# Determination of Linezolid in Human Plasma Using Turbulent Flow Online Extraction and Tandem Mass Spectrometry

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## ABSTRACT :

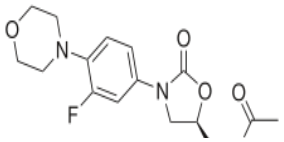
A rapid and sensitive HTLC-ESI-MS/MS method was developed for the determination of Linezolid in human plasma using an internal standard (cetirizine hydrochloride) with an advanced online sample preparation. This HTLC technique reduces the time required for sample cleanup since sample extraction and analysis are performed. A 10µl of prepared sample is directly injected into the HTLC-MS/MS system where analyte was retained on the extraction column (Cyclone P 50 × 0.5mm, 50µm) and washed away the waste with the help of extraction solvent. Then the analyte was eluted from the extraction column and transferred to the analytical column (Zorbex XDB C18 50 × 2.1mm, 5µm) using mobile phase of the mixture of 0.5% formic acid, 10mM ammonium formate and acetonitrile. The eluted analyte was then detected on mass spectrometer with ESI ion source and a positive selective reaction monitoring mode (SRM). The SRM transitions were m/z 383.20 → 337.20 for Linezolid and m/z 389.10 → 201.01 for internal standard. The developed method was validated as per USFDA guidelines. The method was linear over the concentration range of 0.409 – 20.310 ng/ml. The within batch and between batch accuracy for the three concentrations (LQC, MQC and HQC) were ranged from 98 -110.6% and 98 .6 – 108.3% respectively. The % RSD for all the QC samples was ranged from 3.0 – 8.1%. The percentage recovery of linezolid in HQC (16ng/ml), MQC (13ng/ml) and LQC (1,1ng/ml) was 60.3, 73 and 86.45% respectively. Stability studies were also performed and the results were within the acceptance range. This method was applied to the measurement of linezolid in human plasma and pharmacokinetic study.

**Keywords:** HTLC-ESI-MS/MS, selective reaction monitoring.

## INTRODUCTION:

Linezolid is a synthetic antibacterial agent of the oxazolinone class. Chemically it is N-(((5S)-3-(3-fluoro-4-(morpholin-4-yl) phenyl)-2-oxo-1,3-oxazolidin-5-yl)methyl) acetamide. It has the molecular formula of $C_{16}H_{20}FN_3O_4$ and molecular weight of 337.346g/mol. It has Invitro activity against aerobic gram positive bacteria, certain gram negative bacteria and anaerobic organisms. It selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70S initiaton complex, which is an essential component of the bacterial translation process.

Linezolid is rapidly and extensively absorbed after oral dosing. Maximum plasma concentrations (Cmax) are 15-27mg/l, reached approximately 1-2 hrs after dosing. The absolute bioavailability is approx. 100%. It is metabolized via morphine ring oxidation. In humans 30% of the linezolid dose is extracted in the urine as the parent drug. Plasma elimination half-life is 3.4-7.4hrs.



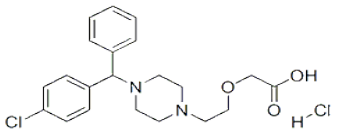




**Figure. 1 Structure of linezolid. Figure: 2 Structure of cetrizine hydrochloride**

Literature survey reveals that very few high performance liquid chromatography with tandem mass spectroscopy (LC-MS/MS) methods were published for the determination of linezolid in human plasma. This method requires effective sample cleanup procedure, such as solid phase extraction or liquid-liquid extraction. In the proposed method, High turbulence liquid chromatography (HTLC), sample cleanup is not necessary and offline time consuming. The challenge at the time was to design a chromatographic platform that would utilize turbulent flow properties to isolate small analytes from macromolecules present in complex matrices such as biological fluids. In this case the macromolecules are rapidly eliminated to waste with a high flow rate of aqueous mobile phase. While the small molecules are retained on extraction column and are subsequently eluted onto an analytical column with organic

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mobile phase for chromatographic separation.

