

PTK 0796 (BAY 73-6944): Effects of Environmental Variation on MICs and Confirmation of Disk Mass

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ABSTRACT

Background PTK0796 (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcycline), is a novel antibacterial agent of the tetracycline family with potent and enhanced activity against resistant gram-positive and gram-negative pathogens.

Methods Using *E. coli* (EC) ATCC 25922, *S. aureus* (SA) ATCC 29213, and *E. faecalis* (EF) ATCC 29212 and NCCLS microbroth dilution (M7-A6) or disk diffusion (M2-A8) methodology, the present study was designed to: 1) determine the effect of varying test conditions (pH 6.0 to 8.0, divalent cation concentrations from 0.25 to 2 X normal range, inoculum size from 10³ to 10⁷ CFU/ml, presence or absence of CO₂, and the presence of blood in the test medium) on the *in vitro* activity of PTK0796; and 2) determine the appropriate disk content of PTK0796 for performing the disk diffusion susceptibility test by comparing zone diameters from 15, 30, and 60 µg disks compared to microbroth dilution MICs against clinical isolates.

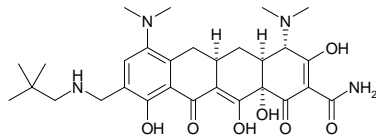
Results No differences in MICs were noted under varying conditions of divalent cation content, or the presence of blood or CO₂. Up to a 4-fold increase in MICs was noted with increases in inoculum size from 10³ to 10⁷ CFU/ml for SA and EF. No change was noted for EC. Although stable over a pH range of 7.2 to 7.4, the MICs increased up to 4-fold at pH 6.0. All gram-positive and *Haemophilus* strains were susceptible at a concentration of ≤4 µg/ml. Zones of inhibition for the 15, 30, and 60 µg disks generally correlate with MIC's. The 30 µg disk could be used for all species tested.

Conclusions When performed within NCCLS guidelines, minor variations in pH, divalent cation concentration, incubation in CO₂, and presence of blood in the test medium had little effect on the *in vitro* activity of PTK0796. Increasing inoculum size increased MICs by 4-fold for SA and EF but not EC. All disk contents gave good correlations to MICs.

INTRODUCTION

The novel aminomethylcycline, PTK 0796 (BAY 73-6944), was tested under several different conditions of susceptibility MIC testing to determine whether factors of the test might influence the degree of activity observed. In addition, a comparison of MIC testing and disk susceptibility testing was undertaken, including an assessment of several possible disk contents.

Structure of PTK 0796 (BAY 73-6944)



METHODS

Microorganisms

- 1084 isolates representing 40 species, majority were recent clinical strains collected in last 2 years.
- *E. coli* (EC) ATCC 25922, *S. aureus* (SA) ATCC 29213, and *E. faecalis* (EF) ATCC 29212

Antibiotics

- PTK 0796 standardized powder was obtained from Paratek Pharmaceutical Inc.
- PTK 0796 15, 30, and 60 µg disks were prepared by CML.
- Comparative agents were obtained from their manufacturers or commercial sources.

Susceptibility Tests

- The NCCLS M7-A6 standard method was followed for both broth and NCCLS M2-A8 was followed for disk diffusion testing.
- MIC panels were prepared in house and stored at -70 degrees C until use.

Quality Control

The following quality control strains were tested for effects of varying the media, inoculum and environment

- *S. aureus* ATCC 29213
- *E. faecalis* ATCC 29212
- *E. coli* ATCC 25922

The above strains plus the following strains were used during the disk mass confirmation studies

- *S. aureus* ATCC 25923
- *S. pneumoniae* ATCC 49619
- *P. aeruginosa* ATCC 27853
- *H. influenzae* ATCC 49247

RESULTS

Table 1
Effects of Lysed Horse Blood in CAMHB on PTK 0796 Microbroth Dilution MICs

