Pharm Methods, 2019;10(2):47-52.
A multifaceted peer reviewed journal in the field of Pharm Analysis and Pharmaceutics
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Original Article

Estimation of Eravacycline Dihydrochloride in Biological Matrices by LC-MS/MS

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ABSTRACT

Objective: The validated protein precipitation method was applied for estimation of Eravacycline dihydrochloride in human plasma with Rolitetracycline hydrochloride as an internal standard (ISTD) by using HPLC-ESI-MS/MS. **Methods:** The chromatographic separation was achieved with 20mM Ammonium acetate (pH-3.0):Methanol:Acetontrile (20:20:60,%v/v) using the TELOS LU C18 (2) 5µm, 100 x 4.6 mm. The total analysis time was 3 min and flow rate was set to 0.5 mL/min. Results: The mass transitions of Eravacycline dihydrochloride and Rolitetracycline hydrochloride obtained were m/z 632.5®84.3 and 436.2®84.3. The standard curve shows correlation coefficient (*s*) greater than 0.999 with a range of 15.00-120.00 pg/ml using the linear regression model. **Conclusion:** The method was suitable and conveniently applicable to pharmacokinetic and bioavailability studies for estimation of Eravacycline in biological matrices by HPLC-ESI-MS/MS.

Key words: Eravacycline, Rolitetracycline, Human plasma, HPLC-ESI-MS/MS, Bioanalysis.

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DOI : 10.5530/phm.2019.2.9

INTRODUCTION

The need for new antibiotics to treat the increasing number of multi-drug-resistant bacteria was recognized most recently in April 2011 by the World Health Organization's call for a six-point global policy package that includes joint planning, surveillance, drug regulation, rational use of medicines, infection prevention and control and innovation and research.[1-6]

In some countries, there is little difference in the incidences of multidrug-resistant pathogens in the community and in the hospital; most notably, extended-spectrum β-lactamase (ESBL)-producing and/or carbapenem-resistant *Enterobacteriaceae* are being isolated in patients with no prior contact with the health care system, resulting in increased hospital stays for otherwise healthy adults with urinary tract infection or pyelonephritis.[3-8]

Eravacycline is a novel fluorocycline antibiotic designed to overcome resistance to common tetracycline-specific efflux and ribosomal protection mechanisms and is impervious to other antibiotic-specific resistance mechanisms.[4-6]

Similar to other members of the tetracycline antibiotic class, eravacycline has been shown to be a potent, mechanism-based inhibitor of the bacterial ribosome. It has modifications at both the C-7 (fluorine) and C-9 [2-(pyrrolidin-1-yl)ethanamido] positions on the tetracyclic core that were made possible by using a totally synthetic route.[7-15]

Eravacycline is a fluorocycline antibacterial within the tetracycline class of antibacterial drugs. Eravacycline disrupts bacterial protein synthesis by binding to the 30S ribosomal subunit thus preventing the incorporation of amino acid residues into elongating peptide chains. In general, eravacycline is bacteriostatic against gram-positive bacteria (e.g., *Staphylococcus aureus* and *Enterococcus faecalis*); however, *in vitro* bactericidal activity has been demonstrated against certain strains of *Escherichia coli and Klebsiella pneumoniae.* eravacycline, a synthetic tetracycline-class antibacterial agent for intravenous administration.

Chemically, eravacycline is a C7-, C9-substituted sancycline derivative. The chemical name of eravacycline dihydrochloride is [(4S,4aS,5aR,12aS)-4-(dimethylamino)-7- fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-[2-(pyrrolidin-1-yl) acetamido]-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide] dihydrochloride.[9-23] The molecular formula for eravacycline dihydrochloride is $C_{27}H_{31}FN_4O_8 \bullet _2HCl$ and its molecular weight is 631.5. The chemical structures of Eravacycline Dihydrochloride and Rolitetracycline dihydrochloride depicted in Figure 1.

