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RESEARCH ARTICLE

STABILITY INDICATING LC-MS/MS METHOD FOR DETERMINATION OF TEICOPLANIN IN HUMAN PLASMA

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ABSTRACT

A rapid, simple, selective and sensitive LC-MS/MS method was developed for the determination of Teicoplanin in human plasma using Imipramine as internal standard (IS). The method was developed with turbid ion spray (TIS) in the positive ion and multiple reaction monitoring (MRM) mode. The assay procedure involves a simple liquid – liquid extraction of Teicoplanin and Imipramine from human plasma by using methyl-tert-butyl ether. The mobile phase was acetonitrile: 10mM ammonium formate, pH 4.5 in the ratio of 90:10%v/v. Chromatographic separation was achieved on Gemini C_{18} (50×4.6mm,5µ) column with a flow rate of 1.0ml/min. The MRM transitions monitored for Teicoplanin and Imipramine were 324.70/108.90 and 280.80/86.00 respectively. The developed method was validated as per FDA guidelines. Linearity was observed from 306.022-199205.354pg/ml with correlation coefficient of 0.9969. The percent recovery for the drug and IS was found to be 78.31 and 64.96% respectively. Stability studies like freeze thaw, bench top, short term and long term were performed and the results were found to be within the acceptance limits according to FDA guidelines.

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INTRODUCTION

Teicoplanin is a semisynthetic glycopeptide antibiotic used in prophylaxis and treatment of serious infections caused by gram positive bacteria, including methicillin resistant staphylococcus aureus and enterococcus faecalis. It acts by inhibiting peptidoglycan polymerization resulting in inhibition of cell wall synthesis and cell death. Teicoplanin (CAS no. 61036-62-2); (Figure 1), glycopeptide antibiotic is a mixture of five major (named Teicoplanin A2-1 through A2-5) and four minor (named Teicoplanin RS-1 through RS-4) compounds. It has a chemical formula of $C_{88}H_{97}Cl_2N_9O_{33}$ and molecular weight of 1879.6580.Targocid (Teicoplanin) is commercially available as injection (200,400mg). It is not absorbed orally, but intravenous and intramuscular administrations are well tolerated. Its plasma protein binding was found to be 90-95%. Teicoplanin is eliminated predominantly by kidneys and only 2-3% of an intravenously administered dose is metabolized. The mean terminal half-life ($t_{1/2}$) is around 70-1000hrs. (http://en.wikipedia.org/wiki/teicoplanin)

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Several analytical procedures areavailable in the literature for the estimation of Teicoplanin in biological fluids: fluorescence polarization immunoassays (FPIA), homogeneous turbidimetric immunoassays, HPLC and LC-MS. An overview of chromatographic methods available for the determination of Teicoplanin can be seen in Table.1. The present research work aims to develop a sensitive, precise, accurate and specific LC-MS/MS method for the analysis of this drug compared to the available methods. (Hanada *et al*., 2005; McCann *et al*., 2002; Mochizuki *et al*., 2007; Reed *et al*., 1997; Fung *et al*., 2012; Tsai I *et al*., 2013)

MATERIALS AND METHODS

Teicoplanin was sourced by matrix laboratories limited, India and Imipramine (IS) was procured from Sigma Aldrich. HPLC grade methanol, acetonitrile and Methyl-tert-butyl ether were purchased from Merck (Darmstadt, Germany). Ammonium formate and formic acid were purchased from S.D. fine chemicals (Mumbai, India). HPLC grade water was prepared from Millipore MilliQ apparatus. Analytes free human plasma was procured from Supratech pathological laboratory at Ahmadabad from different individual sources.

