RESEARCH ARTICLE

Method Development and Validation For Determining Stability of Omadacycline In Biological Matrices By Liquid Chromatography–Mass Spectrometry

Satya Prasad B*, Jaya Kumari S

Department of Pharmacognosy, School Pharmaceutical Sciences
Vel`s Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai – 117, Tamilnadu, India

Received: 17th October, 19; Revised: 21th November, 19, Accepted: 15th December, 19; Available Online: 25th December, 2019

ABSTRACT

The validated protein precipitation method was applied for the estimation of Omadacycline (OM) in human plasma with Omadacycline-D9 (OMD9) as an internal standard (ISTD) by using HPLC-ESI-MS/MS. Zorbax Eclipse Plus C18, 2.1 x 50 mm, 3.5 µm, was selected as the analytical column. The column temperature was set at 45°C. Mobile phase composition was 0.1% formic acid: methanol (80:20 v/v). Source flow rate of 300 µL/min without a split. An injection volume of 10 µL. Omadacycline and Omadacycline-D9 mesylate were eluted at 1.2 ± 0.2 min, with a total run time of 3.0 min for each sample. The mass transitions of Omadacycline and Omadacycline-D9 obtained were m/z 557.6 ® 456.6 and 566.7 ® 456.6, respectively. The standard curve shows a correlation coefficient (r^2) greater than 0.9983 with a range of 5.00 to 12000.00 pg/ml using the linear regression model.

Keywords: Bioanalysis, HPLC-ESI-MS/MS, Human plasma, Omadacycline.

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijpqa.10.4.14

How to cite this article: Prasad, B.S. and Kumari, S.J. (2019). Method Development and Validation For Determining Stability of Omadacycline In Biological Matrices By LC-MS/MS. International Journal of Pharmaceutical Quality Assurance 10(3): 640-645.

Source of support: Nil

Conflict of interest: Authors declare that there is no conflict of interest.

Funding: Not applicable.

Author's Contributions: The work was designed; experimental work carried by BSP, and the results was interpreted by SJK. The manuscript was written by BSP. Both the authors read and approved the final manuscript.

INTRODUCTION

Omadacycline is the first aminomethylcycline antibiotic in late-stage clinical development. Aminomethylcyclines are semisynthetic antibiotics related to tetracyclines.[1-9] Similar to their tetracycline counterparts, aminomethylcyclines inhibit bacterial protein synthesis. Importantly, however, the two main mechanisms of tetracycline resistance, namely, efflux pumps and ribosomal protection, are overcome by modifications present at the C-7 and C-9 positions in the chemical structure of omadacycline.[8-15] Omadacycline has been shown to be active against a variety of bacterial pathogens: Gram-positive aerobes, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant and multidrug-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococcus (VRE); Gram negative aerobes; some anaerobes; and atypical bacteria, such as *Legionella* spp. and *Chlamydia* spp.[10-24]

The chemical name of Omadacycline is ((4S, 4aS, 5aR, 12aR)-4,7- bis (dimethyl amino) – 9-[(2, 2- dimethyl propylamino) methyl]-1, 10, 11, 12 a-tetrahydroxy-3, 12 – dioxo-4a, 5, 5a, 6-tetrahydro-4H – tetracene – 2-carboxamide. The molecular formula is $C_{29}H_{40}N_4O_7$ and the molecular weight is 557.66.[25] The chemical structure of Omadacycline and Omadacycline 9 are given in Figure 1.

The literature survey reveals that one method was reported on the quantification of omadacycline by using LC-MS/ MS.[26-27] There is no method reported for estimation of omadacycline using deuterated internal standard in biological samples.