Date	ATCC #	Species	PTK 0796 MIC	CAMHB LHB PTK 0796 MIC	Change (Log2)	Doxycycline MIC	Doxycycline MIC Change (Log2)	Minecycline MIC	Minecycline MIC Change (Log2)	
3/16/2003	25922	<i>E. coli</i>	1	0.5	1	1	1	0	1	0
3/16/2003	25922	<i>E. coli</i>	0.5	0.5	0	1	1	0	0.5	-1
3/17/2003	25922	<i>E. coli</i>	0.5	0.5	0	1	1	0	0.5	0
3/18/2003	25922	<i>E. coli</i>	0.5	0.5	0	0.5	1	-1	0.25	-1
3/19/2003	25922	<i>E. coli</i>	0.5	0.5	0	1	1	0	0.5	0
3/16/2003	29212	<i>E. faecalis</i>	0.12	0.12	0	4	4	0	2	2
3/16/2003	29212	<i>E. faecalis</i>	0.12	0.12	0	4	4	0	2	2
3/17/2003	29212	<i>E. faecalis</i>	0.12	0.12	0	4	4	0	2	2
3/18/2003	29212	<i>E. faecalis</i>	0.12	0.12	0	4	4	0	2	2
3/19/2003	29212	<i>E. faecalis</i>	0.12	0.12	0	4	4	0	2	2
3/16/2003	29213	<i>S. aureus</i>	0.25	0.25	0	0.25	0.25	0	0.25	0
3/16/2003	29213	<i>S. aureus</i>	0.25	0.25	0	0.25	0.25	0	0.25	0
3/17/2003	29213	<i>S. aureus</i>	0.25	0.25	0	0.25	0.25	0	0.25	0
3/18/2003	29213	<i>S. aureus</i>	0.25	0.25	0	0.25	0.25	0	0.25	0
3/19/2003	29213	<i>S. aureus</i>	0.25	0.25	0	0.25	0.25	0	0.25	0

• No differences in MICs were observed when lysed horse blood was added to the broth

Table 2
Effects of Variation in Inoculum Size

Date	ATCC #	Species	Inoculum of 10 ³ PTK 0796 MIC	Inoculum of 10 ⁴ PTK 0796 MIC	Inoculum of 10 ⁵ PTK 0796 MIC	Inoculum of 10 ⁶ PTK 0796 MIC	Inoculum of 10 ⁷ PTK 0796 MIC
4/7/2003	25922	<i>E. coli</i>	0.5	0.5	0.5	0.5	1
4/8/2003	25922	<i>E. coli</i>	0.25	0.5	0.5	0.5	0.5
4/9/2003	25922	<i>E. coli</i>	0.5	0.5	0.5	0.5	2
4/10/2003	25922	<i>E. coli</i>	0.5	0.5	0.5	0.5	1
4/11/2003	25922	<i>E. coli</i>	0.5	0.5	0.5	0.5	1
4/7/2003	29212	<i>E. faecalis</i>	0.006	0.06	0.12	0.12	0.25
4/8/2003	29212	<i>E. faecalis</i>	0.06	0.12	0.12	0.12	0.25
4/9/2003	29212	<i>E. faecalis</i>	0.06	0.06	0.12	0.12	0.12
4/10/2003	29212	<i>E. faecalis</i>	0.06	0.12	0.12	0.12	0.25
4/11/2003	29212	<i>E. faecalis</i>	0.06	0.12	0.12	0.25	0.25
4/7/2003	29213	<i>S. aureus</i>	0.12	0.12	0.25	0.25	0.25
4/8/2003	29213	<i>S. aureus</i>	0.12	0.12	0.12	0.25	0.25
4/9/2003	29213	<i>S. aureus</i>	0.12	0.12	0.25	0.5	0.25
4/10/2003	29213	<i>S. aureus</i>	0.12	0.12	0.25	0.5	0.5
4/11/2003	29213	<i>S. aureus</i>	0.12	0.12	0.25	0.5	0.5

• MICs increased by 1–2 dilutions when the inoculum was increased from 10³ to 10⁷ cfu/ml