Table 1. Chromatographic methods published for the determination of Teicoplanin

Author	Method	Sample preparation	Mobile phase	Stationary phase	Inaccuracy	Impression	Method comparison
Reed *et al*.	HPLC-UV	PPT/LLE	Acetonitrile+25mM potassium Phosphate buffer pH 6.0	Biophase ODS,5µm,250×4.6mm	90%	<7.6%	nd
McCann *et al*.	HPLC-UV	PPT/LLE	Acetonitrile+ 30mM Ammonium acetate buffer pH 4.4	Sphereclone C_8,5µm,150×50×4.6mm	99.1-101.8%	<2.76%	FPIA
Hanada *et al*.	HPLC-UV	PPT/LLE	Acetonitrile+ 50mM Potassium phosphate buffer pH4.0	L-Column ODS,5µm,250×4.6mm	nd	<12%	FPIA
Fung *et al*.	LC-MS/MS	PPT	1% ammonium acetate+0.1% formic acid in water or methanol	Acquity UPLC BEH C_{18},1.7µm,2.1×50mm	nd	<13.4%	FPIA
Tsai *et al*.	LC-MS/MS	PPT	0.1% formic acid in water or acetonitrile	Kinetex C_{18}, 2.6µm,2.1×50mm	88.0-110.6%	<14.7%	nd
Mochiznki *et al*.	HPLC-ECD	Filtration	Acetonitrile+100mM phosphate buffer pH 4.4	Capcell PAK C_8, 5µm,150×4.6mm	nd	<5.9%	nd
Daniel *et al*.	LC-MS	centrifugation	-	Hypersil gold C_{18} column	99.6-109%	<6.9%	QMS assay

LLE, liquid/liquid extraction; nd, not done; PPT, protein precipitation; FPIA, fluorescence polarization immunoassays

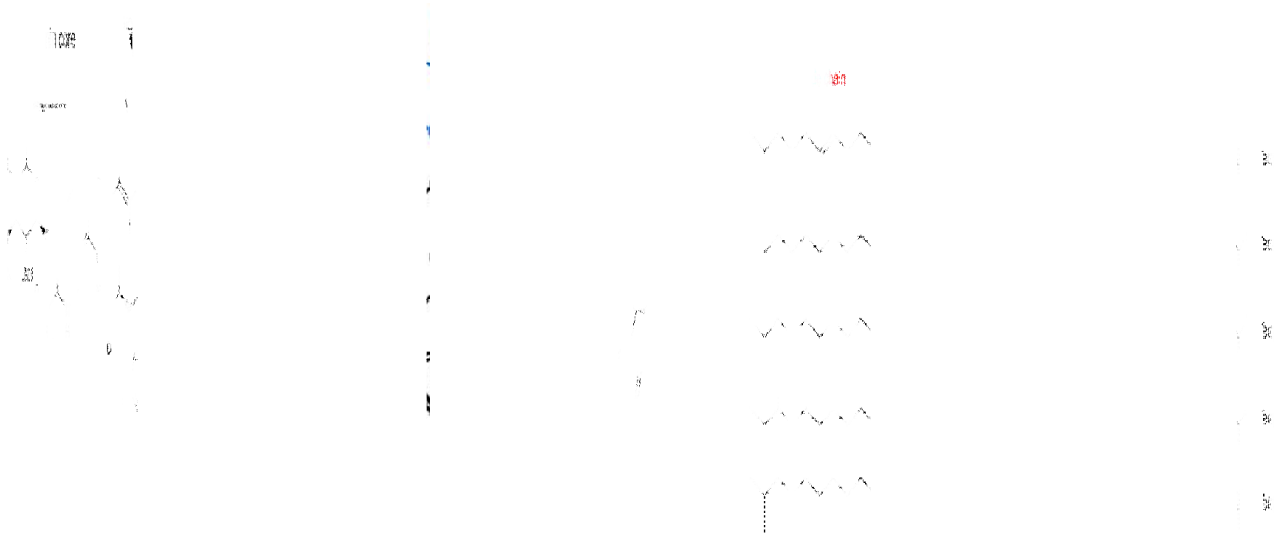


Figure 1. Structure of Teicoplanin. (A) Core structure; (B) Teicoplanin A2–1; (C) Teicoplanin A2–2; (D) Teicoplanin A2–3; (E) Teicoplanin A2–4; (F) Teicoplanin A2–5. R, rest group, displayed under (B–F), T, Teicoplanin core structure, displayed under (A)

Instrumenation and analytical conditions

The LC-MS/MS system consisted of a Schimadzu LC-20AD HPLC system (Schimadzu, Japan) interfaced with an API 4000 Quadrouple mass spectrometer (AppliedBio system, Canada) equipped with a turbo ion spray source. The chromatograms were acquired by using Analyst software version 1.4. Teicoplanin and Imipramine were separated using Gemini C_{18}(50×4.6mm, 5µ) column (Phenomenex, USA). The mobile phase consisted of acetonitrile and ammonium formate 10mM pH 4.5 in the ratio of 90:10%v/v. The flow rate was set at 1.0ml/min.

