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STABILITY OF AG337, A THYMIDYLATE SYNTHASE INHIBITOR, IN PVC INFUSION BAGS

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ABSTRACT

The stability of AG337, a selective thymidylate synthase inhibitor, in 5% Dextrose in Water (D5W) PVC infusion bags and sodium bicarbonate treated D5W infusion bags was studied at 30°C for seven days. AG337 Solution for Injection, 2% was diluted in D5W infusion bags to yield final AG337 concentrations of 4 mg/mL, 1 mg/mL and 0.1 mg/mL. For the sodium bicarbonate treated D5W infusion bags, the final concentrations of AG337 used were 4 mg/mL, 1 mg/mL and 0.2 mg/mL. The bags were prepared in triplicate and stored at 30°C. At predetermined time intervals, each bag was visually examined for presence of precipitates and samples were withdrawn for HPLC assay and pH testing. No precipitation was observed in any of the samples. The pH of the sodium bicarbonate treated D5W infusion bags remained constant throughout the study. The recovery of AG337 was greater than 93% in all samples tested. The recovery of methyl and propylparaben, used as preservatives in the formulation, however, decreased over time probably due to adsorption in the PVC bags. This study demonstrated that AG337 was stable at 30°C for up to seven days, in D5W infusion bags and in the presence of sodium bicarbonate treated D5W infusion bags.

INTRODUCTION

AG337 (Figure 1) is a selective inhibitor of the enzyme, thymidylate synthase (TS)[1]. It has shown potent anti-tumor activity *in vivo* against both intraperitoneal and intramuscularly implanted murine tumors[2]. AG337 is formulated for parenteral administration as a 2% buffered aqueous concentrate solution. The concentrate AG337 solution contains 50% propylene glycol as a co-solvent and parabens (methyl and propyl) as preservatives[3]. The aqueous solution is administered as an infusion in 5% Dextrose in Water (D5W). The concentration of AG337 in the infusion bags may range as high as 4 mg/mL. The pH of the AG337 infusion solution is typically around 3.8. To minimize irritation

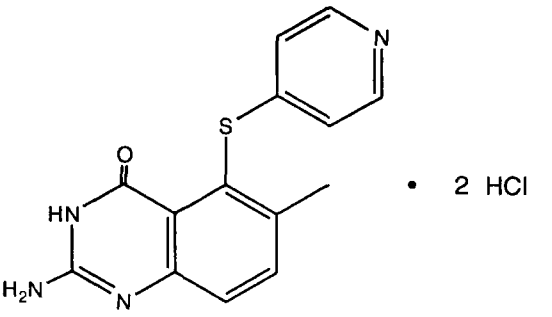

FIGURE 1. Structure of AG337

and phlebitis, commonly observed for peripherally infused fluids, the pH should be as close as possible to the physiological pH. The pH of the AG337 infusion solution may be increased by the addition of a sodium bicarbonate solution[4]. Addition of sodium bicarbonate solution from 0.006% up to 0.113%, to the D5W PVC infusion bag increases the pH to between 4.0 and 4.4.

In these investigations, two studies were undertaken to determine the stability of the AG337 solutions in D5W PVC infusion bags and in the presence of sodium bicarbonate treated D5W infusion bags. The first study tracked the recovery of AG337 and the parabens in D5W infusion bags at 30°C for up to 7 days. The second study was similar in design, following recovery of AG337 and the parabens in sodium bicarbonate treated D5W infusion bags.

MATERIALS AND METHODS:

Materials

AG337 (Agouron Pharmaceuticals, Inc., San Diego, CA), Methylparaben, NF and Propylparaben, NF (Spectrum Chemical, Gardena, CA), 5% Dextrose in Water PVC infusion bags (5% Dextrose Injection, USP, 100 mL Viaflex® bags, Baxter Healthcare Corporation, Deerfield, IL), Sodium bicarbonate Solution, 8.4%, USP (American Reagent Laboratory, Shirley, NY). Methanol and disodium phosphate, HPLC grade, were purchased from Fisher Scientific, Fairlawn, NJ.

