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: L-tartrate hemi-hydrate; solubility; stability; binary mixtures; gelatin; HPMC

Summary

The solubility of L-221869 tartrate hemi-hydrate was determined in water, saline, buffers (pH 2, 4, and 6), methanol, ethanol, and 2-propanol. Chemical stability of the salt in a dry-filled capsule formulation is being assessed by analysis of binary mixtures of the salt with gelatin and HPMC. No instability is noted after 2 weeks, but method development for these samples is ongoing to increase the sensitivity of the assay.

1. Solubility of crystalline L-221869 L-tartrate hemi-hydrate **[NB: (60659:146-147, 151)]**

The solubility of L-221869 tartrate hemi-hydrate (M. Palucki, NB 72061-54) was determined in water, saline, buffers (pH 2, 4, and 6), methanol, ethanol, and 2-propanol. The salt sample was completely soluble in the aliquot of solvent added in all cases except ethanol and 2-propanol, so solubility data is not equilibrium data except in those cases. The ethanol and 2-propanol samples were equilibrated on the rotator for 18 hours, diluted with 0.1% phosphoric acid, and quantitated by HPLC against known standards of the tosylate salt of L-221869. The solubility data are shown in Table 1 below with pH data for the aqueous solutions. The undissolved salts in the ethanol and 2-propanol samples were analyzed by optical microscopy; no amorphous material or new crystal morphologies were observed.

Table 1. Solubility of L-221869 L-tartrate hemi-hydrate

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

2. Chemical stability of L-221869 L-tartrate hemi-hydrate in binary mixtures with gelatin (type B) and HPMC **[NB: (60659:144-145, 149, 152-153, 157-159)]**

In order to investigate the stability of L-221869 tartrate hemi-hydrate (NB72061-54) 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, as were samples of gelatin and HPMC, according to the table below. 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-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 mixture (ca. 20 mg each) were weighed into vials and placed at 5 °C, 40 °C/75% RH, and 80 °C for 1, 2, and 4 weeks. The samples are analyzed by dissolving each in 9.0 mL of 0.1% phosphoric acid, syringe-filtering, and analyzing on the HPLC using the method for the parent compound (L221869_method1). Separate columns are used for gelatin and HPMC samples because gelatin tends to be retained on the column after each run. No chemical degradation was evident after 2 weeks under any of the experimental conditions, but degradation in the gelatin samples may have been masked by the elution of gelatin itself. Method development to circumvent this problem is ongoing.

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Compound #: **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: L-224715; benzenesulfonate; anhydrate form A; chemical stability; L-tartrate; hemi-hydrate; pH; phosphate; solubility; binary mixtures; gelatin; HPMC

Summary

The chemical stability of the benzenesulfonate, tartrate, and phosphate salts is being determined both in the solid state and in solution. Two-week data indicate that the benzenesulfonate and tartrate are stable in the bulk under all conditions studied; the same is true for the phosphate after one week. All of the salts are most stable in solution between pH 2 and 4 and degrade completely in water, pH8 solution, and pH10 solution at 80 °C after 1 week. The phosphate salt shows the best stability in water after one week, but more stability data is needed before salt selection can occur. The solubility of the 3 salts has been determined in water, 0.01N HCl, and alcohols. All three salts are very water-soluble, the phosphate having more than 80-mg/mL parent solubility. All are sparingly soluble in 2-propanol. Experiments are ongoing to determine the chemical stability of binary mixtures of each of the three salts with capsule materials (gelatin, HPMC). Two-week data are available for the benzenesulfonate and the tartrate salts; no degradation has been detected to date. Method development is ongoing to increase the sensitivity of these assays.

1. Bulk and solution chemical stability of L-224715 benzenesulfonate (anhydrate form A) **[NB: (60659:142-143, 149-150, 154, 160)]**

The bulk stability of the benzenesulfonate salt (anhydrate form A) of L-224715 has been assessed after 2 weeks at 40 °C/75% RH and 80 °C by HPLC analysis of solid samples stored under these conditions. No degradation or significant loss of parent was noted under the above conditions. Four-week data (forthcoming) will be needed to confirm this finding.

The solution stability of the benzenesulfonate salt has also been assessed after two weeks at 40 and 80 °C. Like the free base, the benzenesulfonate salt is most stable between pH 2 and 4 at all temperatures studied. At pH 2, ca. 7.7 area % parent is lost after 2 weeks at 80 °C due to hydrolysis of the amide bond, and ca. 9 area % parent is lost after 2 weeks at pH 4 (80 °C) due to both hydrolysis and de-amination. Total loss of parent is observed in water, pH 8 buffer, and pH 10 buffer at 80 °C after 1 week. At 40 °C, the area % degradates (relative to parent) due to hydrolysis and de-amination are similar to those seen for the free base at 1 and 2 weeks:

Salt of L-224715	Rel Area % Hydrolysis			Rel Area % De-amination		
	1wk	2wk	4wk	1wk	2wk	4wk

Free Base	8.9	20.4	47.7	3.8	11.8	28.8
Benzenesulfonate	8.3	18.2		3.6	7.4	

Four-week data (both bulk and solution) for the benzenesulfonate salt are forthcoming.