The mass spectrometer was operated in the positive ion mode with curtain gas, gas 1 and gas 2 flow rates of 15, 10 and 60psi respectively. The ion spray voltage was 5500v and temperature was 500ºc. The precursor to product ion transitions monitored for Teicoplanin and Imipramine were m/z 324.70→108.90 with declustering potential (DP) 32V and Collision energy (CE) 32V and 280.80→86.00 with DP 40V and CE 25V respectively.

Standards

A standard stock solution of Teicoplanin (1.0mg/ml) was prepared by dissolving 25mg of Teicoplanin in 25ml of methanol. Prepare 20µg/ml solution of Teicoplanin was prepared from the standard stock solution by dissolving 0.5ml in to 25ml of methanol. The IS stock solution (150µg/ml) was prepared by dissolving 15mg of Imipramine in methanol. This was further diluted with methanol to get a concentration of 180ng/ml. All the solutions were stored in the refrigerator below 8ºc. Calibration curve standards (CC) were prepared by spiking the respective solutions in screened human plasma in the range of 200013.868pg/ml to 307.264 pg/ml. The QC

samples were prepared at 313.956 pg/ml (LLOQ QC), 872.100 pg/ml (LQC), 51603.578 pg/ml (MQC 2), 103207.156 pg/ml (MQC 1) and 172011.926 pg/ml (HQC) concentrations.

Sample preparation

During method validation, to determine various parameters following procedure was followed for sample preparation. Extracted sample preparation is followed to prepare all samples for each P&A batch and other experiments such as matrix effect, anticoagulant effect, specificity, autosampler carryover, recovery, dilution integrity and stability. Procedure of unextracted sample preparation is followed to prepare aqueous samples to be used in system suitability, autosampler carryover and recovery experiments.

For the preparation of extracted sample preparation required number of plasma samples were retrieved from the deep freezer thawed them at room temperature or in water bath maintained at room temperature and vortexed the tubes to mix. 0.3ml of sample was transferred into prelabelled tubes. Added 50µl of IS dilution to all the samples except STD blank and vortexed for about 10sec. 2.0ml of Methyl-tert-butyl ether was added and extracted for 20min at 40rpm. All samples were centrifuged at 4000rpm for 5min by using refrigerated centrifuge maintained at 10ºc. Approximately 1.0ml of supernatant was transferred into prelabelled tubes and evaporated to dryness under nitrogen at 45±5ºc. Reconstituted the dried samples with 100µl of mobile phase solution and vortexed for 30sec, were transferred into prelabelled autosampler vials, arranged them in the autosampler and injected by using HPLC-MS/MS.

For the preparation of unextracted sample preparation, aliquoted 1.5ml of blank plasma into prelabelled tubes. 10.0ml of Methyl-tert-butyl ether was added and extracted on extractor for 20min at 40rpm. All samples were centrifuged at 4000rpm for 5min by using refrigerated centrifuge maintained at 10ºc.Approximately 8.0ml of supernatant was transferred into prelabelled tubes and evaporated to dryness under nitrogen at 45±5ºc. Added 24µl of respective spiking solution, 200µl of IS dilution, vortex to mix and added 576µl of mobile phase solution as reconstituted solution to the dried samples and vortexed for 30sec. Reconstituted samples were transferred into prelabelled autosampler vials, arranged them in the autosampler and injected by using HPLC-MS/MS.