High Performance Liquid Chromatography (HPLC) Assay

AG337, methyl and propylparaben were assayed by HPLC[5]. Samples were injected neat or diluted to approximately 800 µg/mL of AG337. The dilution was made with HPLC grade water. The chromatographic system consisted of an Hewlett Packard 1050 equipped with a diode array detector (DAD). Chromatographic separation was achieved at room temperature over a Zorbax® C8, 250 x 4.6 mm, 5µ column (Mac-Mod Analytical, Inc., Chadds Ford, PA). The mobile phase used was a 1:1 mixture of methanol and pH 6.5, 0.02M

phosphate buffer. Flow rate was adjusted to 1.0 mL/min and the absorbance monitored at 254 nm with a 4 nm bandwidth. Peak area was integrated by HPChem Station Software (Hewlett-Packard). AG337, methyl and propylparaben eluted from the column at 14.30, 8.35, and 25.41 minutes respectively.

The stability indicating HPLC method was validated for interday variability with a 7 level standard curve. The standards injected covered a range of 76 to 1272 μg/mL for AG337, 11 to 178 μg/mL for methylparaben and 1.5 to 25 μg/mL for propylparaben. The standard curve obtained for the concentration ranges tested was linear with an average $R^2 \geq 0.997$ for each compound. Interday relative standard deviations (RSD) over a 5 day sampling period, for AG337, methyl and propylparaben were less than 0.4, 0.7 and 6.7% respectively. Intraday percent RSD was less than 0.2%, 0.4%, 3.1% for AG337, methyl and propylparaben, respectively.

Preparation of Infusion Solutions and Samples

Preparation of Sodium Bicarbonate Solutions: Two dilute solutions of sodium bicarbonate, 0.84% and 0.084% were obtained after a series dilution of Sodium Bicarbonate Injection, USP, 8.4% in 100 mL D5W Infusion bags for use in Study II.

Study I: Preparation of AG337 Solutions in D5W Infusion Bags: AG337 Solution for Injection, 2% was diluted in 100 mL D5W Infusion Bags to achieve concentrations of 4.0, 1.0 and 0.1 mg/mL of AG337. The 4.0 mg/mL and the 1.0 mg/mL solutions were prepared by removing 20 and 5 mLs of D5W and replacing with 20 and 5 mLs of AG337 Solution for Injection, 2%, in each of 3, 100 mL D5W infusion bags respectively. For the 0.1 mg/mL solutions, 0.5 mL of AG337 Solution for Injection, 2%, was directly added to each of 3, 100 mL D5W infusion bags. All the bags were shaken to mix the solutions and 2 mLs of the prepared infusate were removed for $t_{(0)}$ assay from each bag. The bags were sealed with Parafilm®, and placed at 30°C.

Study II: Preparation of AG337 Solutions in D5W Infusion bags treated with sodium bicarbonate solution: Using the same method as described in Study I, AG337 Solution for Injection, 2% was diluted in 100 mL D5W Infusion bags to achieve AG337 concentrations of 4.0 and 1.0 mg/mL. For the 0.2 mg/mL solution, 1.0 mL of AG337 Solution for Injection, 2%, was directly added to each of three, 100 mL D5W infusion bags. All the infusion bags were treated with the sodium bicarbonate solutions, prior to the addition of AG337 Solution for Injection, 2%. Sodium bicarbonate was added to each of the D5W infusion bags, such that the bags containing 4, 1 and 0.2 mg/mL of AG337 would have a final sodium bicarbonate concentration of 0.113%, 0.028% and 0.006%, respectively, after addition of AG337. All the bags were shaken to mix the solutions and 4 mL samples were removed for $t_{(0)}$ assay. The bags were sealed with Parafilm® and placed at 30°C.

Control Solutions

Test control solutions were prepared in Type I USP borosilicate glass volumetric flasks using the same method described in Study I and II. The flasks were shaken to mix the contents thoroughly and aliquots removed for $t_{(0)}$ assay. The flasks were sealed with Parafilm® and placed at 30°C.

Sampling Procedure

Two milliliter samples were withdrawn through a syringe using a 21 gauge needle at 6 hours, 12 hours, 1 day, 3 day, 5 day and 7 day intervals for HPLC analysis of AG337, methylparaben and propylparaben. For Study IIB, 4 mL samples were withdrawn at similar time intervals to accommodate the measurement of pH.