In this paper we report the method for the determination of linezolid in human plasma by using HTLC-MS/MS. In addition we represent the validation parameters and the stability data for linezolid.

## 2. MATERIALS AND METHODS:

### 2.1 Materials:

Linezolid USP was obtained from Glenmark Pharmaceuticals,Mumbai, India. The internal standard cetirizine hydrochloride was procured from Praveen laboratories, Gujarat, India. HPLC grade acetonitrile, methanol and acetone were purchased from SD fine chemicals, Mumbai, India. Formic acid and propane-2-ol were purchased from Rankem Pharmaceuticals NewDelhi, India. HPLC grade water is used for preparing all the solutions.

### 2.2 Instruments:

A Cohesive 2300 HTLC system (Cohesive Technolgies, Franklin, MA, USA) with quaternary pump as a loading pump and a binary pump as a elution pump. Thermoelectron Finnigan TSQ Quantum Discovery Max (Thermoelectron corporation, USA) mass spectrometer equipped with electron spray ionization was used as detector. The data was collected and analysed using Analyst software version 1.4.

### 2.3 Standard solutions and Quality control (QC) samples:

The standard stock solutions of linezolid and internal standard were prepared at about 649.5µg/ml and 1mg/ml respectively in methanol. From the stock solution the intermediate solution of linezolid (65µg/ml) and internal standard (10µg/ml) were prepared in methanol. Calibration curve (CC) spiking solutions in methanol were prepared in the concentration range of 1000ng/ml to 20ng/ml from the intermediate solutions as shown in the table1. Spiked plasma CC standards were prepared by spiking the respective CC standards in screened plasma in the range of 20ng/ml to 0.4ng/ml. (Table2). Similarly QC spiking solutions were also prepared from the intermediate solutions of linezolid and internal standard as shown in the table3. QC samples were prepared by spiking the respective QC spiking solutions in screened human plasma representing HQC (16ng/ml), MQC (13ng/ml) and LQC (1.1ng/ml) as described in the table 4.

**Table: 1 Spiking solutions for calibration curve of linezolid**

| Stock Conc. (ng/mL) | Vol. Taken (mL) | Diluent's Vol. (mL) | Final Vol.(mL) | SS Conc. (ng/mL) | SS ID |
|---|---|---|---|---|---|
| 650000 | 1.000 | 9.000 | 10.000 | 66500.000 | INTM STK1 |
| 65000 | 1.600 | 8.400 | 10.000 | 10400.000 | INTM STK2 |
| 10400.000 | 1.000 | 9.000 | 10.000 | 1040.000 | SS STD1 |
| 1040.000 | 8.000 | 2.000 | 10.000 | 832.000 | SS STD2 |
| 832.000 | 7.500 | 2.500 | 10.000 | 624.000 | SS STD3 |
| 624.000 | 6.500 | 3.500 | 10.000 | 406.000 | SS STD4 |
| 406.000 | 5.000 | 5.000 | 10.000 | 203.000 | SS STD5 |
| 203.000 | 5.000 | 5.000 | 10.000 | 102.000 | SS STD6 |
| 102.000 | 4.000 | 6.000 | 10.000 | 41.000 | SS STD7 |
| 41.000 | 4.900 | 5.100 | 10.000 | 20.000 | SS STD8 |