Method validation parameters (Tsai *et al*., 2013; www.ema.europa.eu/docs/en_GB/document_library/Report/2013/04/WC500142229.pdf)

The developed method was validated according to the US FDA guidelines. System suitability experiment was performed by injecting six consecutive injections using aqueous standard mixture of drug and IS during the start of the validation. The autosampler carryover was performed to check any carryover in the blank sample. It was done by injecting standard, reconstituted solution, standard blank and extracted standard equivalent to highest concentration in the CC in a sequence. The linearity of the method was determined by using $1/x^2$ weighed least square regression analysis of standard plots associated with a 10 point standard curve. Accuracy should be measured using a minimum of five determinations per concentration. The mean value should be within 15% of the actual value except for LLOQ, where it should not deviate by more than 20%.

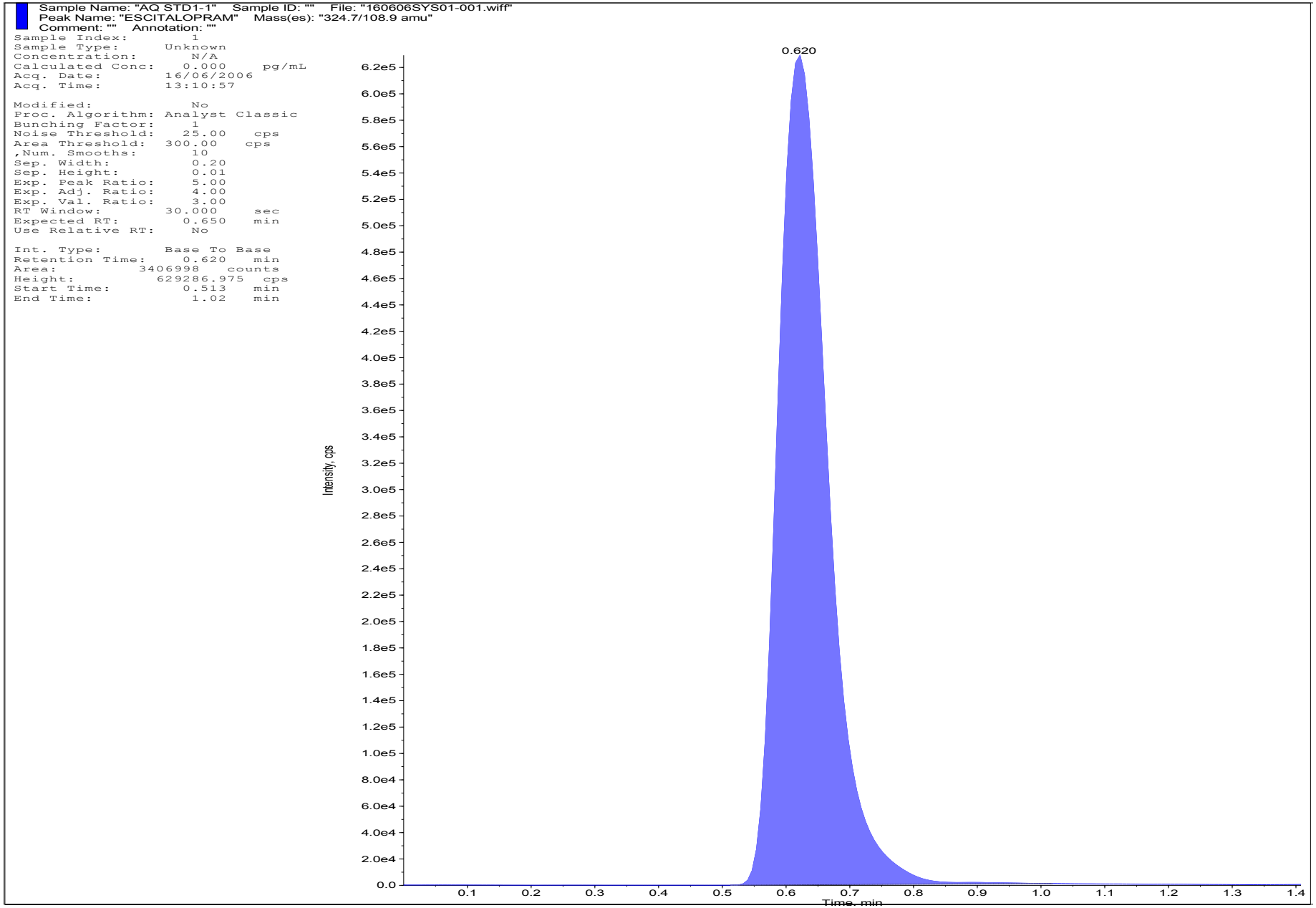
Intra batch precision should be measured using a minimum of five determinations per concentration. The precision determined at each concentration level should not exceed 15% of the correlation coefficient (CV) except for the LLOQ, where it should not exceed 20% of the CV. For inter batch precision, intrabatch experiments are repeated on four different days by different analysts. Precision from the four day experiments was compared with the intra batch precision. Ruggedness was performed by using three precision and accuracy batches. One batch was analyzed by different analyst; second batch was analyzed by using a different column and third batch by using different extractor. Selectivity was proved by determining three different parameters, namely matrix effect, specificity and recovery. Matrix effect was done by processing HQC and LQC in six different human plasma lots. Specificity was proved by processing standard blanks and LLOQ in six different human plasma lots by following the procedure for extracted sample preparation. Area of the peak at the retention time of analyte and IS in standard blank sample was compared with area of the analyte and IS in the LLOQ sample. Recovery was determined by analyzing six replicates of HQC, MQC and LQC by following the procedure for the preparation of unextracted sample preparation and compared with same concentration level QC samples processed by following the procedure for extracted sample preparation.