RESULTS AND DISCUSSION

Study I: AG337 Solution in D5W Infusion Bags - 4 mg/mL Solutions

Table 1 shows the assay results for recovery of AG337 in D5W infusion test solutions stored at 30°C for the 7 day period. For theoretical AG337 concentrations of 4 mg/mL, the test samples showed AG337 recoveries ranging between 98 and 101%, which were similar to the control solutions in glass. Table 2 shows the recovery of methylparaben from the same D5W infusion solutions. At an AG337 concentration of 4 mg/mL, the theoretical methylparaben content is 360 µg/mL. The assayed value of methylparaben at $t_{(0)}$ was between 320 and 325 µg/mL for the test samples and the percent recovery of methylparaben dropped progressively to 73 - 75% by day 7 for all the samples. The assay values for the control solutions ($t_{(0)}$ = 333 µg/mL) showed recovery of around 93% through the 7 day test period. The propylparaben concentration in the test samples dropped rapidly and by day 3 was not detectable (Table 2). The propylparaben values for the control solution remained at 101% recovery. The low recovery of the parabens from the PVC bags indicated an interaction or adsorption with the PVC infusion bag. The low recovery of the parabens from PVC bags has been reported previously[6].

AG337 Solution in D5W Infusion Bags - 1 mg/mL Solutions

The test samples containing 1 mg of AG337/mL demonstrated AG337 recoveries ranging between 98 and 101% (Table 1). The control solutions also showed recovery values for AG337 (100%). The 1 mg/mL AG337 infusion solutions contained between 78 and 81 µg/mL methylparaben at $t_{(0)}$. Recovery dropped to between 65 and 68% by day seven (Table 2). The recovery of propylparaben from the 1 mg AG337/mL test samples demonstrated a rapid decrease in the concentration of propylparaben, though the levels remained measurable throughout the study period (Table 2). The recovery of propylparaben in solution dropped to between 16 and 20% by day seven, for concentrations of between 7 and 9 µg/mL. The control solution stored in glass bottles, showed 100% recovery for both methyl and propylparaben throughout the study period, indicating adsorption of the parabens with PVC bags.

AG337 Solution in D5W Infusion Bags - 0.1 mg/mL Solutions

For the solutions containing 0.1 mg/mL of AG337, when $t_{(0)}$ and $t_{(7\ day)}$ values were compared, the sample solution bags showed recoveries of 90 and 95% (Table 1). At $t_{(0)}$, two of the bags assayed at 0.11 mg/mL while the third bag assayed at 0.12 mg/mL. The percent recovery at day 7 was at 96%. The low recovery values and the variation for the third sample bag is attributed to a high

TABLE 1.
Assay Recovery of AG337 from Infusion Solutions

Time(day) —→	Sample Concn (Theoretical)	Experimental Data 0	1d	3d	5d	7d
STUDY I	4 mg/mL	3.76 ± 0.03 (3.83)	3.75 ± 0.03 (3.84)	3.73 ± 0.01 (3.81)	3.76 ± 0.03 (3.83)	3.77 ± 0.02 (3.83)
	1 mg/mL	0.94 ± 0.01 (0.94)	0.93 ± 0.01 (0.93)	0.93 ± 0.02 (0.93)	0.93 ± 0.01 (0.93)	0.93 ± 0.01 (0.93)
	0.1 mg/mL	0.11 ± 0.01 (0.18)	0.10 ± 0.01 (0.18)	0.10 ± 0.00 (0.18)	0.10 ± 0.00 (0.18)	0.10 ± 0.00 (0.18)
STUDY IIB	4 mg/mL	3.89 ± 0.05 (3.85)	3.85 ± 0.04 (3.86)	3.83 ± 0.07 (3.87)	3.84 ± 0.06 (3.81)	3.88 ± 0.04 (3.82)
	1 mg/mL	0.91 ± 0.05 (1.01)	0.90 ± 0.05 (1.01)	0.90 ± 0.05 (1.01)	0.90 ± 0.05 (1.01)	0.90 ± 0.05 (1.01)
	0.2 mg/mL	*0.19 ± 0.01 (0.19)	0.19 ± 0.01 (0.19)	0.19 ± 0.01 (0.19)	0.19 ± 0.01 (0.19)	0.19 ± 0.01 (0.19)

(The numbers in parenthesis are for assay values for the control solution in glass)
* 6 hr time point

$t_{(0)}$ assay and not due to degradation or adsorption. The reason for the large variation is attributed to the assay method being at its lowest detection levels for this series of studies. The methylparaben content of 0.1 mg/mL AG337 test samples started with a concentration between 9 and 10.1 µg/mL compared to a theoretical of 9 µg/mL. The recovery from the samples over time decreased to between 54 and 61% (Table 2). Recovery of the control at 7 days remained at 99%. The concentration of propylparaben for the 0.1 mg/mL AG337 test samples was below the detection level throughout the study (Table 2). Even at $t_{(0)}$, none of the samples gave a measurable peak for propylparaben. However, the recovery of propylparaben from the control solutions remained close to 100% over the 7 day test period.