**Table: 2 spiked plasma calibration curve standards of linezolid**

| SS ID | SSConc. (ng/mL) | Spiking Vol. (mL) | Plasma Vol. (mL) | Final Vol. (mL) | Spiked Plasma Conc. (ng/mL) | STD ID |
|---|---|---|---|---|---|---|
| Diluent | 0.000 | 0.200 | 9.800 | 10.000 | 0.000 | STDBL |
| SS STD1 | 1040.000 | 0.200 | 9.800 | 10.000 | 21.000 | STD8 |
| SS STD2 | 832.000 | 0.200 | 9.800 | 10.000 | 17.000 | STD7 |
| SS STD3 | 624.000 | 0.200 | 9.800 | 10.000 | 13.000 | STD6 |
| SS STD4 | 406.000 | 0.200 | 9.800 | 10.000 | 8.000 | STD5 |
| SS STD5 | 203.000 | 0.200 | 9.800 | 10.000 | 4.000 | STD4 |
| SS STD6 | 102.000 | 0.200 | 9.800 | 10.000 | 2.000 | STD3 |
| SS STD7 | 41.000 | 0.200 | 9.800 | 10.000 | 0.800 | STD2 |
| SS STD8 | 20.000 | 0.200 | 9.800 | 10.000 | 0.400 | STD1 |

**Table:3 Spiking solutions for quality control samples of linezolid**

| Conc.(ng/mL) | Stock Vol.(mL) | Diluent's Vol. (mL) | Final Vol.(mL) | Conc. (ng/mL) | SS ID |
|---|---|---|---|---|---|
| 10400.000 | 0.750 | 9.250 | 10.000 | 780.000 | SS HQC |
| 780.000 | 8.000 | 2.000 | 10.000 | 624.000 | SS MQC |
| 624.000 | 3.500 | 6.500 | 10.000 | 219.000 | SS INT QC |
| 219.000 | 2.500 | 7.500 | 10.000 | 55.000 | SS LQC |

**Table:4 Spiked plasma quality control samples of linezolid**

| SS ID | SS Conc. (ng/mL) | Spiking Volume (mL) | Plasma Vol. (mL) | Final Vol. (mL) | Spiked Plasma Conc. (ng/mL) | QC ID |
|---|---|---|---|---|---|---|
| SS HQC | 780 | 2 | 8 | 100 | 16 | HQC |
| SS MQC | 624 | 2 | 8 | 100 | 13 | MQC |
| SS LQC | 55 | 2 | 8 | 100 | 1.1 | LQC |

**2.4 Sample preparation:**

For sample preparation, the required number of spiked plasma samples (STD blank, CC, QC samples) were taken out from the deep freezer thaw them at room temperature and vortex the tubes to mix. Remove the caps from the polypropylene tubes, aliquot 0.5ml of CC, QC into prelabelled Eppendorf vials. Add 50µl of ISTD dilution (10µg/ml) to all the samples except STD blank and vortex for about 10 seconds. Centrifuge the Eppendorf vials at 14000rpm and 10degrees for 20min, transfer the 0.4ml of supernatant to prelabelled HPLC vials. Add 0.4ml of 10mm ammonium formate and vortex to mix and subsequently transfer the HPLC vials to the autosampler.

**2.5 Chromatographic conditions:**

A cyclone P HTLC column (50mm × 0.5mm, 50µm) from cohesive technologies Inc (Franklin,MA,USA) and a Zorbex XDB $C_{18}$ column (50 × 2.1mm, 5µm) from Agilent technologies Ltd, Bangalore were used as extraction and analytical columns respectively. Mobile phases used as follows

Mobile phase A : 10mM ammonium formate, 0.5% formic acid aqueous solution

B : 0.5% formic acid acetonitrile solution

C : acetonitrile solution

D : acetonitrile/IPA/acetone (60:25:15%vv/v)

Rinsing cycle conditions:

Rinsing volume: 100µl

Needle stroke: 38.2mm

Rinsing speed: 35µl/sec

Sampling speed : 15µl/sec

Purge time : 2min

Rinse dip time: 2sec

Rinse mode : before and after inspiration

The online extraction method consists of four general steps: loading, transfer, elution and equilibration. First the sample is applied during the loading step by the loading pump on to the turboflow column at a flow rate of 2ml/min. where the matrix components are rapidly washed away and the analytes are retained with mobile phase A. at the transfer step, the analytes are eluted from the extracted column and transferred on to the reverse phase analytical column, where the analytes are separated conventionally, at a flow rate of 0.2ml/min with a mobile phase that is stored in the transfer loop and filled before the loading step. At the same time, a mobile phase composed of 50%A and 50% B is delivered at a flow rate of 0.4ml/min from the eluting pump. At the elution step, the analytes are separated on to the analytical column and eluted to the mass spectrometer with the same mobile phase. Then the extraction column is washed to reduce the carry over. At the equilibration step, the loop is filled with the mobile phase of 100%B and then both extraction and analytical columns are equilibrated with 100%A for the next injection.