Stability of Teicoplanin and Imipramine was assessed in different conditions. Bench top stability was performed by analyzing six replicates of HQC and LQC placed on bench for about six hrs by following the procedure for extracted sample preparation and these samples were compared with freshly retrieved P&A batch. Freeze taw stability of the spiked QC samples was determined during three freeze thaw cycles stored below -20ºc, comparing against the freshly thawed QC samples. Wet extract stability of the spiked QC samples was determined for about 26hrs by storing in autosampler maintained at temperature of 10ºc. Stability was assessed by comparing the stability samples against the samples injected at zero hour. Short term stock solution stability was determined by storing the QC samples at room temperature on the bench for a period of about six and half hours and stored below 8ºc in the refrigerator. Long term stock solution stability was assessed by storing the QC samples below 8ºc in the refrigerator for a period of about 7 days.

RESULTS AND DISCUSSION

Optimization of liquid chromatography and mass spectrometry conditions

Complete resolution of Teicoplanin and Imipramine was achieved with a mixture of acetonitrile and 10mM ammonium formate pH 4.5 in the ratio of 90:10%v/v with a flow rate of 1.0ml/min on Gemini C_{18} (50×4.6mm,5µ) column.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Peak Name: "ESCITALOPRAM" Mass(es): "324.7/108.9 amu"
Comment: "" Annotation: ""
Sample Index: 1
Sample Type: Unknown
Concentration: N/A
Calculated Conc: 0.000 pg/mL
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Acq. Time: 13:10:57
Modified: No
Proc. Algorithm: Analyst Classic