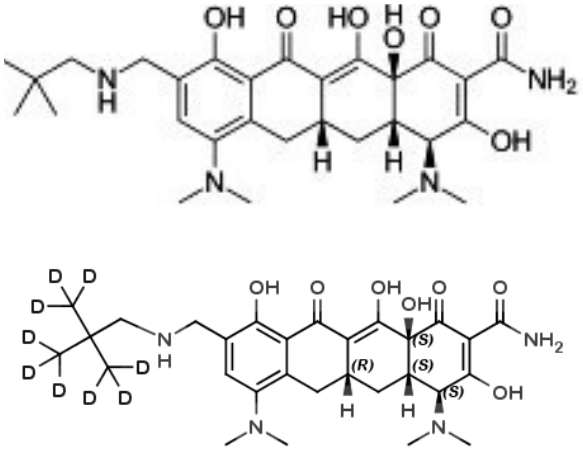

Figure 1: Chemical structures of A) Omadacycline B) Omadacycline-D9

**Author for Correspondence: satyaprasadsri@gmail.com*

The main goal of the present study is to develop and validate the novel simple, sensitive, selective, rapid, rugged and reproducible analytical method for the quantitative determination of omadacycline in human plasma by HPLC-ESI-MS/MS.

MATERIALS AND METHODS

Chemical Resources

Omadacycline and Omadacycline-D9 were obtained by TLC Pharma chem, Canada. LC grade methanol, Methyl t-butyl ether, and Dichloromethane were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Analytical Reagent grade formic acid and sodium carbonate were procured from Merck (Mumbai, India). Human plasma (K_2EDTA) was obtained from Doctors pathological Lab, Hyderabad. Ultrapure water from the Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study. All other chemicals in this study were of analytical grade.

Instrument Resources

An API 4000 HPLC-ESI-MS/MS system (Applied Biosystems), 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany), data acquisition and processing were accomplished using Analyst® Software 1.4.1.

Chromatographic conditions

Zorbax Eclipse Plus C18, 2.1 x 50 mm, 3.5 µm, was selected as the analytical column. The column temperature was set at 45°C. Mobile phase composition was 0.1% formic acid: methanol (80:20 v/v). Source flow rate at 300 mL/min without a split. An injection volume of 10 µL. OM and OMD9 were eluted at 1.2 ± 0.2 min, with a total run time of 3.0 min for each sample.

Detection

The pure drug solutions of OM and OMD9 were prepared in methanol (10.00 ng/mL) and injected with a flow rate of 5 µL/min into positive ion mode mass spectrometer for optimization of mass parameters like source temperature, IS, heater gas, nebulizer gas, curtain gas, CAD gas (all gas channels were purged with ultra-high pure nitrogen gas), EP, DP, CE, FP, and CXP were optimized. The analysis was performed using MRM positive ion mode with mass transitions of m/z (amu) 557.6 ® 456.6 and 566.7 ® 456.6 for OM and OMD9, respectively. The mass fragmentation pattern of parent and product ions mass spectra were depicted in Figure 2.

Standard calibration and quality control samples preparation

Stock solutions of OM (1000.00 µg/mL) and OMD9 (1000.00 µg/ml) were prepared in methanol. The internal standard (OMD9) spiking solution (500.00 ng/mL) was prepared in 75% methanol from OMD9 stock solution. Stock solutions of OM, OMD9 and intermediate spiking solutions were stored in refrigerated conditions (2–8°C) until analysis.

Calibration standards 5.00 to 12000.00 pg/mL, quality control samples of lower limit QC, low QC, mid-QC, high QC (5.00, 15.00, 4500.00, 9000.00 pg/mL) were used by spiking the appropriate amount of standard solution in the drug-free plasma and stored at –30°C till analysis.

Sample extraction

The liquid-liquid extraction procedure was used for the isolation of OM from the plasma samples. For this purpose, 50µL of OMD9 (IS) concentration of 10ng|mL) 100µL plasma (respective concentration of plasma sample) was added into ria vials then vortexed approximately. Followed by 200µL of 1M Na_2C0_3 solution, 3mL of Extraction solvent (MTBE: DCM (3:1,v/v) was added to each tube and vortexed for 10 minutes. After that, the samples were centrifuged at 4000 rpm for approximately 10 minutes at 20°C temperature and transferred the supernatant into respective ria vials. These samples were allowed to dry under a nitrogen stream at 25°C. Finally, the residue was reconstituted with 200µL of reconstitution solution (MeOH : 0.1% formic acid (1:4). Further samples were centrifuged at 4000 rpm for approximately 2 minutes, and at 20°C and supernatant were transferred into autosampler vials with caps, and 10 µL of sample was injected onto the LC-MS/MS system.