Study II: AG337 Solution in D5W Infusion Bags Treated with Sodium Bicarbonate - 4.0 mg/mL Solutions

The stability of AG337 in D5W treated with sodium bicarbonate was monitored over a 7 day period. The recovery of AG337 and the parabens was determined by HPLC assay and the pH was monitored using a pH meter (Model 220, Corning Science Products, Corning, NY). At 4 mg/mL, the pH values for

TABLE 2.
Assay Recovery of Methyl and Propylparaben from Infusion Solutions

Time	(day) ⟶	Sample Concn (Theoretical)	Experimental Data 0	1d	3d	5d	7d
S	Methylparaben	360 µg/mL	321.93 ± 2.93 (333.10)	290.00 ± 3.52 (339.50)	264.37 ± 2.58 (336.70)	240.20 ± 3.84 (331.30)	238.30 ± 2.78 (338.10)
T	Propylparaben	40 µg/mL	36.15 ± 0.92 (41.00)	19.73 ± 0.65 (42.30)	n/d (41.10)	n/d (40.40)	n/d (41.80)
U	Methylparaben	90 µg/mL	80.00 ± 1.64 (81.40)	68.90 ± 1.08 (81.50)	60.90 ± 1.45 (81.50	56.40 ± 0.70 (81.40)	53.10 ± 1.61 (81.90)
D	Propylparaben	10 µg/mL	8.60 ± 0.87 (10.20)	3.80 ± 0.20 (10.20)	2.13 ± 0.15 (10.00)	1.83 ± 0.06 (9.70)	1.57 ± 0.06 (10.30)
Y	Methylparaben	9 µg/mL	9.33 ± 0.67 (16.10)	7.97 ± 0.06 (16.40)	6.80 ± 0.00 16.30)	6.37 ± 0.06 (16.20)	5.40 ±0.10 (16.00)
I	Propylparaben	1 µg/mL	n/d (2.10)	n/d (1.60)	n/d (2.00)	n/d (2.10)	n/d (2.10)
S	Methylparaben	360 µg/mL	329.80 ± 3.12 (328.70)	293.57 ± 3.41 (330.90)	266.63 ± 4.05 (333.40)	252.77 ± 3.22 (328.80)	244.63 ± 4.76 (328.20)
T	Propylparaben	40 µg/mL	40.17 ± 1.08 (39.50)	20.03 ± 0.42 (40.40)	13.77 ± 0.45 (41.30)	10.97 ± 1.25 (39.50)	9.07 ± 0.42 (40.70)
U	Methylparaben	90 µg/mL	76.90 ± 3.82 (86.30)	65.50 ± 2.91 (86.30)	59.00 ± 3.15 (86.50)	54.27 ± 2.89 (86.30)	50.40 ± 2.33 (86.60)
D	Propylparaben	10 µg/mL	9.10 ± 0.50 (11.10)	3.80 ± 0.17 (10.70)	2.43 ± 0.12 (10.50)	1.83 ± 0.12 (10.70)	0.80 ± 0.00 (10.80)
Y	Methylparaben	18 µg/mL	*16.20 (16.50)	13.73 ± 0.40 (16.70)	11.90 ± 0.36 (16.60)	11.10 ± 0.35 (16.70)	10.50 ± 0.61 (16.80)
II B	Propylparaben	2 µg/mL	*2.0 (2.0)	0.8 ± 0.1 (2.0)	0.6 ± 0.1 (2.2)	n/d (2.1)	n/d (1.4)

(The numbers in parenthesis are for assay values for the control solution in glass)
* 6 hr time point

n/d= none detected

the test samples ranged between 4.1 and 4.2 and did not differ significantly from the control solution.