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Area Threshold: 300.00 cps
,Num. Smooths: 10
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Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
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Expected RT: 0.650 min
Use Relative RT: No
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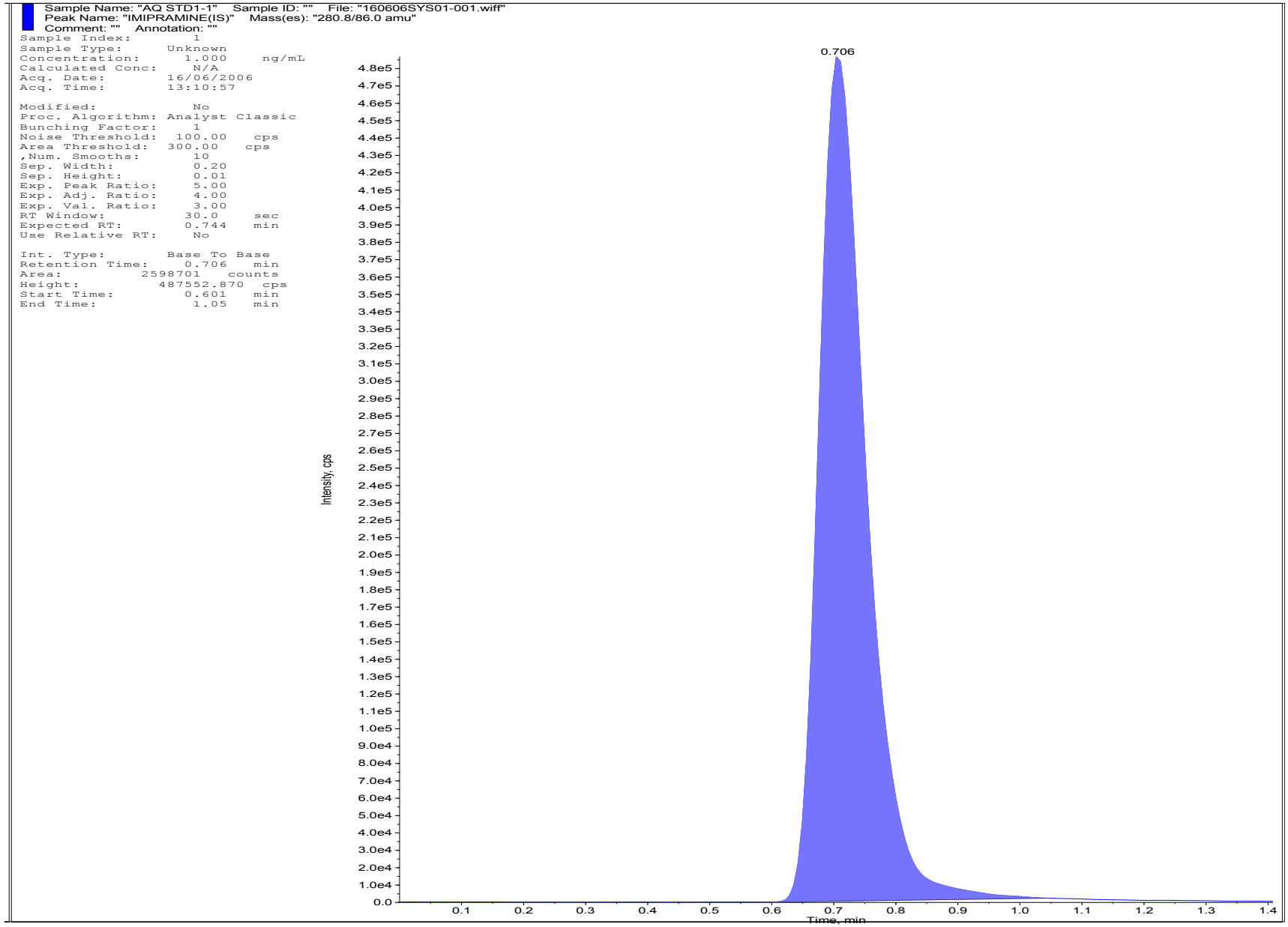


Figure 2. A representative chromatogram of drug and internal standard

Following detailed optimization of mass spectrometry conditions m/z 324.70 precursor ion to the m/z 108.90 was used for quantification of Teicoplanin. Similarly for IS m/z 280.80 precursor ion to the m/z 86.00 was used for quantification purpose. (The fragmentation pattern of Teicoplanin and the data pertaining to this was not presented here) A representative chromatogram of drug and IS was shown in the following Figure 2.

Method validation procedures

System suitability: System suitability experiment was performed by injecting six consecutive injections using aqueous standard mixture of drug and internal standard during the start of the validation. The %CV of system suitability was observed in the range of 0.88 to 3.71% for response of drug and IS which is not more than 5% as per the acceptance criteria (Table 2).

Autosampler carryover

The carryover effect of the auto sampler was performed by injecting sequence of injections. There was no significant carry over observed during the experiment i.e. Area of the peak at the retention time of drug and IS in reconstituted solution and standard blank samples was not more than 2% of the area of the drug and IS in aqueous and extracted samples.

