IN THE UNITED STATES PATENT OFFICE

In re Application of:	)
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Calderari, et al.	)
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Serial No.: 11/186,311	)
	)
Filing Date: July 21, 2005	)
	)
Title: **LIQUID PHARMACEUTICAL FORMULATIONS OF PALONOSETRON**	)

DECLARATION OF DANIELE BONADEO, M. Chem. Pharm.
37 C.F.R. § 1.132

I, Daniele Bonadeo, hereby give this declaration.

1) My name is Daniele Bonadeo.

2) I am an inventor for this application.

3) I am also Director, Head of Corporate Technical Affairs, Manufacturing Operations, for Helsinn Healthcare SA ("Helsinn"), the assignee of the above-referenced patent application.

4) One of my job responsibilities at Helsinn is to ensure that Helsinn's drug products are stable, and that adequate testing is performed to ensure such stability.

5) One of Helsinn's main products is palonosetron hydrochloride, which is marketed in the United States as Aloxi®. The product is marketed in injectable form and marketing authorization has been obtained for gel-cap dosage form for the treatment of nausea and vomiting from chemotherapy, radiotherapy and general surgery.

6) Numerous stability studies have been performed on the injectable formulation of palonosetron over the years to evaluate how changes to the formulation and manufacturing process would impact stability. These studies were performed by Helsinn, our contract manufacturers, and the owner of palonosetron before we acquired the drug.

7) All of our studies, regardless of the formulation parameter which was varied, show that the stability of palonosetron generally improves as the concentration of palonosetron is reduced, and that concentration is the most important determinant of product stability.

8) Table 1 contains the results of one of our stability studies, conducted in a phosphate-buffered, saline solution at pH 7.4.

TABLE 1. Palonosetron HCl Concentration-Stability Study (pH 7.4, 40 °C)

Palonosetron HCl Conc. (mg/ml, as free base)	% Palonosetron HCl Remaining at			
	1 week	2 weeks	5 weeks	8 weeks
0.01				100
0.1	100	100		101
1.0		101		99
10	99	93	57	23
50	102	73		49

9) As can be seen, the stability of the molecule improves in this formulation as its concentration decreased, with greatest stability seen below 0.1 mg/ml. We made this same observation in other studies, as discussed in greater detail below.

10) We also performed a pH-stability study to determine the best pH at which to formulate the molecule. The study was conducted with 60 mcg/ml palonosetron aqueous solutions, buffered at pH 2.0, 5.0, 7.4 and 10.0. No ingredients were present other than the pH adjusting agent, pH buffer, and palonosetron. The results are reported in Table 2.

TABLE 2. Palonosetron HCl 80 °C pH-Stability Study

pH at Room Temp.	pH at Reaction Temp.	Buffer	T_{90} (days)
2.0	2.0	0.01 M HCl	76
5.0	5.0	Acetate	Not determined. 99.2% remaining at 252 days
7.4	7.3	Phosphate	180
10	9.4	Carbonate	270

11) The results demonstrate that the molecule is extremely stable at a pH of 5.0, when maintained at a low palonosetron concentration such 60 mcg/ml, and that stabilizers and the like are unnecessary to maintain that stability.

12) We also conducted additional studies to evaluate the impact of various excipients on stability, and improve the stability even further.

13) After settling on mannitol and a citrate buffer for the formulation for practical reasons, we studied the effect of palonosetron and EDTA concentration on stability, maintaining the pH constant at approximately 5.0, and keeping the same tonicifying agent (mannitol) and buffering agent (trisodium citrate). Stability was measured based on the percentage of palonosetron that remained undegraded at 1, 2, 3 and 6 months, under standard conditions of accelerated stability testing (i.e. 40 °C).

14) The results are reported below in Table 3.