The percent recovery of AG337 over the 7 days ranged between 98 and 101% for the test samples at 4 mg/mL (Table 1). The recovery of the control solutions remained at 100%. The percent recovery of methylparaben over the 7 days is shown in Table 2. At an AG337 concentration of 4 mg/mL, the methylparaben concentration is 360 µg/mL. The $t_{(0)}$ values of the test samples ranged between 326 and 332 µg/mL. The control samples assayed at 329 µg/mL. However, as observed in the previous samples, the methylparaben concentration for the test samples decreased over the seven days to between 73 and 76% at day 7. The percent recovery of propylparaben over the 7 days is shown in Table 2. At an AG337 concentration of 4 mg/mL, propylparaben has a theoretical concentration of 40 µg/mL. The test samples ranged between 39 and 41 µg/mL with the control samples at 39 µg/mL. By day 7, the control sample was still at 39 µg/mL. The test samples dropped to 9 µg/mL for a percent recovery between 22 and 24%. These results again indicate the adsorption of parabens to PVC bags.

AG337 Solution in D_5W Infusion Bags Treated with Sodium Bicarbonate - 1.0 mg/mL Solutions

For the solutions containing 1 mg/mL of AG337, the pH values ranged between 4.1 and 4.4 with no change occurring over the 7 day test period.

The percent recovery of AG337 from the solutions containing 1 mg/mL of AG337 showed results similar to the previous 4 mg/mL samples, with recovery ranging between 99 and 100% (Table 1). The theoretical methylparaben content of the 1 mg/mL AG337 samples is 90 µg/mL. The measured concentration at $t_{(0)}$ for the test samples ranged between 73 and 80 µg/mL. The control samples assayed at 87 µg/mL for $t_{(0)}$. By day 7, the methylparaben concentration in the PVC infusion bags dropped to between 47 and 52 µg/mL; a recovery of 65 - 66% (Table 2). The control samples remained at a 100% recovery of 87 µg/mL. The 1 mg/mL AG337 solutions started with a theoretical propylparaben content of 10 µg/mL. The test samples assayed between 9 and 10 µg/mL with the control sample at 11.1 µg/mL (Table 2). The control samples maintained close to 100% recovery dropping to 10.8 µg/mL. The test samples dropped to 0.8 µg/mL for a recovery of between 8 and 9%.

AG337 Solution in D_5W Infusion Bags Treated with Sodium Bicarbonate - 0.2 mg/mL Solutions

At 0.2 mg/mL of AG337, the pH value on day 3 dropped from 4.1 - 4.3 to a value of 3.9. However, for all the other days, the pH value of the infusion solutions treated with sodium bicarbonate ranged between 4.0 and 4.4 over the 7 day test period for all samples.

Two of the samples bags showed values for AG337 recovery at 0.41 and 0.32 mg/mL, while the third bag assayed at 0.19 mg/mL. The values for the 6 hour ($t_{(6\ hr)}$) samples were between 0.18 and 0.19 mg/mL for all the bags and since there was consistency in these 3 values, compared to the $t_{(0)}$ values, the 6 hour samples were used to compare the later time points. At day 3, recovery for the test samples averaged 96.7% with the control samples at 98.3% (Table 1). The 0.2 mg/mL AG337 samples assayed with a theoretical methylparaben content

of 18 μg/mL for $t_{(0)}$. The assay values for methylparaben, for the 6 hour samples were between 14.6 and 15.4 μg/mL for the 3 bags and were used as the comparison concentration. The test samples decreased from 15 μg/mL of methylparaben to between 10 and 11 μg/mL (66 - 73% recovery) at day 7 (Table 2). The control samples remained constant at 17 μg/mL. The 0.2 mg/mL AG337 solutions started with a theoretical propylparaben concentration of 2 μg/mL. At $t_{(6\ hr)}$, the test samples were below the quantifiable limits for the assay and were below detectable levels by day 5 (Table 2). The control samples assayed at 2.0 μg/mL (100% recovery) through day 5.

CONCLUSION

AG337 is stable at 30°C for up to seven days in D5W infusion bags at concentrations of 0.1 mg/mL to 4 mg/mL. Increasing the pH of the D5W infusion with sodium bicarbonate does not adversely affect stability. No precipitation was evident in any of the samples. The percent recovery of AG337 remained greater than 93% in all samples in the concentration range tested. The recovery of methyl and propylparaben decreased over time when stored in PVC infusion bags probably due to adsorption of parabens on to the bags.

The limit of quantitation for the HPLC method was a limiting factor for the determination of AG337, methyl and propylparaben at the lower concentration range of 0.2 mg/mL to 0.1 mg/mL of AG337. The wider variation in the recovery values was attributed to this limitation. However, based on the current clinical dosing of AG337, the assay method was not modified any further to detect lowered concentration levels.

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