A literature survey on Eravacycline revealed that, until now no analytical method was reported for its determination in biological samples by HPLC-MS/MS. However, clinical[3-10] and pharmacological[11-23] results were published for determination of antibiotic activity in human volunteers.

Since literature did not cite any method for determination of Eravacycline in biological samples using deuterated internal standard in biological samples by HPLC-MS/MS.

The main goal of the present study is to develop and validate the novel simple, sensitive, selective, rapid, rugged and reproducible analytical method for quantitative determination of Eravacycline in human plasma by HPLC-ESI-MS/MS.[24-25]

MATERIALS AND METHODS

Materials

Chemical Resources

Eravacycline dihydrochloride (EC) (Clearsynth, Mumbai, India) and Rolitetracycline hydrochloride (RC) (Clearsynth, France), Methanol (J.T Baker, USA), Ammonium acetate (Merck, Mumbai, India), Ultra pure water (Milli-Q system, Millipore, Bedford, MA, USA), human plasma (Doctors Pathological labs, hyderabad, India). The chemicals and solvents were used in this study analytical and HPLC 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.

Methods

Chromatographic conditions

The chromatographic separation was achieved with 20mM Ammonium acetate (pH-3.0):Methanol:Acetontrile (20:20:60,%v/v), gave the best peak shape and low baseline noise was observed using the TELOS LU C_{18} (2) 5µm 100 x 4.6mm. The total analysis time was 3 min and flow rate was set to 0.5 mL/min.. The temperature was set to 38°C for the column oven. The peak elution times for the Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) (Internal standard) were found at 0.83 and 0.78 min. Figure 2 and 3.

Detection

Analysis was performed using MRM positive ion mode with mass transitions of m/z (amu) 632.5®84.3 and 436.2®84.3 for Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) (Internal standard).

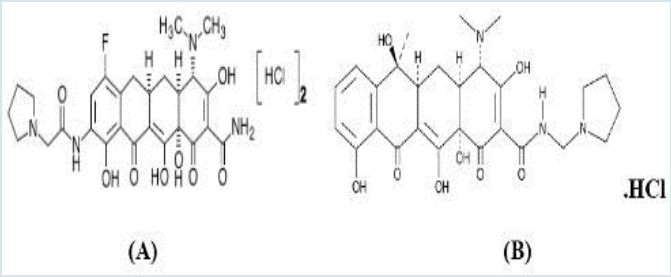

Figure 1: Chemical structures of A) Eravacycline dihydrochloride B) Rolitetracycline hydrochloride.

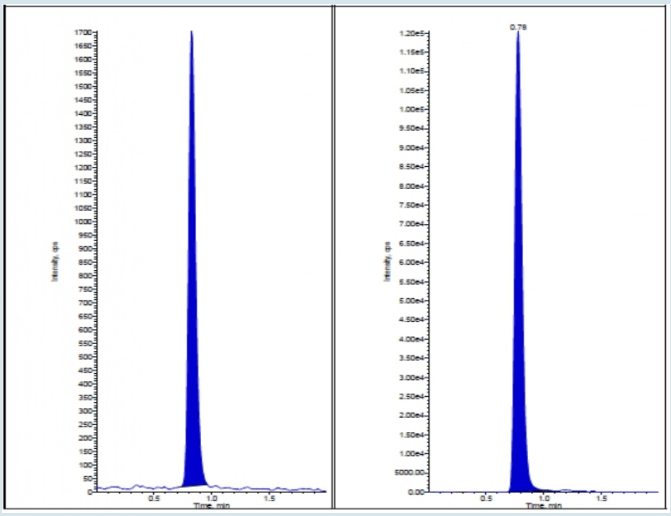

Figure 2: Chromatogram of LLOQ sample (Eravacycline dihydrochloride and Rolitetracycline hydrochloride).

