

MERCK RESEARCH LABORATORIES PROGRESS REPORT

JANUARY 2002



Category: Endocrine/Metabolic	Title: L-221869 (dipeptidyl-peptidase IV)
Compound #: 0726	Target Class: dipeptidyl-peptidase IV
Disease: noninsulin-dependent diabetes mellitus	Dosage Form/Potency: To Be Determined
Cross Project Function:	
Project Team:	Reporting Area: Pharmaceutical Research & Development
Dept. : 854 – Pharmaceutical Research	Sub-Group: Pharmaceutical Chemistry – Rahway
Department Head: Michael Kaufman	Author(s): Shultz, Leigh
Key Words: Bulk and solution stability; HCl salt; p-toluenesulfonate salt; benzenesulfonate salt; tartrate salt; hydrolysis and de-amination; solubility; pKa; stoichiometry (tartaric acid salt); 1:1 salt; buffer; hemihydrate	
Summary	
<p><i>Bulk and solution stability studies (1, 2, and 4 week) have been completed for the HCl, p-toluenesulfonate, benzenesulfonate, and L-tartrate salts of L-221869. Limited solubility data is also available for these salts, with the exception of the HCl salt. The pKa of L-221869 has been measured by titration as 7.98. HPLC, VTI, TGA, and DSC data indicate that the L-tartrate salt of L-221869 is a 1:1 salt and exists as a hemihydrate.</i></p>	

1. Bulk and solution stability of L-221869 HCl salt [NB: (60659:101, 104, 107, 122, 136)]

Bulk stability of the HCl salt was ascertained by HPLC analysis of samples stored at 40 °C/75% RH and at 80 °C/amb RH for 1, 2, and 4 weeks. The stability data are shown in the table below and are reported in area % relative to samples stored at -20 °C.

Table 1. 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 (data from Y. Wang, Analytical Research). The stability of the HCl salt of L-221869 in solution was evaluated by HPLC analysis of samples stored at 40 and 80 °C in aqueous solutions of varying pH. The solution stability data are shown in the table below. Data are reported in area % relative to samples stored at -20 °C.

Table 2. 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.

2. Bulk and solution stability of L-221869 p-toluenesulfonate salt [NB: (60659:101, 104, 107, 122, 135)]

The stability of bulk p-toluenesulfonate salt was evaluated by HPLC analysis of samples stored at 40 °C/75% RH and at 80 °C/amb RH for 1, 2, and 4 weeks. Data for the salt are shown the table below and are reported in area % relative to samples stored at -20 °C.

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. Solution stability was ascertained by HPLC analysis of samples stored at 40 and 80 °C in aqueous buffers of varying pH. The data are shown in Table 4 below and are reported in area % relative to samples stored at -20 °C.

Table 4. 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.

3. Bulk and solution stability of L-221869 benzenesulfonate salt [NB: (60659:106, 114, 119, 124-125, 137)]

The bulk stability data for the benzenesulfonate salt of L-221869 are shown in the table below. Data were obtained by HPLC analysis of samples stored at 40 °C/75% RH and 80 °C/amb RH for 1, 2, and 4 weeks. Data are reported in area % relative to samples stored at -20 °C.

Table 5. 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 stability of the benzenesulfonate salt in solution was evaluated by HPLC analysis of samples stored at 40 and 80 °C in a variety of aqueous buffer solutions (20 mM). The solution stability data are shown in the table below and are reported in area % relative to samples stored at -20 °C.

Table 6. 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 the native pH of the salt in water is greater than 6. The salt is most stable toward hydrolysis between pH 2 and 4. No de-amination is observed at pH

4. Bulk and solution stability of L-221869 L-tartrate salt [NB: (60659:106, 114, 119, 124-125, 138)]

The solid-state thermal stability of the tartrate salt of L-221869 was evaluated by HPLC analysis of samples stored at 40 °C/75% RH and 80 °C for 1, 2, and 4 weeks. The bulk stability data are shown in the table below and are reported in area % relative to samples stored at -20 °C.