Method validation

The developed method was validated over a linear concentration range of 5.00 to 12000.00 pg/mL. The validation parameters include selectivity and specificity, LoQ, linearity, precision and accuracy, matrix effect, recovery, stability (freeze-thaw, autosampler, benchtop, long term) was evaluated under validation section.[28-30]

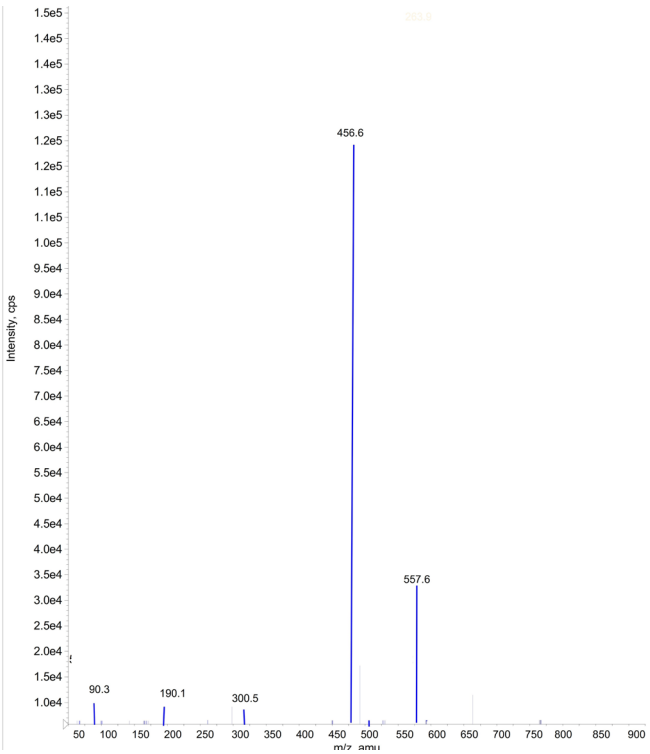

Figure 2: Mass fragmentation pattern of omadacycline (OM)

Selectivity and Specificity

Ten lots of blank plasma samples were analyzed, out of which six lots free from interference were selected for assessing the selectivity and specificity. The endogenous/potential interfering peak areas for blank samples must be less than 20% of the LLoQ peak area of OM retention time and less than 5% for OMD9 retention time.

Limit of Quantification (LoQ)

Six LLOQ standards were prepared in screened plasma lot along with IS (500 pg/mL), and signal to noise ratio (S/N) was calculated using analyst software.

Linearity

Calibration standards were prepared to obtain linearity range of 5, 10, 100, 600, 1200, 2400, 4800, 7200, 9600 & 12000 pg/ml and assayed in five replicates on five different days.

Precision and Accuracy

One set of calibration standards and one set contains four different concentrations of quality control standards of lower limit QC (5.00 pg/mL), low QC (15.00 pg/mL), mid QC (4500.00 pg/mL) and high QC (9000.00 pg/mL) concentrations were prepared in screened plasma and analyzed each quality control (QC) standards in six replicates on the same day (Intra-day) and five different days (Inter-day).

Matrix Effect

Six extracted blank plasma samples in three replicates were spiked with the un-extracted concentration of mid-QC (4500.00 pg/mL) and compared with un-extracted standards of the same concentration.

Recovery

The recovery of samples was performed by protein precipitation method. The extraction recovery was determined in six replicate by comparing the extracted QC standards with un-extracted QC standards at three different concentrations of low (15.00 pg/mL), medium (4500.00 pg/mL), high (9000.00 pg/mL).

Stability studies

- *Benchtop Stability (Room Temperature Stability, 24hours)*

Six replicates of spiked low and high concentrations (Benchtop stability samples) were set aside at ambient temperature up to 24-hour. Samples were processed and compared with newly prepared low and high concentrations (comparison samples).