2. Bulk and solution chemical stability of L-224715 L-tartrate (hemi-hydrate) **[NB: (60659:143, 149, 152, 154)]**

The bulk stability of the tartrate salt (hemi-hydrate) of L-224715 has been assessed after 2 weeks at 40 °C/75% RH and 80 °C by HPLC analysis of solid samples stored under these conditions. No degradation or significant loss of parent was noted under the above conditions. Four-week data (forthcoming) will be needed to confirm this finding.

The solution stability of the tartrate salt has also been assessed after two weeks at 40 and 80 °C. Like the benzenesulfonate salt, the tartrate salt is most stable between pH 2 and 4 at all temperatures studied. At pH 2, ca. 2.5 area % parent is lost after 2 weeks at 80 °C due to hydrolysis of the amide bond, and ca. 7 area % parent is lost after 2 weeks at pH 4 (80 °C) due to both hydrolysis and de-amination. Total loss of parent is observed in water, pH 8 buffer, and pH 10 buffer at 80 °C after 1 week. At 40 °C, the area % degradates (relative to parent) observed due to hydrolysis and de-amination are slightly lower than those seen for the benzenesulfonate salt at 1 and 2 weeks:

Salt of L-224715	Rel Area % Hydrolysis			Rel Area % De-amination		
	1wk	2wk	4wk	1wk	2wk	4wk
Free Base	8.9	20.4	47.7	3.8	11.8	28.8
Benzenesulfonate	8.3	18.2		3.6	7.4	
L-Tartrate Hemihydrate	6.3	13.7		4.2	6.1	

The amount of degradation observed in solution after 2 weeks is unexpected based on the initial pH of the stability samples (pH = 5.1 at 0.1 mg/mL salt). After the data was acquired, the pH of the 40-°C stability samples was measured. The pH of the 1-week sample was 7.5, and the pH of the 2-week sample was 7.6. To see if the rise in pH upon storage at 40 °C was reproducible, a fresh sample of the tartrate salt in water was prepared using the same stock solution used for the stability samples. This fresh solution had a pH of 5.1. The solution was placed in at 40 °C for 48 hours, during which time the pH of the solution rose to 7.54. HPLC analysis of this solution showed relative area % degradates to be low (hydrolysis, 0.4%; de-amination, 0.6%), indicating that the degradation of the tartrate salt observed on stability is the result of the rise in pH, rather than the rise in pH being due to degradation. The cause of the pH increase at 40 °C is still under investigation.

Four-week bulk and solution stability data for the tartrate salt are forthcoming.

3. Bulk and solution chemical stability of L-224715 phosphate **[NB: (60659:153, 157, 159)]**

The bulk stability of the phosphate salt of L-224715 has been assessed after 1 week at 40 °C/75% RH and 80 °C by HPLC analysis of solid samples stored under these conditions. No degradation or significant loss of parent was noted under the above conditions. Two- and four-week data (forthcoming) will be needed to confirm this finding.

The solution stability of the phosphate salt has also been assessed after one week at 40 and 80 °C. Like the other salts studied, the phosphate salt is most stable between pH 2 and 4 at all temperatures studied. At pH 2, ca. 1 area % parent is lost after 1 week at 80 °C due to hydrolysis of the amide bond, and ca. 1.5 area % parent is lost after 1 week at pH 4 (80 °C) due to both hydrolysis and de-amination. Total loss of parent is observed in water, pH 8 buffer, and pH 10 buffer at 80 °C after 1 week. At 40 °C, the area % degradates (relative to parent) observed due to hydrolysis and de-amination are lower than those seen for the benzenesulfonate salt after 1 week:

Salt of L-224715	Rel Area % Hydrolysis			Rel Area % De-amination		
	1wk	2wk	4wk	1wk	2wk	4wk
Free Base	8.9	20.4	47.7	3.8	11.8	28.8
Phosphate	3.8			2.3		

Benzenesulfonate	8.3	18.2	3.6	7.4
L-Tartrate Hemihydrate	6.3	13.7	4.2	6.1

Two- and four-week stability data (both solution and bulk) will be needed to confirm the stability of the phosphate salt. Given that all of the salts of L-224715 show good bulk stability thus far, solution stability data (especially stability in water) will be used to differentiate them; stability in water is expected to be important for such a water-soluble salt which may go amorphous in a formulation.