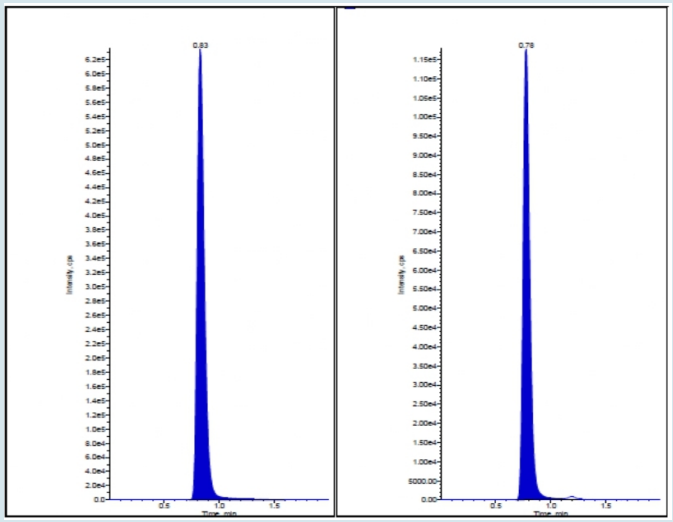

Figure 3: Chromatogram of ULOQ sample (Eravacycline dihydrochloride and Rolitetracycline hydrochloride).

Standard calibration and quality control samples preparation

Stock solutions of Eravacycline dihydrochloride (1000.00 µg/ml) and Rolitetracycline hydrochloride (1000.00 µg/ml) were prepared in methanol. The internal standard (Rolitetracycline hydrochloride) spiking solution (30.00 pg/ml) was prepared in Acetonitrile: Methanol (50:50%,v/v) from internal standard stock solution. Stock solutions of Eravacycline dihydrochloride, Rolitetracycline di hydrochloride and intermediate spiking solutions were stored in refrigerated conditions (2-8°C) until analysis.

Calibration standards (15, 30, 45, 60, 75, 90, 105 and 120 pg/mL), quality control samples of lower limit QC, low QC, mid QC, high QC (15.00, 55.00, 65.00, 100.00 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

To each labeled polypropylene tube 50 µL of Rolitetracycline hydrochloride (RC) (Internal standard) (30.00 pg/mL) was mixed with the 50 µL plasma sample, then 1.0 mL of Acetonitrile: Methanol (50:50%,v/v) were added, vortexed for 10 min and centrifuged at 15000 rpm for 5 min at 25°C. The organic phase was transferred to auto sampler vials and injected into the HPLC-ESI-MS/MS for analysis.

Method validation

The developed method was validated over a linear concentration range of 15.0-120.0 pg/ml. The validation parameters include selectivity and specificity, LOQ, Linearity, precision and accuracy, matrix effect, recovery, stability (freeze–thaw, auto sampler, bench top, long term) was evaluated under validation section.

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 Eravacycline dihydrochloride retention time and less than 5% for Rolitetracycline hydrochloride (RC) (Internal standard) retention time.

Limit of Quantification (LOQ)

Six LLOQ standards were prepared in screened plasma lot along with IS (30.00 pg/ml) and signal to noise ratio (S/N) was calculated using analyst software.

Linearity

Calibration standards were prepared to obtain linearity range of 15, 30, 45, 60, 75, 90, 105 and 120 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 (15.00 pg/mL), Low QC (45.00 pg/mL), Mid QC (55.00 pg/mL) and High QC (110.00 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 (45.00 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 sextuplicate by comparing the extracted QC standards with un-extracted QC standards at three different concentrations of Low QC (45.00 pg/mL), Mid QC (55.00 pg/mL) and High QC (110.00 pg/mL).

Stability studies

Bench top Stability (Room Temperature Stability, 48 h)

Six replicates of spiked low and high concentrations (Bench top stability samples) were set aside at ambient temperature up to 48 h. 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 h (-30°C to room temperature) and compared with newly prepared low and high concentrations (comparison samples).