**Table:5 Rinsing cycle by gradient programme**

| step | | | Turboflow column | | | | | Cut in loop | | Analytical column | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Description | Start (min) | sec | Flow rate (ml/min) | A% | B% | C% | D% | Tee | loop | Flow (ml/min) | step | A% | B% |
| Loading | 0 | 60 | 2 | 0 | 100 | 0 | 0 | - | Out | 0.6 | Step | 50 | 50 |
| Trasfering | 1 | 60 | 0.2 | 50 | 0 | 50 | 0 | T | In | 0.4 | Step | 50 | 50 |
| Washing | 2 | 60 | 2 | 0 | 0 | 0 | 100 | - | In | 0.6 | Ramp | 50 | 50 |
| washing | 3 | 60 | 2 | 0 | 0 | 0 | 100 | - | Out | 0.6 | Step | 50 | 50 |
| Washing | 3.5 | 60 | 2 | 0 | 0 | 0 | 100 | - | In | 0.6 | Step | 50 | 50 |
| Filling loop | 4 | 60 | 2 | 100 | 0 | 0 | 0 | - | in | 0.6 | Step | 50 | 50 |
| equilibrating | 4.5 | 60 | 2 | 0 | 100 | 0 | 0 | - | out | 0.6 | step | 50 | 50 |

**2.6 Mass spectrometry conditions:**

Mass spectrometric analysis was carried out using Finnigan TSQ quantum discovery max from Thermo electron corporation, USA. Data acquisition for quantification and confirmation are performed in SRM mode (selective reaction monitoring) using analyst software version 1.4.
Ionization mode: ESI (electron spray ionization)
Scan type: SRM
Polarity: positive ion mode
SRM transitions: Drug – $Q_1$ mass 383.20; $Q_3$ mass 337.20 (m/z)
ISTD - $Q_1$ mass 389.10; $Q_3$ mass 201.0 (m/z)
Spray voltage: 3000
Sheath gas pressure (arb): 40
Auxillary gas pressure (arb) : 10
Capillary temperature: 350°c
Collision energy: drug – 22; ISTD – 26

**3. METHOD VALIDATION:**

The present method was validated as per US FDA guidelines. The system suitability was performed by injecting 6 consecutive injections of AQ standard mixture of drug and ISTD. Autosampler carry over was determined by injecting one blank, STD along with AQ STD and RS in the sequence, to check any carry over in the blank sample. Specificity was proved by processing standard blanks and LLOQ in six different human plasma lots. A linear equation was established to provide the best fit for the concentration versus detector response using $1/x^2$ weighed least square regression analysis of standard associated with eight point calibration curve. Accuracy was evaluated by measuring % mean accuracy at each concentration level of QC . Precision was measured by percent coefficient of variation over the concentration range of QC samples of linezolid during the course of validation. Recovery was determined by comparing the areas of extracted QC samples against the areas of respective aqueous QC samples.
Stability studies like short term stock solution stability (after 6 hrs), long term stock solution stability (after 10 days), bench top stability (22±4°c), autosampler (wet extraction) stability(ambient temperature for 24hrs) and freeze thaw stability (-86°c) were also performed on the QC samples of linezolid.

**4. RESULTS AND DISCUSSION:**

**4.1 Optimization of liquid chromatography and mass spectrometry conditions:**

Complete resolution of linezolid and ISTD was achieved by using a cyclone P HTLC column (50mm × 0.5mm, 50µm) from cohesive technologies Inc (Franklin,MA,USA) as extraction column and a Zorbex XDB $C_{18}$ column (50 × 2.1mm, 5µm) from Agilent technologies Ltd, Bangalore, as analytical column with required mobile phase. Following detailed optimization of mass spectrometry conditions m/z 383.20 precursor ion to the m/z 337.20 for ISTD m/z 389.10 precursor ion to the m/z 201.01 was used for quantification purpose.

