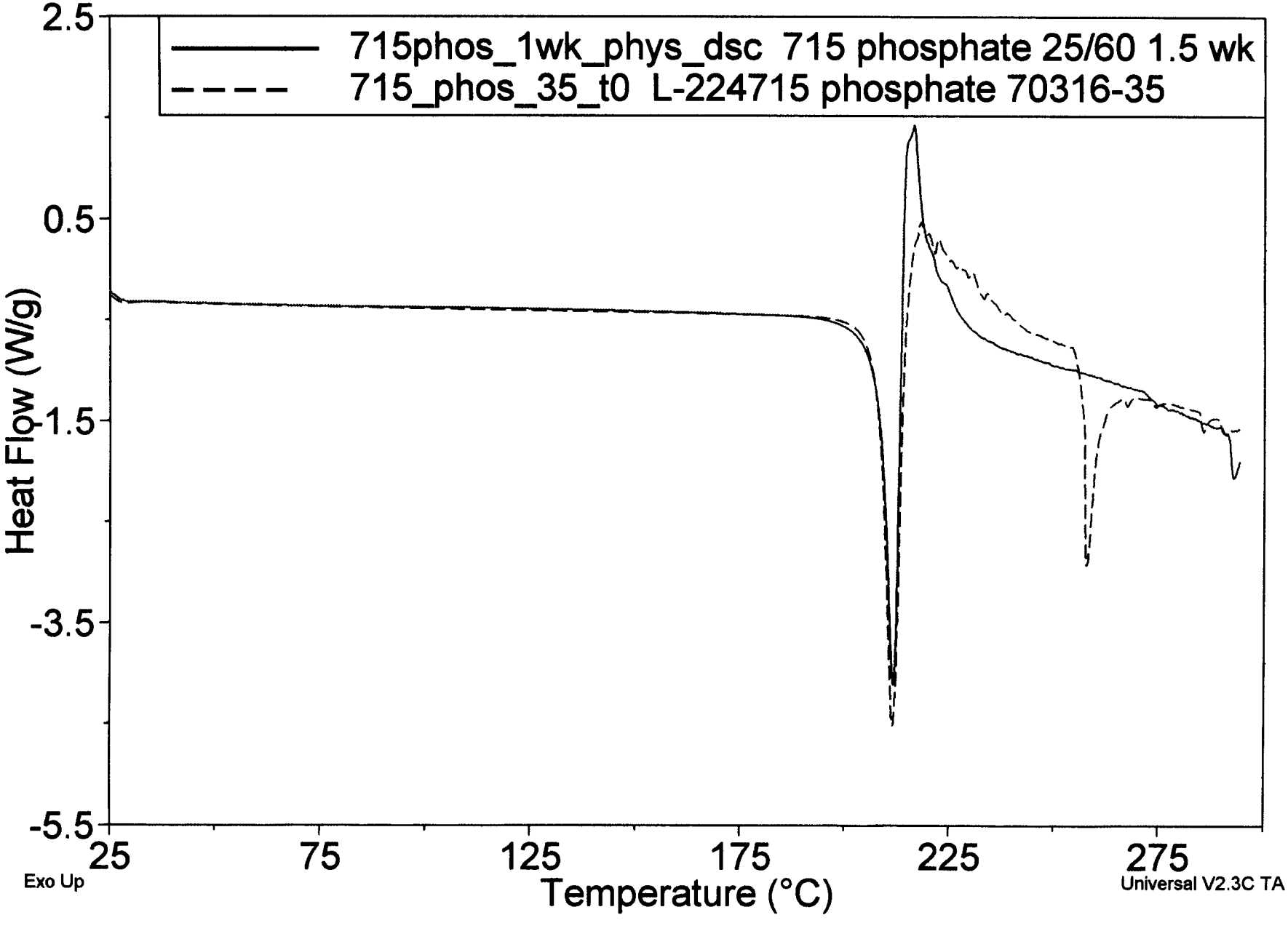
Sample: 715 phosphate 25/60 1.5 wk
Size: 1.3200 mg
Method: standard