Autosampler stability (2-8°C, 72 h)

Six replicates of low and high concentrations (AS stability samples) were stored in auto-sampler up to 72 h at 2-8°C. Stability samples were compared with newly prepared low and high concentrations (comparison samples).

Long-term Stability (-30°C, 60 Days)

After completion of the stability period stored at -30°C (60 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

On the way to develop a simple and easy applicable method for determination of Eravacycline dihydrochloride in human plasma, HPLC-MS/MS was selected as the method of choice. During method development process chromatographic (mobile phase composition, column, flow rate, injection volume, sample volume), mass spectrometric, sample extraction and internal standard parameters were optimized in logical and sequential manner to achieve the best results.

Separation of the Eravacycline dihydrochloride was performed with different branded RP-HPLC C_{18} columns. Initial separation was performed with isocratic elution of 20mM Ammonium acetate (pH-3.0):Methanol:Acetontrile (10:30:60,%v/v) was selected as a mobile phase in varying combinations were tried, but a low response was observed. A mobile phase consisting of 20mM Ammonium acetate (pH-3.0):Methanol:Acetontrile (05:35:60,%v/v) gave the best response, but poor peak shape was observed.

After a series of trials a mobile phase consisting of 20mM Ammonium acetate (pH-3.0) in combination with methanol and acetonitrile in varying combinations were tried. Using a mobile phase containing 20mM Ammonium acetate (pH-3.0):Methanol:Acetontrile (20:20:60,%v/v), gave the best signal along with a marked improvement in the peak shape and low baseline noise was observed using the TELOS LU C_{18} (2) 5µm 100 x 4.6mm analytical column with a flow rate of 0.5 ml/min and reduced runtime to 3 min. The column oven temperature was kept at a constant temperature of about 38°C and temperature of auto sampler was maintained at 4°C. Injection volume of 5 µl sample was adjusted for better ionization and chromatography. For selection of internal standard; Doxycycline, Demeclocycline were tried with optimized mobile phase and column conditions. Finally Rolitetracycline hydrochloride (RC) was selected as IS (internal standard) due to its compatibility with analyte chromatographic conditions.

The peak elution times for the Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) (Internal standard) were found at 0.83 and 0.78 min.

Different procedures like PPT (Protein precipitation), SPE (solid phase extraction) and LLE (liquid-liquid extraction) methods were optimized. Out of all, it was observed that the PPT was suitable due to simple extraction, high recovery and the less ion suppression effect on drug and internal standard.

Electro spray ionization (ESI) provided a maximum response over atmospheric pressure chemical ionization (APCI) mode and was chosen for this method. The instrument was optimized to obtain sensitivity and signal stability during infusion of the analyte in the continuous flow of mobile phase to electrospray ion source operated at a flow rate of 20 µl/min. the Eravacycline dihydrochloride (EC) gave more response in positive ion mode as compare to the negative ion mode.

To get high intense productions source dependent parameters were optimized like nebulizer gas flow 30 psi, CAD gas and curtain gas flow 25 psi, ion spray voltage 5500 V and temperature 500°C. The compound dependent parameters such as the declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), cell exit potential (CXP) were optimized during tuning as 35, 25, 10, 20, 12 eV for Eravacycline dihydrochloride and Rolitetracycline hydrochloride, respectively. The collision activated dissociation (CAD) gas was set at 4 psi using nitrogen gas. Quadrupole-1 and quadrupole-3 were both maintained at a unit resolution and dwell time was set at 200 ms for Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) (Internal standard).

The predominant peaks in the primary ESI spectra of Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) correspond to the MH^+ ions at m/z 632.5 and 436.2 respectively. Productions of Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) scanned in quadrupole-3 after a collision with nitrogen in quadrupole-2

had a m/z of 83.4 for both respectively. The parent and productions mass spectrums of Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) were shown in Figure 4 and 5.