- *Freeze and thaw stability (after 3rd cycle at –30°C)*

Six replicates of low and high concentrations (FT stability samples) were frozen at -30°C and subjected to three freeze-thaw cycles of 24, 36, and 48-hour (-30°C to room temperature) and compared with newly prepared low and high concentrations (comparison samples).

- *Autosampler stability (2–8°C, 65-hour)*

Six replicates of low and high concentrations (AS stability samples) were stored in auto-sampler up to 65 h at 2-8°C. Stability samples were compared with newly prepared low and high concentrations (comparison samples).

- *Long-term Stability (-30°C, 64 Days)*

After completion of the stability period stored at -30 °C (64 days) six replicates of low and high concentrations (LT stability samples) were compared with newly prepared low and high concentrations (comparison samples).

RESULTS AND DISCUSSION

Method development

Initially, a mobile phase consisting of ammonium acetate and acetonitrile in varying combinations were tried, but a low response was observed. The mobile phase containing acetic acid: acetonitrile (20:80 v/v) and acetic acid: methanol (20:80 v/v) gives a better response, but poor peak shape was observed. A mobile phase of 0.1% formic acid in water in combination with methanol and acetonitrile with varying combinations were tried using a mobile phase containing 0.1% formic acid in water in combination with methanol (20:80 v/v), the best signal along with a marked improvement in the peak shape was observed for OM and OMD9. Short length columns, such as symmetry shield RP18 (50mm x 2.1 mm, 3.5 m), Inertsil ODS-2V (50mm x 4.6 mm,5 m),Hypurity C18 (50mm x 4.6 mm, 5 m) and Rypurity Advance (50 mm x 4.0 mm, 5 m), YMC basic (50 mm x2 mm, 5 m), Zorbax Eclipse Plus C18, (2.1mm x 50 mm, 3.5 µm) were tried during the method development. The best signal and good peak shape was obtained using the Zorbax Eclipse Plus C18, 2.1 x 50 mm, 3.5 mm, column. It gave satisfactory peak shapes for both OM and OMD9. A flow rate of 0.3mL/min without splitter as used and reduced the run time to 3.0 min. Both Analyte and IS were eluted with a shorter time at 2.0 min. For an LC-MS/MS analysis, utilization of stable isotope-labeledd or suitable analog drugs as an internal standard proves helpful when a significant matrix effect is possible. In our case, OMD9 was found to be best for the present purpose. The column oven temperature was maintained at a constant temperature of about 45°C.

An injection volume of a 10µL sample is adjusted for better ionization and chromatography. Prior to load the sample for LC injection, the co-extracted proteins should be removed from the prepared solution. For this purpose, initially, we tested with different extraction procedures like PPT (Protein Precipitation), LLE (liquid extraction), and SPE (Solid Phase extraction). We found ion suppression effect in protein precipitation method for drug and internal standard. Further, we tried with SPE and LLE. Out of all, we observed LLE is suitable for the extraction of drugs and IS. We tried with several organic solvents (ethyl acetate, chloroform, n-hexane, dichloromethane, and methyl tertiary butyl ether) individually as well with combinations in LLE to extract analyte from the plasma sample. In our case methyl tertiary butyl ether: dichloromethane (75:25) combination served as suitable extraction solvent. Autosampler wash is optimized as 80% methanol. Several compounds were investigated to find a suitable IS, and finally, OMD9 found

the most appropriate internal standard for the present purpose. There was no significant effect of IS on analyte recovery, sensitivity, or ion suppression. Righ recovery and selectivity were observed in the Liquid-Liquid extraction method. These optimized detection parameters, chromatographic conditions, and extraction procedure resulted in reduced analysis time with accurate and precise detection of omadacycline in human plasma. The parent and production mass spectrums of OM and OMD9 were shown in Figure 3.