**4.2 Method validation procedures:**

**4.2.1 System suitability:**

System suitability was performed by injecting six consecutive injections using aqueous standard mixture of drug and ISTD during the start of method validation. The % CV of system suitability was observed in the range of 3.7 – 4.2% for response of the drug and ISTD, which is not more than 5% as per the acceptance criteria. The results were shown in the following table 6.

**Table:6 Results of system suitability**

| Injection . | Drug Area | Drug Retention Time | ISTD Area | ISTD Retention Time | Area Ratio |
|---|---|---|---|---|---|
| 1 | 4124427 | 2.2 | 126430 | 2.1 | 32.62222 |
| 2 | 4392474 | 2.2 | 112588 | 2.1 | 36.0137 |
| 3 | 4374485 | 2.2 | 122032 | 2.1 | 35.84703 |
| 4 | 4587745 | 2.2 | 125753 | 2.1 | 36.48219 |
| 5 | 4450798 | 2.2 | 122467 | 2.1 | 36.34284 |
| 6 | 4550489 | 2.2 | 125257 | 2.1 | 36.32922 |
| Mean | 4413403 | 2.2 | 122421.2 | 2.1 | 35.61 |
| S.D | 164952 | 0 | 5139.232 | 0 | 1.480 |
| % CV | 3.7 | 0 | 4.2 | 0 | 4.2 |

**4.2.2 Specificity:**

To demonstrate that no interfering compounds elute at the retention times of linezolid or ISTD, the specificity was evaluated by testing six different lots of human plasma. No endogenous interferences were found at the retention times of linezolid or ISTD as seen in the table 7. Representative chromatogram of blank was shown in the figure3.

**Table: 7 Results showing specificity of linezolid**

| S.No | Area of interference at Rt of analyte | Area observed for extracted LOQ | % interference at Rt of Analyte | Area of interference at Rt of ISTD | Area observed for extracted LOQ | % interference at Rt of ISTD |
|---|---|---|---|---|---|---|
| 1 | 0 | 20340 | 0 | 1689 | 751695 | 0.26 |
| 2 | 0 | 17898 | 0 | 2643 | 702759 | 0.41 |
| 3 | 0 | 21824 | 0 | 2399 | 608705 | 0.37 |
| 4 | 0 | 16635 | 0 | 3078 | 656531 | 0.48 |
| 5 | 0 | 17864 | 0 | 2229 | 551921 | 0.34 |
| 6 | 0 | 13073 | 0 | 3010 | 572453 | 0.47 |
| Mean | | 17939 | 0 | | 640677 | 0.39 |