Method validation

Selectivity and Specificity, Limit of Quantification (LOQ)

No significant response was observed at retention times of Eravacycline dihydrochloride (EC) and Rolitetracycline hydrochloride (RC) 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 15.0 pg/ml. Represent chromatograms were shown in Figure 6.

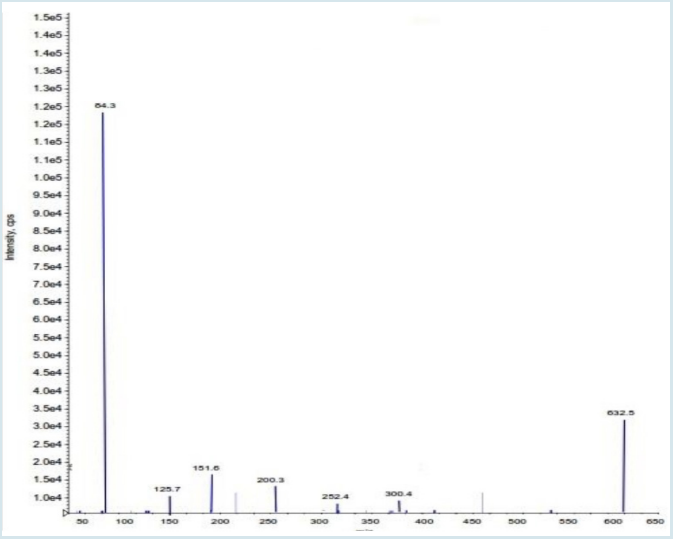

Figure 4: Mass fragmentation pattren of Eravacycline dihydrochloride (EC).

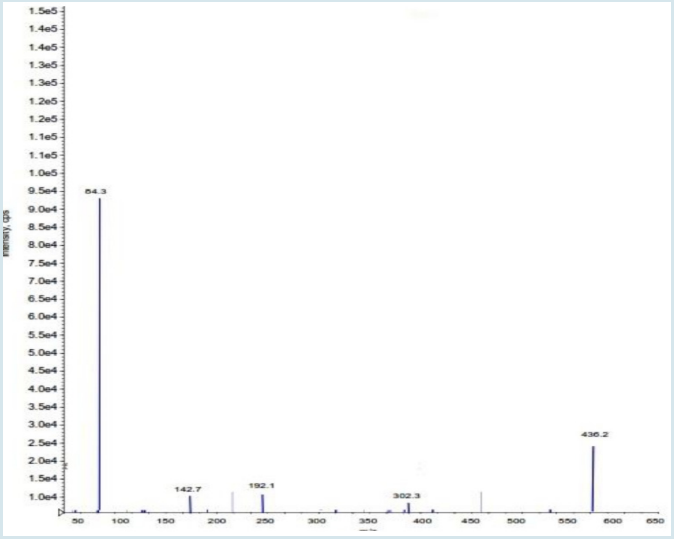

Figure 5: Mass fragmentation pattren of Rolitetracycline hydrochloride (RC) (Internal standard).

Linearity

Linearity was plotted as a peak area ratio (EC peak area / RC peak area) on the y-axis against EC concentration (pg/ml) on the x-axis. Calibration curves were found to be consistently accurate and precise for EC over a linearity range of 15 to 120.00 pg/ml. The correlation coefficient was greater than 0.99980 for Eravacycline dihydrochloride. The %CV was less than 15% and mean %accuracy was ranged between 98.23 - 103.33%. Results were presented in Table 1.

Precision and Accuracy

Intra and inter batch %accuracy for Eravacycline dihydrochloride was ranged between 99.74-103.74 and 99.54 to 101.78. %CV is 0.65 to 1.04 and 0.21% - 1.31%. Results are presented in Table 2.