TABLE 3. Formulation Optimization Study

Formulation (1)			Potency (% Label Strength)						Degradation Products (Total % Label Strength)					
Palo.Conc.	Buffer Conc.	EDTA Conc.	Storage Time (mo.) at 40C					Rate Const.	Storage Time (mo.) at 40 C					Rate Const.
			0	1	2	3	6		0	1	2	3	6	
(2)	(3)	(% w/v)						(4)						(4)
0.10	20	0.000	101	103	99	101	100	-0.29		0.30	0.68	0.61	0.97	0.15
0.10	40	0.000	101	106	103	106	104	-0.61		0.24	0.49	0.46	0.70	0.11
0.40	60	0.000	99	99	98	100	95	-0.75		0.07	0.14	0.46	1.92	0.33
0.40	10	0.000	99	98	97	97	95	-0.59		0.13	0.09	0.22	0.71	0.12
0.40	35	0.050	102	103	103	103	102	-0.15		0.07	0.06	0.21	0.16	0.03
0.40	10	0.100	101	102	101	101	100	-0.29		0.13	0.17	0.06	0.10	0.01
0.40	60	0.100	101	99	99	100	99	-0.27		0.00	0.00	0.02	0.13	0.02
1.00	20	0.050	101	102	102	100	99	-0.35		0.21	0.08	0.28	0.37	0.06
1.00	20	0.050	99	100	99	99	98	-0.31		0.15	0.07	0.20	0.22	0.03
1.20	40	0.000	97	99	96	97	88	-1.73		0.36	1.00	1.59	8.77	1.49
1.20	20	0.000	98	99	97	98	95	-0.55		0.03	0.37	0.61	1.73	0.30
2.50	80	0.000	101		93	91		-3.25			4.04	7.81		2.52
2.50	40	0.000	101		97	94		-1.75			2.77	4.39		1.45
2.50	20	0.000	100		97	95		-1.50			2.65	4.04		1.34
2.50	80	0.050	100		99	97		-1.11			0.79	1.07		0.36
2.70	35	0.000	100	100	102	99	91	-1.58		1.22	2.72	2.76	7.21	1.17
2.70	10	0.050	99	98	99	100	96	-0.48		1.40	1.22	1.17	3.03	0.43
5.00	60	0.000	100	100	98	97		-1.16		2.25	2.90	5.56		
5.00	10	0.000	100	102	100	99		-.075		1.24	2.02	2.86		
5.00	0	0.000	99	99	98	93		-1.92		1.93	3.95	7.83		
5.00	60	0.050	101	99	98	94		-2.41		3.10	3.56	8.14		
5.00	60	0.050	101	101	97	96		-1.66		2.86	4.19	7.10		
5.00	60	0.100	99	98	93	91		-2.82		2.65	4.29	7.55		
5.00	10	0.100	98	98	95	85		-4.37		2.19	4.23	13.87		

1. Lots are sorted by Palonosetron HCl concentration, EDTA concentration and buffer concentration. All lots contain mannitol as the tonicifying agent.
2. Palonosetron concentrations are in mg/mL free base equivalents.
3. Citrate buffer concentrations are millimolar. All formulations are pH 5.0.
4. Rate constants are calculated with degradation product concentrations in two significant digits.

15) One notable observation from these results is that the presence of EDTA improves stability at low palonosetron concentrations, but actually decreases stability at high palonosetron HCl concentrations.

16) The fact that EDTA improves the stability of palonosetron at all is somewhat surprising, given our earliest work with the molecule, in which palonosetron demonstrated comparable stability at 5 °C as it did at 60-100 °C. If the molecule were undergoing auto-oxidation (the typical reason for adding a chelating agent), one would expect the higher temperature to produce more radical initiators and a faster reaction and degradation.

17) The fact that the chelating agent consistently improves the molecule's stability only at lower concentrations is also an intriguing discovery.

18) Based on the results in Table 3, we prepared several graphs to inform our analysis, including the response surface plot depicted below as Figure 1. This graph plots degradation rate constant as a function of EDTA concentration and palonosetron HCl concentration, holding the buffer strength constant at 20 mM. A lower degradation rate constant indicates a more stable formulation.

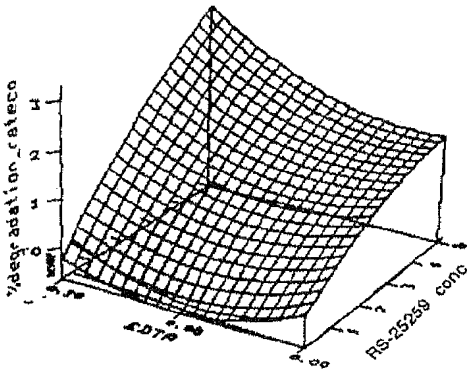

FIGURE 1

19) As can be seen, the stability of palonosetron improved as its concentration was reduced across the entire range of conditions studied. This same result can be seen when the buffer strength is varied, as shown below in Table 4. These results are consistent with the results reported above in Table 1, and reinforce our opinion that palonosetron concentration is a critical factor for the stability of palonosetron formulations.

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