Method validation

Selectivity and Specificity, Limit of Quantification (LoQ)

No significant response was observed at retention times of OM and OMD9 in blank plasma as compared to LLoQ and blank with IS samples. The limit of quantification for this method was proven as the lowest concentration of the calibration curve, which was proven as 5.0 ng/mL. Representative chromatograms were shown in Figure 4.

Linearity

Linearity was plotted as a peak area ratio (OM peak area/ OMD9 peak area) on the y-axis against OM concentration (pg/mL) on the x-axis. Calibration curves were found to be consistently accurate and precise for OM over a linearity range of 5.00 to 10000.00 pg/mL.

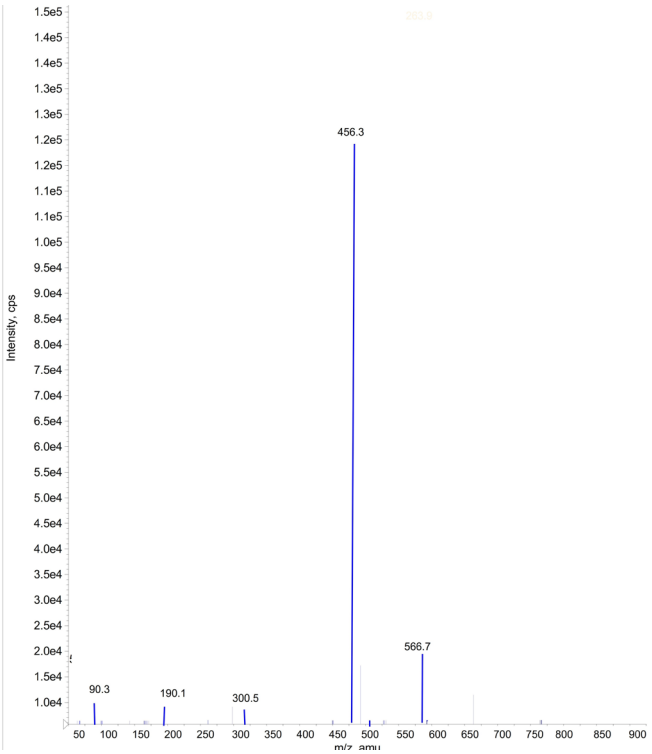

Figure 3: Mass fragmentation pattern of Omadacycline D9 (OMD9)

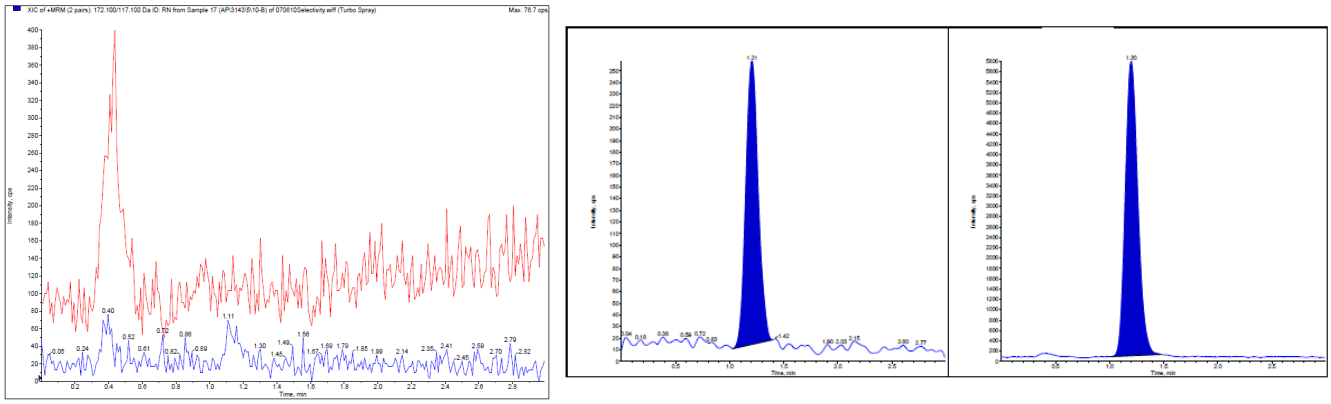

Figure 4: Representative chromatograms of Omadacycline in plasma a) Blank plasma chromatogram for interference-free OM and OMD9 b) Chromatogram of LLOQ sample (OM with OMD9).