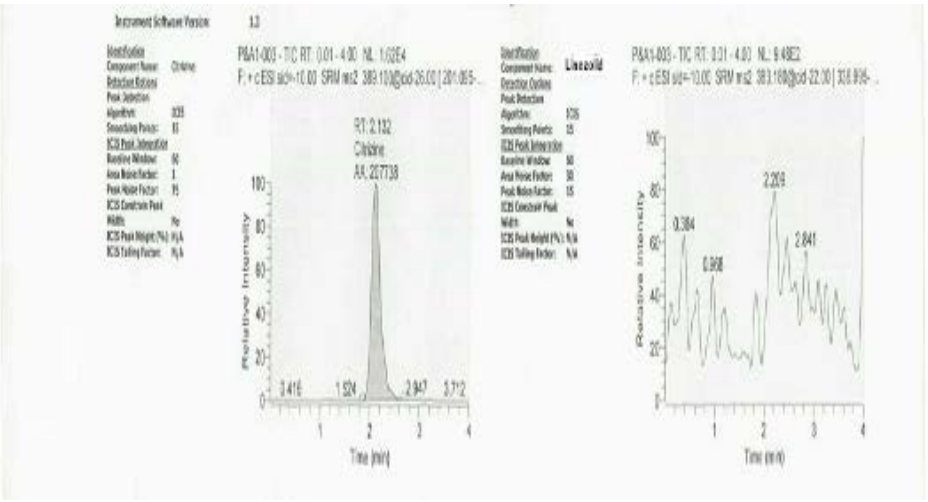


**Figure: 3 A representative chromatogram of blank with ISTD**

### 4.2.3 Autosampler carryover:

Autosampler carryover was tested by injecting one blank and one extracted STD in the chromatographic system along with aqueous standard and reconstituted solution in the sequence. No significant carry over was found for linezolid while 0.006% of carryover was found for ISTD which was shown in the following table 8.

**Table:8 Responses of linezolid with ISTD for autosampler carry over**

| Sample ID. | Response (Area Count) | | | |
|---|---|---|---|---|
| | Linezolid | Limits | ISTD | Limits |
| RS | 0 | | 1256 | |
| AQ STD | 912018 | | 575203 | |
| RS | 0 | 18240 | 1534 | 11504 |
| AQ STD | 881911 | | 566324 | |
| RS | 0 | 17638 | 0 | 11326 |
| RS | 0 | | 833 | |
| Extracted STD Blank | 0 | | 11865 | |
| EXT STD 1 | 624394 | | 552922 | |
| Extracted STD Blank | 0 | 12487 | 3711 | 11058 |
| EXT STD 1 | 612607 | | 555147 | |
| Extracted STD Blank | 0 | 12252 | 2127 | 11102 |

### 4.2.4 Linearity and calibration curve:

A linear equation was established to provide the best fit for the concentration versus detector response using $1/x^2$ as weighting factor. Calibration curve was drawn and it was found to be linear in the concentration range of 0.409 to 20.310ng/ml with the coefficient of 0.997 as seen in the figure 4.

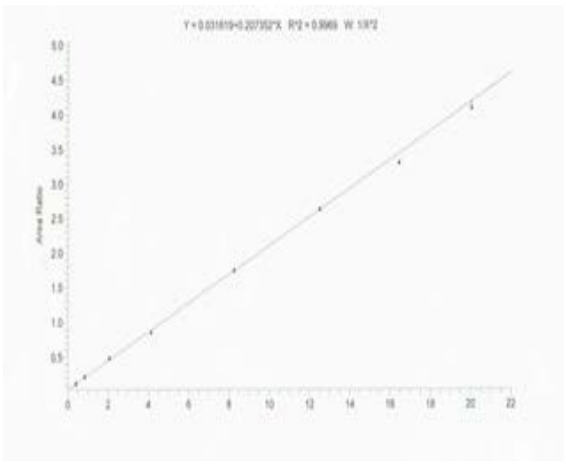

Figure: 4 Calibration curve of linezolid

**4.2.5 Precision:**

Precision is measured by percent coefficient of variation over the concentration range of QC samples of linezolid during the course of validation. The within batch precision for all low, middle, high and LOQ QC samples were ranged from 3.0% to 8.1% and 6.9 to 12.3% which is within the acceptance limits of 15% and ±20% for LOQ QC samples respectively. The between batch precision for all low, middle, high and LOQ QC samples were ranged from 4.4% to 6.9% and 9.6% which is within the acceptance limits of 15% and ±20% for LOQ QC samples respectively.

**4.2.6 Accuracy**:

The accuracy was defined as the absolute value of the ratio of back calculated mean values of QC samples to their respective nominal values expressed as percentage. The within batch accuracy for all low, middle, high and LOQ QC samples were ranged from 98% to 110.6% and 98.8 to 108.3% which is within the acceptance limits of 15% and ±20% for LOQ QC samples of nominal concentrations respectively. The between batch precision for all low, middle, high and LOQ QC samples were ranged from 98.6% to 108.3% and 99.2 % which is within the acceptance limits of 15% and ±20% for LOQ QC samples of nominal concentrations respectively. The representative chromatograms of HQC,MQC, LQC and LOQ QC samples were shown in the figures 5,6,7,8 respectively.