Linearity, accuracy and precision

The linearity of the method was determined by using a $1/x^2$ weighed least square regression analysis of standard plots associated with a ten point standard curve. A representative calibration curve is shown in the Figure 3. The correlation coefficient ® observed more than 0.99. The mean accuracy observed for the CC standards were ranged from 92.48 to 109.08% which is within the acceptance limits of 85 to 115% except for LLOQ standard, which is 80 to 120%.

Table 2. Aqueous responses of Teicoplanin and Imipramine for system suitability

Analyte		Response of Drug and ISTD					
		SYS 01	SYS 02	SYS 03	SYS 04	SYS 05	SYS 06
Teicoplanin	1.	5616479	5924073	4430387	4683529	4028646	3120841
	2.	5558028	6105072	4525266	4764820	4103621	3158855
	3.	5517195	6084589	4385316	4690003	4099424	3328713
	4.	5530770	6072249	4456648	4727971	4196981	3283605
	5.	5684650	5921465	4233534	4706351	4202760	3336845
	6.	5665037	6114810	4347041	4643908	3967732	3346487
Mean		5595359.8	6037043.0	4396365.3	4702763.7	4099860.7	3262557.7
SD		70638.39	89773.30	100474.75	41221.51	92265.35	98203.99
%CV		1.26	1.49	2.29	0.88	2.25	3.01
Imipramine	1.	2998187	2310778	2228056	2272399	1478817	2481451
	2.	2882615	2309794	2330905	2124455	1397474	2466654
	3.	2883530	2321526	2277177	2211048	1461300	2578375
	4.	3059746	2329726	2311855	2145797	1345062	2524846
	5.	3024108	2326306	2222364	2078052	1455138	2544140
	6.	2936012	2385560	2244565	2154023	1476302	2554057
Mean		2964033.0	2330615.0	2269153.7	2164295.7	1435682.2	2524920.5
SD		74607.35	28099.61	45130.70	68330.21	53324.04	43267.30
%CV		2.52	1.21	1.99	3.16	3.71	1.71