4. Solubility and pH of L-224715 benzenesulfonate **[NB: (60659:143, 146-147, 151, 155, 158)]**

The solubility of the anhydrate (form A) of the benzenesulfonate salt (K. Hansen, NB 70130-347) of L-224715 was determined in water, 0.01N HCl, methanol, ethanol, and 2-propanol. The samples of the salt dissolved immediately in all of the above solvents except 2-propanol; this sample was diluted with 0.1% phosphoric acid and analyzed by HPLC against known standards of the free base. The solubility data obtained are shown in the table below:

Solvent	Sol. Salt (mg/mL)	Sol. Parent (mg/mL)	$pH_{initial}$	pH_{final}
water	>53.3	>38.4	6.13	*
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

* volume of solution too low to measure pH

The pH of the water sample was not obtained due to sample limitations, but the pH of a 10.0-mg/mL solution of the salt was measured at 5.75, while the pH of a 1.0 mg/mL solution was 6.66. The pH of a saturated solution should be lower than that of the 10.0-mg/mL solution.

The solid remaining in the 2-propanol sample was analyzed by microscopy; no amorphous material or morphology changes were observed. The solubility values is aqueous solution are likely those of the hemi-hydrate, which forms above 85% RH (C. Lindemann, Analytical Research).

5. Solubility and pH of L-224715 L-tartrate hemi-hydrate **[NB: (60659:143, 146-147, 150-151, 155)]**

The solubility of the hemi-hydrate of the tartrate salt (K. Hansen, NB 70130-359) of L-224715 was determined in water, 0.01N HCl, methanol, ethanol, and 2-propanol. The samples of the salt dissolved immediately in all of the above solvents except ethanol and 2-propanol; these samples were diluted with 0.1% phosphoric acid and analyzed by HPLC against known standards of the free base. The solubility data obtained are shown in the table below:

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

The pH of a 1.0-mg/mL solution of the salt was 3.98, while the pH of a 0.1-mg/mL solution was measured at 5.1.

The solids remaining in the ethanol and 2-propanol samples were analyzed by microscopy; no amorphous material or morphology changes were observed.

6. Solubility and pH of L-224715 phosphate **[NB: (60659:158-159)]**

The solubility of the phosphate salt (K. Hansen, NB 70316-25) of L-224715 was determined in water, 0.9% NaCl, 0.01N HCl, methanol, ethanol, and 2-propanol. The samples of the salt dissolved immediately in all of the aqueous solvents; the supernatants from the alcoholic samples were diluted with 0.1% phosphoric acid and analyzed by HPLC against known standards of the free base. The solubility data obtained are shown in the table below:

Solvent	Sol. Salt (mg/mL)	Sol. Parent (mg/mL)	$pH_{initial}$	pH_{final}
water	>100, <150	>81, <121	6.13	*
saline	>72	>58	5.57	*
0.01 N HCl	>75	>61	2.08	*
methanol	0.41	0.33	n/a	n/a
ethanol	0.045	0.036	n/a	n/a
2-propanol	0.098	0.079	n/a	n/a

* volume of solution too low to measure pH

The volume of solution resulting from the aqueous samples was in each case too low to measure pH. The pH of a 1.0-mg/mL solution of the salt was 5.61, suggesting a monobasic salt.

The solids remaining in the ethanol and 2-propanol samples have not yet been analyzed by microscopy; the higher solubility in 2-propanol (with respect to ethanol) may be due to the formation of amorphous material in that solvent.

7. Solubility of L-224715-000T001 (crystalline free base) **[NB: (60659:146-147, 150)]**

The solubility of the crystalline free base of L-224715 was determined in PEG 400, glycerol, methanol, ethanol, and 2-propanol. The samples of the salt dissolved immediately in methanol and ethanol and slowly in 2-propanol and PEG 400. Heating for several minutes with a heat gun was required to dissolve the drug in glycerol. Aliquots of the PEG 400 and glycerol samples were diluted with 0.1% phosphoric acid and analyzed by HPLC to determine if any degradation of the drug occurred upon dissolution in these media; none was observed. The solubility data obtained 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.

8. Chemical stability of L-224715 benzenesulfonate (anhydrate form A) in binary mixtures with gelatin (type B) and HPMC **[NB: (60659:144, 149, 152-153, 157-159)]**

In order to investigate the stability of L-224715 benzenesulfonate (anhydrate form A, NB70130-347) 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, as were samples of gelatin and HPMC, according to the table below. 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 mixture (ca. 20 mg each) were weighed into vials and placed at 5 °C, 40 °C/75% RH, and 80 °C for 1, 2, and 4 weeks. The samples are analyzed by dissolving each in 9.0 mL of 0.1% phosphoric acid, syringe-filtering, and analyzing on the

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