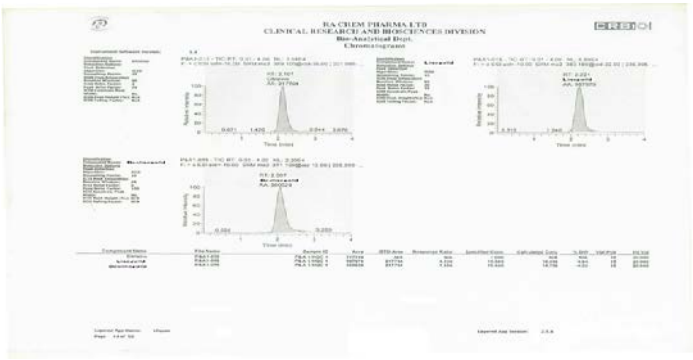

**Figure:5 A representative chromatogram of HQC sample**

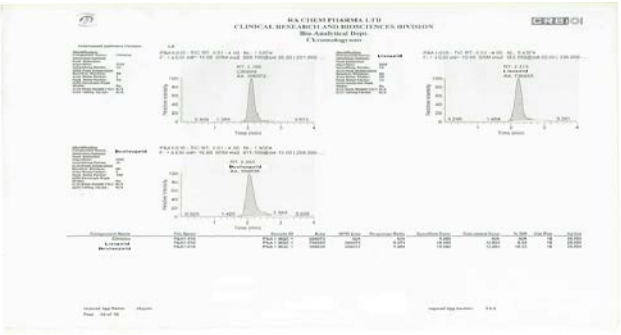

**Figure:6 A representative chromatogram of MQC sample**

### 4.2.7 Recovery:

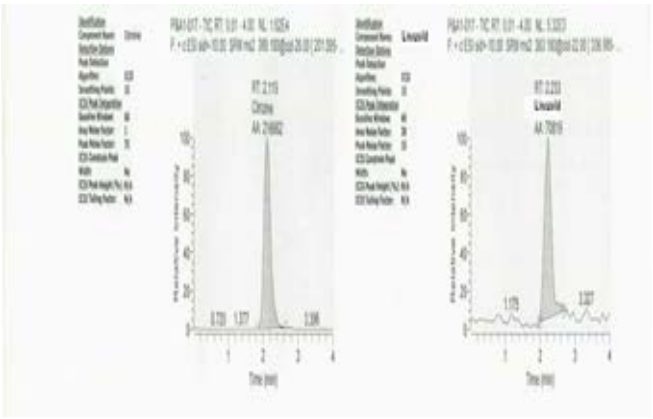

**Figure:7 A representative chromatogram of LQC sample**

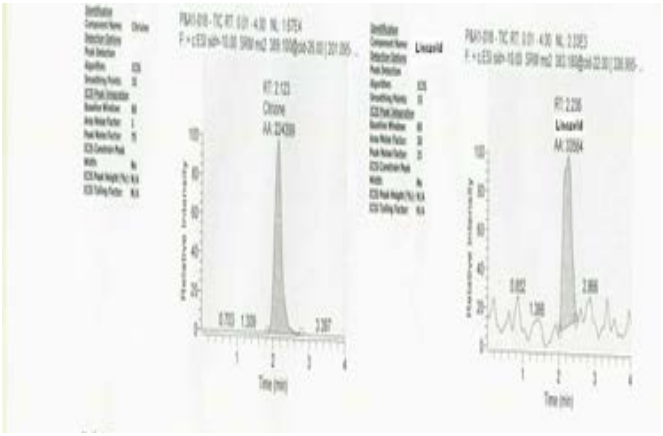

**Figure:8 A representative chromatogram of LOQ QC sample**

Recovery was determined by comparing the areas of extracted QC samples against the areas of respective aqueous QC samples. The % mean recovery of linezolid in HQC, MQC and LQC was 60.73,73 and 86.45% respectively. Similarly the % mean recovery of ISTD in the same QC samples was 71.3, 58.5 and 54.3% respectively. The results of recovery of drug and ISTD were shown in the following tables 9,10.