Table 7. 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. Solution stability was measured by HPLC analysis of samples stored at 40 and 80 °C in aqueous buffers of varying pH for 1, 2, and 4 weeks. The data are shown in Table 8 below and are reported in area % relative to samples stored at -20 °C.

Table 8. 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 crystalline salts 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. Table 9 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.

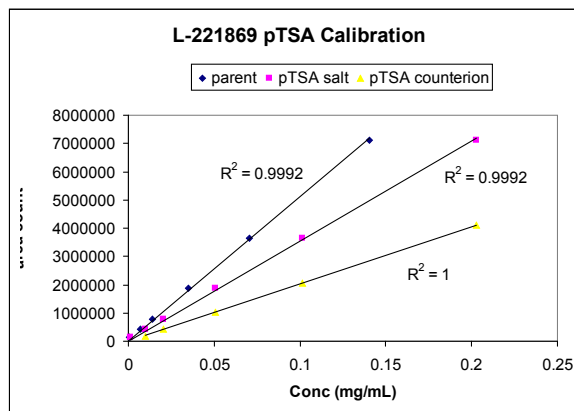
Table 9. Comparison of degradation in salts of L-221869

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	1.9	5.0	9.5	1.0	2.6	4.1

The data clearly indicate that the tartrate salt is more stable in unbuffered water than the benzenesulfonate salt.

5. Solubility of L-221869 p-toluenesulfonate salt [NB: (60659:102-103, 139)]

The solubility of the p-toluenesulfonate salt of L-221869 was investigated in water, saline, 0.01 N HCl, and buffers from pH 4 to pH 10. The HPLC was calibrated using standards of the p-toluenesulfonate salt in water, and the calibration plot is shown below.



The calibration data were used to determine the solubility of the p-toluenesulfonate salt in a variety of solvents. The solubility data are shown in Table 10 below.

Table 10. Solubility of L-221869 p-toluenesulfonate salt

Solvent	Sol. Salt (mg/mL)	Sol L-221869 (mg/mL)	pH _{initial}	pH _{final}
water	15.05	10.43	6.25	6.38
saline	12.44	8.62	5.90	6.20
0.01 N HCl	11.55	8.01	2.00	2.00
20 mM sodium acetate	15.02	10.41	4.02	3.95
20 mM sodium phosphate	13.35	9.25	6.04	5.78
20 mM sodium phosphate	13.51	9.36	7.97	7.27
20 mM sodium carbonate	10.58	7.33	9.95	8.19

Some of the samples (pH 2, 4, 6, 8, and 10) contained little to no solid after equilibration, so the solubility of the p-toluenesulfonate in these media may be higher. The solubilities in water and saline can be considered as equilibrium data. The final pHs of the solubility samples indicate that the salt does not dissociate to a high degree in these solvents.

6. Solubility of L-221869 benzenesulfonate salt and L-221869 L-tartrate salt [NB: (60659:115-116, 139)]

The solubilities of the benzenesulfonic acid and tartaric acid salts were also investigated in water and 0.01 N HCl. The data are shown in the table below along with the data for the free base (J. Remenar) and the p-toluenesulfonate for comparison.

Table 11. Comparison of solubility data for crystalline salts and free base of L-221869

Salt	Solvent	Sol. Salt (mg/mL)	Sol. L-221869 (mg/mL)	pH _{initial}	pH _{final}
Free Base	water	n/a	2.2		
p-Toluenesulfonic acid	water	15.1	10.4	6.25	6.38
Benzenesulfonic acid	water	>20.0	>14.2	5.85	6.17
L-Tartaric acid	water	>19.5	>14.1	5.85	3.62
Free Base	0.01 N HCl	n/a	>20		
p-Toluenesulfonic acid	0.01 N HCl	>11.6	>8.0	2.00	2.00
Benzenesulfonic acid	0.01 N HCl	>10.0	>7.1	2.17	2.20
L-Tartaric acid	0.01 N HCl	>10.0	>7.2	2.17	3.02

The benzenesulfonate and tartrate salts are more soluble in water than the p-toluenesulfonate salt. The data for the benzenesulfonate and tartrate cannot be considered equilibrium values, as the entire solid sample dissolved in the course of the equilibration. The final pH of the tartrate salt solution is of note, as this is in the most stable pH range for L-221869.