The correlation coefficient was greater than 0.9980 for OM. The %CV was less than 15%, and mean %accuracy was ranged between 95.58-100.53%. The results were presented in Table 1.

Precision and Accuracy

Intra and inter batch % accuracy for OM was ranged between 95.83 to 101.44 and 89.10 to 101.59. % CV is 1.46 to 4.51 and

Table 1: Calibration curve details

Spiked plasma Concentration (pg/mL)	*Concentration measured (pg/ml) (Mean ± S.D)*	*% CV (n = 5)*	*% Accuracy*
5	4.96 ± 0.09	1.80	99.20
10	8.81 ± 0.35	3.95	88.10
100	95.58 ± 3.33	3.48	95.58
600	599.88 ± 1.97	0.33	99.98
1200	1184.00 ± 16.70	1.41	98.67
2400	2408.00 ± 63.00	2.62	100.33
4800	4676.00 ± 152.72	3.27	97.42
7200	6904.00 ± 254.76	3.69	95.89
9600	9032.00 ± 210.85	2.33	94.08
12000	11893.40 ± 139.27	1.17	99.11

Table 2: Precision and accuracy (Analysis with spiked samples at three different concentrations)

Spiked Plasma Concentration (pg/ml)	*Within-run (Intra-day)*			*Between-run (Inter-Day)*		
	Concentration measured (n = 6;pg/mL;mean ± S.D)	*%CV*	*% Accuracy*	*Concentration measured (n = 6;pg/mL;mean ± S.D)*	*%CV*	*%Accuracy*
15.00	14.92 ± 0.23	1.54	99.44	14.58 ± 0.26	1.77	97.21
4500.00	4497.45 ± 65.87	1.46	99.94	4529.36 ± 73.48	1.62	100.65
9000.00	9129.42 ± 411.56	4.51	101.44	9143.43 ± 161.07	1.76	101.59

Table 3: Stability studies of omadacycline in plasma

	Room temperature Stability		*Processed sample Stability*		*Long term stability*		*Freeze and thaw stability*	
	24h		*65h*		*64 days*		*Cycle (48-hour)*	
Spiked Plasma concentration (pg/mL)	*Concentration measured (n = 6;pg/mL; mean ± S.D)*	*% CV (n = 6)*	*Concentration measured (n = 6;pg/mL; mean ± S.D)*	*%CV (n = 6)*	*Concentration measured (n = 6;pg/mL; mean ± S.D)*	*%CV (n = 6)*	*Concentration measured (n = 6;pg/mL; mean ± S.D)*	*%CV (n = 6)*
15.00	14.99 ± 0.77	4.12	14.79 ± 0.30	2.01	15.45 ± 0.42	2.69	16.20 ± 2.92	5.8
9000.00	9571.86 ± 394.82	5.12	8552.23 ± 266.65	3.12	8579.36 ± 249.51	2.91	9486.43 ± 520.63	5.49

1.62-7.09. Results are presented in Table 2.

Recovery

The mean % recovery for LQC, MQC, HQC samples of OM were 94.12%, 97.76%, and 96.06%, respectively. The overall mean %recovery and %CV of OM across QC levels is 94.12% and 5.12%. For the OMD9 (internal standard), the mean % recovery and % CV is 91.68% and 7.09%, respectively.

Matrix Effect

No significant matrix effect found in different sources of rat plasma tested for OM, OMD6. The % CV was found to be 3.71.

Stability (freeze-thaw, autosampler, benchtop, long term)

Quantification of the OM in plasma subjected to three freeze-thaw cycles (−30°C to room temperature), autosampler (processed), room temperature (Benchtop), long-term stability details was shown in Table 3.