Recovery

The mean %recovery for LQC, MQC, HQC samples of Eravacycline dihydrochloride were 97.86%, 95.90% and 97.53% respectively. The overall mean %recovery and %CV of Eravacycline dihydrochloride across QC levels is 97.10% and 2.30%. For the Rolitetracycline hydrochloride (internal standard) the mean %recovery and %CV is 91.18% and 4.30%.

Table 1: Calibration curve details.

Spiked plasma Concentration (pg/ml)	Concentration measured (pg/ml) (Mean±S.D)	%CV (*n*=5)	%Accuracy
15.00	15.50±0.21	1.33	103.33
30.00	29.47±1.09	3.70	98.23
45.00	45.23±0.28	0.61	100.52
60.00	60.22±2.11	3.51	100.37
75.00	75.30±0.34	0.45	100.40
90.00	90.45±0.14	0.61	100.50
105.00	105.70±0.09	0.09	100.67
120.00	120.50±0.32	0.26	100.42

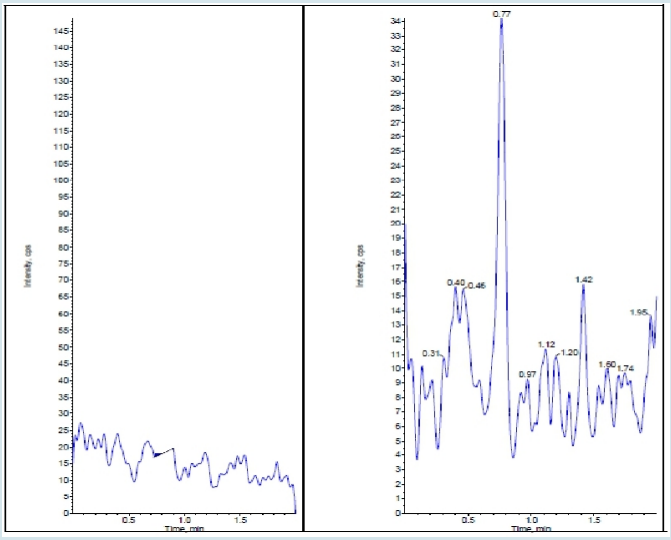

Figure 6: Representative chromatograms of in Blank plasma interference free Eravacycline dihydrochloride and Rolitetracycline hydrochloride.

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
45.00	45.57±0.16	1.04	101.26	45.80±0.60	1.31	101.78
55.00	54.57±0.49	0.89	99.74	55.36±0.26	0.48	100.66
110.00	110.67±0.55	0.49	100.61	109.78±0.94	0.85	99.80

Table 3: Stability studies of Eravacycline dihydrochloride in plasma.

Spiked Plasma concentration (pg/ml)	Room temperature Stability		Processed sample Stability		Long term stability		Freeze and thaw stability	
	48h		24h		60 days		Cycle (48h)	
	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)
55.00	44.93±0.72	1.61	44.87±0.55	1.24	55.67±0.08	4.6	44.85±0.78	1.74
100.00	109.54±1.44	1.31	111.04±0.56	0.51	100.43±0.65	7.3	110.57±0.24	2.2

Matrix Effect

No significant matrix effect found in different sources of rat plasma tested for Eravacycline dihydrochloride, Rolitetracycline hydrochloride. The %CV was found to be 1.82.

Stability (freeze–thaw, auto sampler, bench top, long term)

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

CONCLUSION

The method described in this manuscript has been developed and validated over the concentration range of 15.0–120.0 pg/ml in human plasma. The intra and inter-batch precision (%CV) was less than 15.0% and %accuracy ranged from 99.54-103.74%. The overall % recovery for Eravacycline dihydrochloride, Rolitetracycline hydrochloride was greater than 90%. The selectivity, sensitivity, precision and accuracy obtained with this method make it suitable for the purpose of the present study. In conclusion, the method used in the present study is easy and fast to perform; it is also characterized with an 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 Eravacycline dihydrochloride.