**Table: 9 Recovery of linezolid**

| Replicate No. | HQC | | MQC | | LQC | |
|---|---|---|---|---|---|---|
| | Aqueou response | Extract response | Aqueous response | Extract response | Aqueous response | Extract response |
| 1 | 306132 | 360629 | 230072 | 306636 | 24089 | 25260 |
| 2 | 362551 | 426187 | 225158 | 323926 | 13966 | 29954 |
| 3 | 329471 | 363265 | 212962 | 342796 | 15171 | 26364 |
| 4 | 308896 | 368184 | 233893 | 289800 | 15790 | 28092 |
| 5 | 340985 | 457161 | 202959 | 308275 | 15153 | 23859 |
| 6 | 332468 | 410432 | 189034 | 318414 | 14641 | 37328 |
| Mean | 330083.8 | 397643 | 215679.7 | 314974.5 | 16468.3 | 28476.2 |
| SD | 20983.93 | 39850.79 | 17366.04 | 17967.55 | 3782.82 | 4833.42 |
| % CV | 6.4 | 10 | 8.1 | 5.7 | 23 | 17 |
| % Mean Recovery | 60.3 | | 73 | | 86.45 | |

**Table:10 Results of recovery of ISTD**

| Replicate No. | HQC | | MQC | | LQC | |
|---|---|---|---|---|---|---|
| | Aqueous response | Extract response | Aqueous response | Extract response | Aqueous response | Extract response |
| 1 | 299185 | 218147 | 353291 | 207004 | 379907 | 221375 |
| 2 | 311989 | 212549 | 340135 | 225420 | 413790 | 212676 |
| 3 | 297127 | 215132 | 342572 | 208676 | 370244 | 214687 |
| 4 | 302104 | 209983 | 365395 | 198558 | 410395 | 213150 |
| 5 | 295633 | 225750 | 383602 | 222087 | 379513 | 208193 |
| 6 | 300461 | 204017 | 391051 | 211995 | 383242 | 199703 |
| Mean | 301083.2 | 214263 | 362674.3 | 212290 | 389515.2 | 211630.7 |
| SD | 5819.36 | 7403.09 | 21216.86 | 9980.34 | 18046.19 | 7235.76 |
| % CV | 1.9 | 3.5 | 5.9 | 4.7 | 4.6 | 3.4 |
| % Mean Recovery | 71.3 | | 58.5 | | 54.3 | |

**4.2.8 Stability studies:**

Stability studies like short term, long term stock solution stability, bench top, autosampler and freeze thaw stability were determined by keeping the QC samples at different conditions. The results were within the acceptance criteria which were shown in the following table11.

**Table:11 Results showing stability of linezolid at different conditions**

| S.NO | PARAMETER | CONDITION | RESULTS | ACCEPTANCE CRITERIA |
|---|---|---|---|---|
| 1 | Short term stock solution stability | 6hrs at ambient temperature | 97.1% | 90-110% |
| 2 | Long term stock solution stability | After storage of 10 days below 8°c | 98.5% | 90-110% |
| 3 | Bench top | 6hrs at room temperature(22±4°c) | 89-99.3% | 85-115% |
| 4 | Autosampler | Stored in autosampler at ambient temperature for 24hrs | 85.6-95.5% | 85-115% |
| 5 | Freeze thaw | Stored at -86°c | 92.1-92.3% | 85-115% |

## 5. CONCLUSION:

The use of turbulent flow chromatography combined with LC-MS/MS detection for analytical separation serves as a significant amount of time in sample preparation and increases sample throughput. The validation results reflected that this method is suitable for regulatory purposes. This method can be strongly recommended for use because it significantly speeds up sample analysis compared to traditional methods and is applicable for regulatory purposes.

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