CONCLUSION

The method described in this manuscript has been developed and validated over the concentration range of 5.00 –12000.00 pg/ml in human plasma. The intra and inter-batch precision (% CV) was less than 15.0%, and % accuracy ranged from 95.83%–101.59%. The overall % recovery for OM, OMD9, was greater than 90%. The selectivity, sensitivity, precision, and accuracy obtained with this method make it suitable for the present study. In conclusion, the method used in the present study is easy and fast to perform; it is also characterized by adequate accuracy, precision, selectivity, and stability. The simplicity of the method, and using rapid protein precipitation extraction with less run time of 3.0 min per sample, make it an attractive procedure in high-throughput bioanalysis of Omadacycline.

ACKNOWLEDGMENTS

The authors wish to thank the support received from Sipra Labs, Hyderabad, India, for providing a literature survey and carrying out this research work.

REFERENCES

1. Villano S, Steenbergen J, Loh E. (2016). Omadacycline: development of a novel amino methyl cycline antibiotic for treating drug-resistant bacterial infections. Future Microbiol 11:1421-1434.
2. Heidrich CG, Mitova S, Schedlbauer A, Connell SR, Fucini P, Steenbergen JN, Berens C. (2016). The novel aminomethylcycline omadacycline has high specificity for the primary tetracycline-binding site on the bacterial ribosome. Antibiotics (Basel). 22: doi:10.3390/antibiotics5040032.
3. Draper MP, Weir S, Macone A, Donatelli J, Trieber CA, Tanaka SK, Levy SB. (2014). Mechanism of action of the novel aminomethycycline antibiotic omadacycline. Antimicrob Agents Chemother 58:1279-1283.
4. Macone AB, Caruso BK, Leahy RG, Donatelli J, Weir S, Draper MP, Tanaka SK, Levy SB. (2014). *In vitro* and *in vivo* antibacterial activities of omadacycline, a novel aminomethycycline. Antimicrob Agents Chemother 58:1127-1135.
5. Pfaller MA, Rhomberg PR, Huband MD, Flamm RK. (2017). Surveillance of omadacycline activity tested against clinical isolates from a global (North American, Europe, Latin American and Asia-Western Pacific) collection (2010-2011). Antimicrob Agents Chemother 61: doi:10.1128/AAC.00018-17.
6. Pfaller MA, Rhomberg PR, Huband MD, Flamm RK. (2017). Activities of omadacycline and comparator agents against *Staphylococcus aureus* isolates from a surveillance program conducted in North American and Europe. Antimicrob Agents Chemother 61: doi:10.1128/AAC.02411-16.
7. Waites KB, Crabb DM, Liu Y, Duffy LB. 2016. *In vitro* activities of omadacycline (PTK 0796) and other antimicrobial agents against human mycoplasmas and ureaplasmas. Antimicrob Agents Chemother 60:7502-7504
8. Dubois J, Dubois M, Martel JF, Tanaka 576 SK. (2016). *In vitro* intracellular activity of omadacycline against *Legionella pneumophila*. Abstr at American Society of Microbiology, Microbe, New Orleans, Louisiana, abstr. 551.
9. Honeyman L, Ismail M, Nelson ML, Bhatia B, Bowser TE, Chen J, Mechiche R, Ohemeng K, Verma AK, Cannon EP, Macone A, Tanaka SK, Levy S. (2015). Structure activity relationship of the aminomethycyclines and the discovery of omadacycline. Antimicrob Agents Chemother 59:7044-7053.