DSC

File: C:...\715phos_1wk_phys_dsc
Operator: LS
Run Date: 25-Feb-02 11:34



60659-166-1
RFS
25 Feb 2002

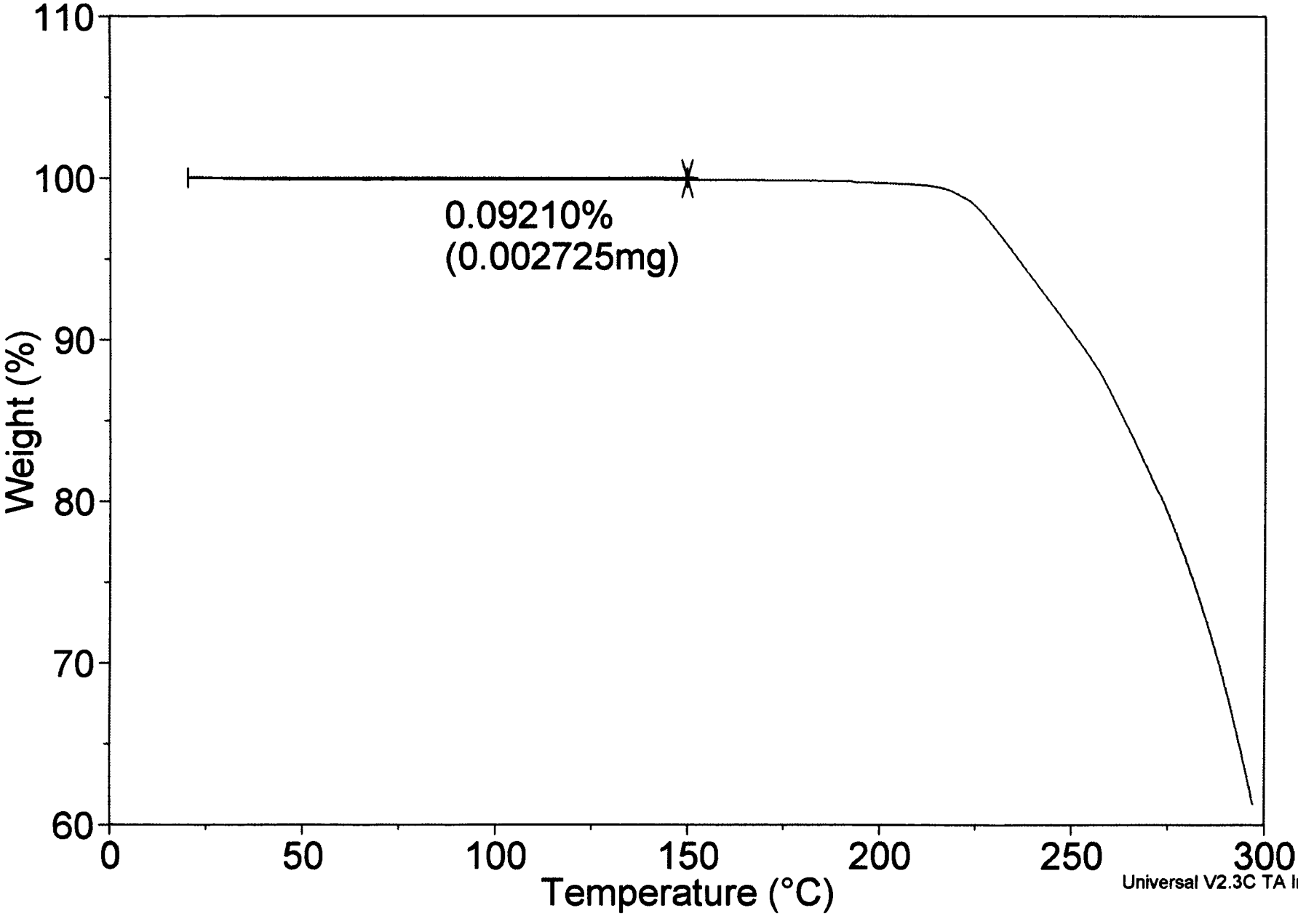


60659-166-2
RJS
25 Feb 2002

Sample: 715 phosphate 25/60 1.5 wk
Size: 2.9590 mg
Method: standard method

TGA

File: C:...\715_phos_1wk_tga
Operator: LS
Run Date: 25-Feb-02 11:43



60659-166-3
RFS
25 Feb 2002