Table 3
Effects of CO₂ on PTK 0796 Geomean MICs by Agar and Microbroth Dilution

ATCC #	SPECIES	BROTH MIC AIR			BROTH MIC CO2		
		PTK 0796 MIC	DOXY MIC	MINO MIC	PTK 0796 MIC	DOXY MIC	MINO MIC
25922	<i>E. coli</i>	0.5	1	0.5	1	1	0.28
29212	<i>E. faecalis</i>	0.12	4	1.78	0.17	2.24	1
29213	<i>S. aureus</i>	0.25	0.25	0.25	0.25	0.25	0.25

• No differences in MICs were observed when MIC's were incubated in CO₂

Table 4
Effects of pH on PTK 0796 Geomean MICs by Microbroth Dilution

ATCC #	SPECIES	PTK 0796 MIC PH 6	PTK 0796 MIC PH 6.5	PTK 0796 MIC PH 7	PTK 0796 MIC PH 7.2	PTK 0796 MIC PH 7.4	PTK 0796 MIC PH 8
25922	<i>E. coli</i>	1.74	0.87	0.76	0.50	0.50	0.87
29212	<i>E. faecalis</i>	0.50	0.25	0.16	0.12	0.12	0.14
29213	<i>S. aureus</i>	0.87	0.44	0.29	0.25	0.25	0.25

• MICs were stable over the pH range of 7.2 to 7.4, MICs were within 1 doubling dilution for pHs of 6.5, 7.0, 7.6, and 8.0

• MICs increased by 1 - 2 dilutions at pH 6.0

Table 5
Effects of Divalent Cation Concentration on PTK 0796 Geomean MICs by Microbroth Dilution

ATCC #	SPECIES	PTK 0796 MIC µg/ml Ca-5 Mg-5	PTK 0796 MIC µg/ml Ca-20 Mg-10	PTK 0796 MIC µg/ml Ca-25 Mg-12.5	PTK 0796 MIC µg/ml Ca-50 Mg-12.5
25922	<i>E. coli</i>	0.60	0.50	0.87	1.00
29212	<i>E. faecalis</i>	0.12	0.12	0.12	0.22
29213	<i>S. aureus</i>	0.25	0.25	0.29	0.33

• No differences in MICs were observed under varying conditions of divalent cation content

Figure 1. PTK 0796 MICs vs. Zone Diameter (30 µg Disks) *Staphylococci, Enterococci & Miscellaneous Gram-positive Species (n=632)*

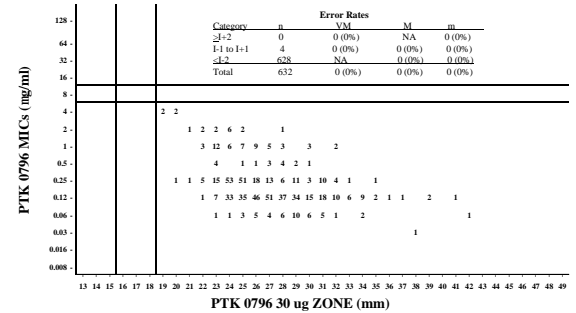


Figure 2. PTK 0796 MICs vs. Zone Diameter (30 µg Disks) *Streptococcus Species (n=183)*

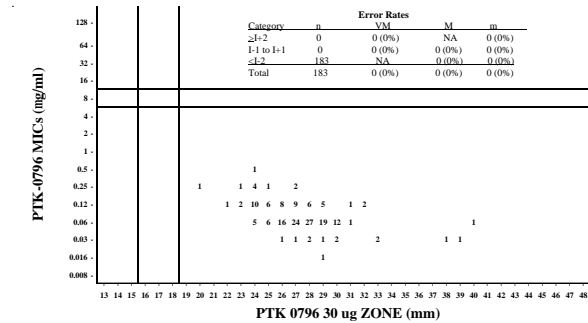


Figure 3. PTK 0796 MICs vs. Zone Diameter (30 µg Disks) *Enterobacteriaceae (n=131)*

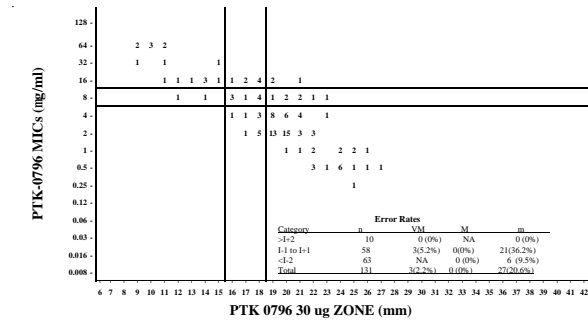


Figure 4. PTK 0796 MICs vs. Zone Diameter (30 µg Disks) *Haemophilus Species (n=74)*