10. Sun H, Ting L, Machneni S, Praestgaard J, Kuemmell A, Stein DS< Sunkara G, Kovacs SJ, Villano S, Tanaka SK. (2016). Randomized, open-label study of the pharmacokinetics and safety of oral and intravenous administration of omadacycline to healthy subjects. Antimicrob Agents Chemother 60:7431-7435.
11. Tzanis E, Manley A, Villano S, Tanaka SK, Bai S, Loh E. (2017). Effect of food on the bioavailability of omadacycline in healthy participants. J Clin Pharmacol 57:321-327.
12. Noel GJ, Draper MP, Halt H, Tanaka SK, Arbeit RD. (2012). A randomized, evaluator 590 blind, phase 2 study comparing the safety and efficacy of omadacycline to those of linezolid for treatment of complicated skin and skin structure infections. Antimicrob Agents Chemother 56:5650-5654.
13. Flarakos J, Du Y, Gu H, Wang L, Einolf HJ, Chun DY, Zhu B, Alexander N, Natrillo A, Hanna I, Ting L, Zhou W, Dole K, Sun H, Kovacs SJ, Stein DS, Tanaka SK, Villano S, Mangold JB. (2016). Clinical disposition, metabolism and in vitro drug-drug interaction properties of omadacycline. Xenobiotica. doi:10.108 0/00498254.2016.1213465.
14. Rodvold KA, George JM, Yoo L. (2011). Penetration of anti-infective agents into pulmonary epithelial lining fluid: focus of antibacterial agents. Clin Pharmacokinet 50:637-664.
15. Ambrose PG, Bhavnani SM, Ellis-Grosse EJ, Drusano GL. (2010). Pharmacokinetic600 pharmacodynamic considerations in the design of hospital-acquired or ventilator-acquired bacterial pneumonia studies: look before you leap! Clin Infect Dis 51(Suppl. 1):S103-S110.
16. Nix DE. (1998). Intrapulmonary concentrations of antimicrobial agents. Infect Dis Clin North Am 12:631-646.
17. Baldwin DR, Honeybourne D, Wise R. (1992). Pulmonary disposition of antimicrobial agents: in vivo observations and clinical relevance. Antimicrob Agents Chemother 36:1176- 1180.
18. Rodvold KA, Gotfried MH, Danziger LH, Servi RJ. (1997). Intrapulmonary steady-state concentrations of clarithromycin and azithromycin in healthy adult volunteers. Antimicrob Agents Chemother 41:1399-1402.
19. Gotfried MH, Danziger LH, Rodvold KA. (2003). Steady-state plasma and bronchopulmonary characteristics of clarithromycin extended-release tablets in normal healthy adult subjects. J Antimicrob Chemother 52:450-456.
20. Rodvold KA, Danziger LH, Gotfried MH. (2003). Steady-state plasma and bronchopulmonary concentrations of intravenous levofloxacin and azithromycin in healthy adults. Antimicrob Agents Chemother 47:2450-2457.
21. Rodvold KA, Gotfried MH, Still JG, Clark K, Fernandes P. (2012). Comparison of plasma, epithelial lining fluid, and alveolar macrophage concentrations of solithromycin (CEM-101) in healthy adult subjects. Antimicrob Agents Chemother 56:5076-5081.
22. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG. (1986). Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J Appl Physiol 60:532-538.
23. Baldwin DR, Wise R, Andrews JM, Ashby P, Honeybourne D. (1990). Azithromycin concentrations at the sites of pulmonary infection. Eur Respir J 3:886-890.
24. Tenero D, Bowers G, Rodvold KA, Patel A, Kurtinecz M, Dumont E, Tomayko J, Patel P. (2013). Intrapulmonary pharmacokinetics of GSK2251052 in healthy volunteers. Antimicrob Agents Chemother, 57:3334-3339.
25. Mark H. Gotfrieda, *et al*., (2017) Comparison of Omadacycline and Tigecycline Pharmacokinetics in the 2 Plasma, Epithelial Lining Fluid, and Alveolar Cells in Healthy Adult Subjects. Antimicrob. Agents Chemother. doi:10.1128/AAC.01135-17, AAC Accepted Manuscript Posted Online. 1-29.
26. Guidance for industry. (2001), Bioanalytical method validation, U.S. Department of Health and Human Services, food and drug administration, center for drug evaluation and research (CDER), Center for biologics evaluation and research (CBER).
27. Guidance for industry. (2002), Food-effect bio availability and fed bio equivalence studies: U.S. Department of Health and Human Services food and drug administration centre for drug evaluation and research (CDER).
28. Guidance for industry. (2003), Bio availability and fed bio equivalence studies for orally administered drug products-general considerations: U.S.Department of Health and Human Services food and drug administration centre for drug evaluation and research (CDER).