7. pK_a determination for L-221869 free base [NB: (60659:131-133)]

The pK_a of L-221869 was determined by titration of a sample of the crystalline free base (39.22 mg) in water (50.0 mL) with 0.10 N HCl. The pH of the solution was measured using a glass electrode and pH meter after the addition of each of 10 aliquots of HCl. Data analysis produced a pK_a value of 7.98 (scatter 0.13).

8. Stoichiometry of L-221869 L-tartrate salt [NB: (60659:116, 126, 128-130)]

HPLC data from two different concentration of the tartaric acid salt were analyzed against known standards of the p-toluenesulfonate salt (see calibration plot above) to determine the stoichiometry of the tartaric acid salt. L-221869 forms a hemifumarate salt and might also form 2:1 salts with other dicarboxylic acids such as L-tartaric acid. The concentrations of L-221869 parent in the two samples were determined from the calibration and were compared to the theoretical L-221869 concentrations for both a 1:1 salt and a 2:1 salt (hemitartrate).

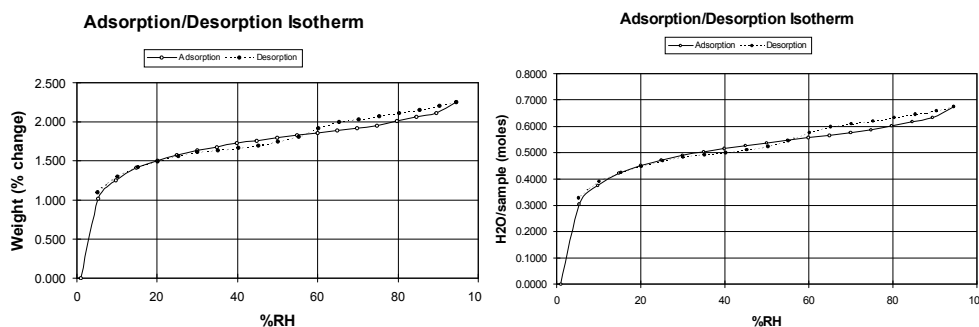
Table 12. Stoichiometry of the L-tartrate salt of L-221869

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 suggest that the tartaric acid salt of L-221869 is a 1:1 salt. This conclusion is supported by solution ¹H NMR data (M. Palucki, Process Research). The native pH of a concentrated solution of L-221869 tartrate in water (see solubility data above) is 3.62, suggesting the presence of a free carboxylic acid group and consistent with a 1:1 salt. The tartaric acid in this salt is half protonated and would be expected to buffer an aqueous solution of the salt; this is evident in the raising of the pH value when the tartrate salt of L-221869 is added to 0.01 N HCl (see solubility data above).

Reports from Analytical Research suggested that the tartrate salt of L-221869 is slightly hygroscopic. TG results (Y. Wang) indicated 1.6% weight loss, and moisture content was estimated at 2% by Karl-Fischer titration of the salt. Dynamic vapor sorption analysis (Y. Wang) of the salt from 5-95% RH at 25 °C indicated that the salt gained 1.0 wt % water by 10% RH and gained a total of 1.8 wt % water by 95% RH.

Given these results, the hygroscopicity of the tartrate salt was measured at 40 °C, producing the data shown in the moisture sorption isotherms below:



These results are nearly identical to the results obtained at 25 °C in Analytical Research. The presence of a hydrated form was considered, and the hemihydrate was calculated to be 1.64 wt % water. The VTI data at both 25 and 40 °C suggest that the salt exists as a hemihydrate under ambient conditions. The material from the VTI run at 40 °C was analyzed by DSC and TGA. The thermograms are shown below.

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