ACKNOWLEDGEMENT

The authors wish to thank the support received from Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamilnadu, India for providing necessary facilities and carrying out this research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HPLC-ESI-MS/MS: High Performance Liquid Chromatography- Electron Spray Ionization/ Tandem Mass Spectrometry; **MRM:** Multiple Reaction Monitoring; **ISTD:** Internal Standard; **ESBL:** Extended-spectrum β-lactamase; **U.S-FDA:** United State Food and Drug Administration; **EC:** Eravacycline dihydrochloride; **RC:** Rolitetracycline hydrochloride; **K2-EDTA:** Di Potassium Ethylene Diamine tetra Acetic acid; **DP:** Declustering Potential; **FP:** Focussing Potential; **EP:** Entrance Potential; **CE:** Collision Cell Entrance Potential; **CXP:** Collision Cell Exit Potential; **CAD:** Collisionally Activated Dissociation; **QC:** Quality control; **LLE:** Liquid-Liquid Extraction; **LLOQ:** Lower Limit of Quantification; **RSD:** Relative Standard Deviation; **S/N:** Signal to Noise Ratio; **ODS:** Octa Decyl Silane; **FT:** Freeze-thaw.

REFERENCES

1. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, *et al.* Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1-12.
2. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, *et al.* Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: A new threat to our antibiotic armamentarium. Arch Intern Med. 2005;165(12):1430-5.
3. Duin DV, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant *Enterobacteriaceae*: A review of treatment and outcomes. Diagn Microbiol Infect Dis. 2013;75(2):115-20.
4. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, Palmore TN, *et al.* Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Science Translational Medicine. 2012;4(148):148ra116-.
5. Roberts RR, Hota B, Ahmad I, Scott RD, Foster SD, Abbasi F, *et al.* Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: Implications for antibiotic stewardship. Clin Infect Dis. 2009;49(8):1175-84.

6. IDSA. Combating antimicrobial resistance: Policy recommendations to save lives. Clin Infect Dis. 2011;52(Suppl 5):S397-428.
7. World Health Organization. World Health Day 2011: Policy briefs. World Health Organization, Geneva. 2011. Switzerland. http://www.who.int/world-health-day/2011/policybriefs/en/index.html.
8. Xiao XY, Hunt DK, Zhou J, Clark RB, Dunwoody N, Fyfe C, *et al.* Fluorocyclines. 1. 7-Fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline: A potent, broad spectrum antibacterial agent. J Med Chem. 2012;55(2):597-605.
9. Clark RB, Hunt DK, He M, Achorn C, Chen CL, Deng Y, *et al.* Fluorocyclines. 2. Optimization of the C-9 side-chain for antibacterial activity and oral efficacy. J Med Chem. 2012;55(2):606-22.
10. Dallenne C, Costa AD, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother. 2010;65(3):490-5.
11. Otto M. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu Rev Microbiol. 2010;64:143-62.
12. Hersh AL, Newland JG, Beekmann SE, Polgreen PM, Gilbert DN. Unmet medical need in infectious diseases. Clin Infect Dis. 2012;54(11):1677-8.
13. Sunenshine RH, Wright MO, Maragakis LL, Hariss AD, Song X, Hebden J, *et al.* Multidrug-resistant Acinetobacter infection mortality rate and length of hospitalization. Drugs. 2007;67(16):2355-82.
14. Evans HL, Lefrak SN, Lyman J, Smith RL, Chong TW, McElearney ST, *et al.* Cost of Gram-negative resistance. Crit Care Med. 2007;35(1):89-95.
15. Maragakis LL, Perencevich EN, Cosgrove SE. Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther. 2008;6(5):751-63.
16. Qureshi ZA, Paterson DL, Peleg AY, Adams-Haduch JM, Shutt KA, Pakstis DL, *et al.* Clinical characteristics of bacteraemia caused by extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the era of CTX-M-type and KPC-type beta-lactamases. Clin Microbiol Infect. 2012;18(9):887-93.
17. Hirsch EB, Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.
18. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010;362(19):1804-13.
19. Marchaim D, Chopra T, Perez F, Hayakawa K, Lephart PR, Bheemreddy S, *et al.* Outcomes and genetic relatedness of carbapenem-resistant *Enterobacteriaceae* at Detroit medical center. Infect Control Hosp Epidemiol. 2011;32(9):861-71.
20. Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, *et al.* Carbapenem-resistant Acinetobacter baumannii and Klebsiella pneumoniae across a hospital system: Impact of post-acute care facilities on dissemination. J Antimicrob Chemother. 2010;65(8):1807-18.
21. Avery TR, Kleinman KP, Klompas M, Aschengrau A, Huang SS. Inclusion of 30-day postdischarge detection triples the incidence of hospital-onset methicillin-resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol. 2012;33(2):114-21.
22. Bambeke FV, Reinert RR, Appelbaum PC, Tulkens PM, Peetermans WE. Multidrug-resistant Streptococcus pneumoniae infections: Current and future therapeutic options. Drugs. 2007;67(16):2355-82.
23. Calfee DP. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci and other Gram-positives in healthcare. Curr Opin Infect Dis. 2012;25(4):385-94.
24. Guidance for industry. 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). 2001.
25. Guidance for industry. 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). 2003.