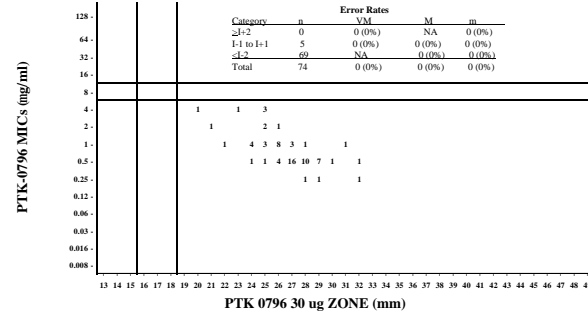
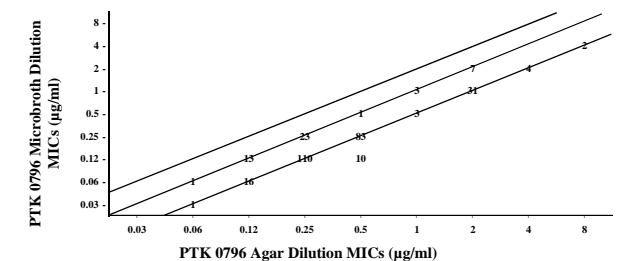


Table 6
Relative Accuracy of 15,30, and 60 µg PTK 0796 Disks

Species Category	Number	Disk Potency	Error Rates No. (%)		
			Minor	Major	Very Major
<i>Staphylococcus</i> spp.	320	15 µg	0	0	0
207 meth- resistant		30 µg	0	0	0
		60 µg	0	0	0
<i>Enterococcus</i> spp.	259	15 µg	0	0	0
135 Vancomycin R		30 µg	0	0	0
		60 µg	0	0	0
<i>Streptococcus</i> spp.	183	15 µg	0	0	0
107 <i>S. pneumoniae</i>		30 µg	0	0	0
		60 µg	0	0	0
<i>Haemophilus influenzae</i>	74	15 µg	0	1 (1.4%)	0
		30 µg	0	0	0
		60 µg	0	0	0
<i>Enterobacteriaceae</i>	131	15 µg	27 (20.6%)	0	0
		30 µg	27 (20.6%)	0	3 (2.2%)
		60 µg	44 (33.6%)	0	4 (3.1%)
non-enteric bacilli	53	15 µg	2 (6.2%)	0	0
		30 µg	7 (13.3%)	0	0
		60 µg	8 (15.9%)	0	0

Figure 5. PTK 0796 Microbroth Dilution MICs vs. Agar Dilution MICs (µg/ml) All Non-Fastidious Species Combined (n=308)



• Comparison of PTK 0796 agar dilution vs. broth microdilution MICs showed a definite skewing toward higher MICs by agar dilution for all species tested.

CONCLUSIONS

- PTK 0796 (BAY 73-6944) is a potent new aminomethylcycline antimicrobial agent.
- All gram positive strains tested and all *H. influenzae* were susceptible at < 4.0 µg/ml.
- Many enteric and some non enteric species were also susceptible at < 4.0 µg/ml.
- Microbroth susceptibility tests on PTK 0796 (BAY 73-6944) were not affected by varying divalent cation content, the addition of lysed horse blood, incubation in a 5% CO₂ environment or by pH changes in the broth from pH 7.0 to pH 8.0.
- Increasing the standard inoculum size from 10³ to 10⁷ caused an increase in the MICs.
- The 30 µg disk performed satisfactorily for all gram positive species and *Haemophilus influenzae*.
- The 30 µg disk also performed satisfactorily for *Enterobacteriaceae* and non-enteric bacilli, however there was a slightly higher percentage of minor errors with these species.
- Agar dilution resulted in somewhat higher MICs compared to microbroth.