PICTORIAL ABSTRACT

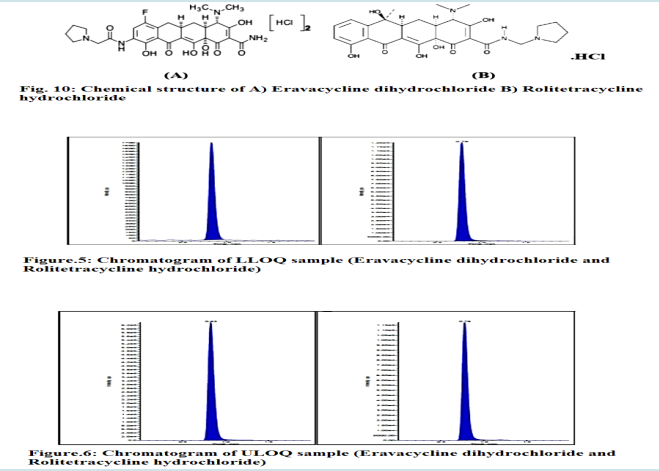

Fig. 10: Chemical structure of A) Eravacycline dihydrochloride B) Rolitetracycline hydrochloride

Figure.5: Chromatogram of LLOQ sample (Eravacycline dihydrochloride and Rolitetracycline hydrochloride)

Figure.6: Chromatogram of ULOQ sample (Eravacycline dihydrochloride and Rolitetracycline hydrochloride)

SUMMARY

- The present research work was designed to a validated a High-throughput HPLC-ESI-MS/MS method for the quantification of Eravacycline Dihydrochloride in Human plasma by using Rolitetracycline Hydrochloride as ISTD.
- The chromatographic separations was achieved using TELOS LU C18 (2) 5µm, 100 x 4.6 mm column. The mobile phase was used a mixture of 20mM Ammonium acetate (pH-3.0), Methanol & Acetontrile in the ratio of 20:20:60%v/v.
- The peak elution times for the Eravacycline dihydrochloride & Rolitetracycline hydrochloride were found at 0.83 and 0.78 min. The mass transitions of m/z (amu) 632.5®84.3 and 436.2®84.3 for Eravacycline dihydrochloride & Rolitetracycline hydrochloride.
- The present developed method has many advantages with respect to short time run analysis (3.0min), less mobile phase consumption and 0.1µL sample is enough for method & it can be applied for the routine pharmacokinetic evaluation of Eravacycline dihydrochloride in human subjects after oral administration of the same.

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