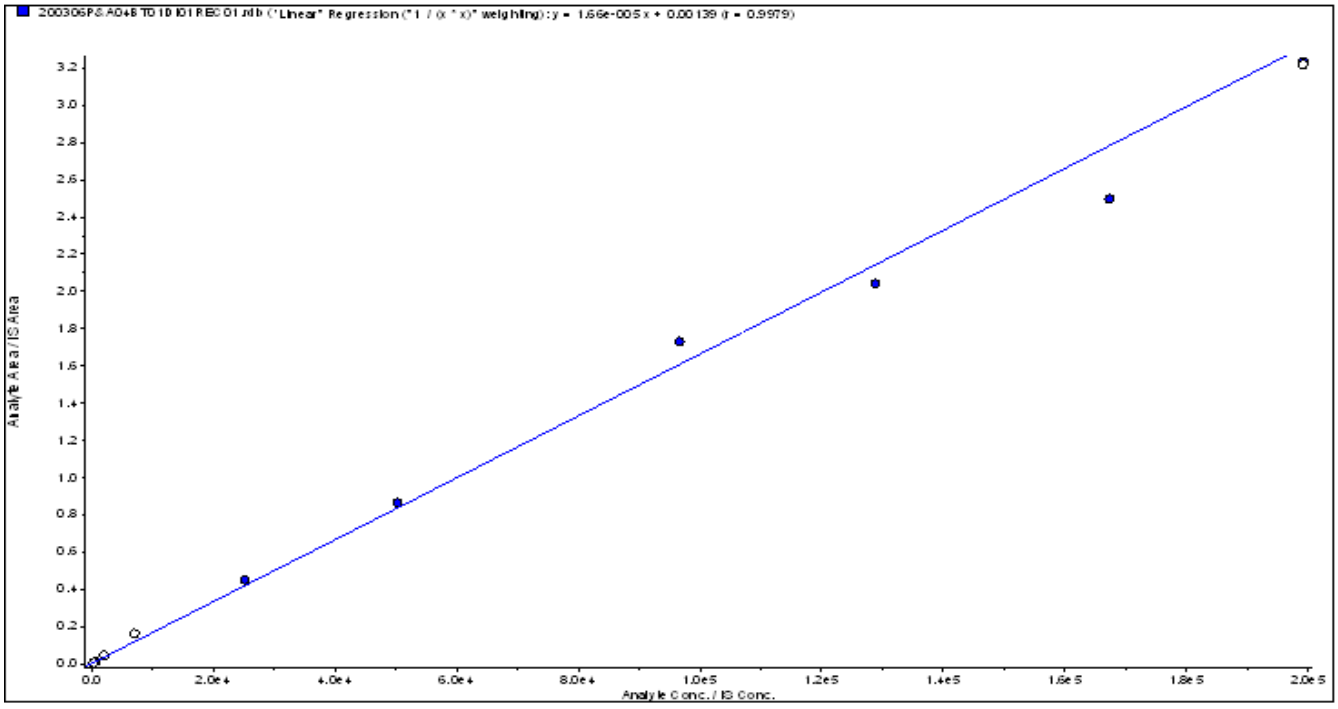


Figure 3. A representative calibration curve

The precision observed for the CC standards were ranged from 2.82 to 6.18% which is within the acceptance limits of 15% except for LLOQ standard which is 20%. The within and between batch accuracy and precision for LQC, MQC1, MQC2 AND HQC samples were ranged from 88.81 to 111.30%,93.01 to 107.55% and 0.41 to 12.23%,2.87 to 6.63% respectively. The results were within the acceptance limits of 85 to 115% for accuracy and 15% for precision. For LLOQ QC samples accuracy and precision were ranged from 88.41 to 97.90%, 92.89 and 1.49 to 17.16%, 3.76% which were within the acceptance limits of 80 to 120% and 20% respectively.

precision and accuracy for CC standards, QC samples and LLOQ samples were within the acceptance limits as shown in the following Table 3 & Table 4.

Selectivity (matrix effect)

The matrix effect for the intended method was assessed by analyzing LQC and HQC of drug prepared with six different batches. The % CV for HQC and LQC was observed 4.65 and 6.68% respectively which were within the acceptance criteria of 15% (Table 5).

Table 3. Back calculated concentrations of calibration curve standards of Drug for Ruggedness showing linearity, accuracy, and precision of three P&A batches analyzed by different column, different analyst with different extractor

STD ID	Nominal Conc. (ng/mL)	P&A (Different Column)	P&A (Different Analyst)	P&A (Different Extractor)	Mean	SD	% CV	% Mean Accuracy
		Back Calculated Concentration for Drug						
STD 1	199205.35	190562.321	190953.156	185327.338	188947.6050	3141.32740	1.66	94.85
STD 2	167332.50	159687.509	151104.073	163212.906	158001.4960	6227.99639	3.94	94.42
STD 3	128846.02	129908.48	138861.636	125144.543	131304.8863	6964.34657	5.30	101.91
STD 4	96634.52	92957.959	99662.926	103761.4	98794.0950	5453.87338	5.52	102.23
STD 5	50249.95	51024.626	54651.592	48456.699	51377.6390	3112.49714	6.06	102.24
STD 6	25124.97	25826.793	24660.048	25268.56	25251.8003	583.55303	2.31	100.50
STD 7	7034.99	8553.970*	6792.842	7391.885	7092.3635	423.58737	5.97	100.82
STD 8	2040.15	2318.623	2150.200	2417.641*	2234.4115	119.09305	5.33	109.52
STD 9	612.04	562.696	547.987	649.472	586.7183	54.84164	9.35	95.86
STD 10	306.02	312.037	319.911	296.031	309.3263	12.16858	3.93	101.08

Table 4. Back calculated concentration of QC samples of Drug for Ruggedness showing accuracy and precision of three P&A batches analyzed by different column, different analyst and with different extractor

QC ID	HQC	MQC1	MQC2	LQC	LLOQ QC
Nominal Conc. (pg/mL)	171316.604	102789.963	51394.981	868.575	312.687
Back Calculated Concentration (pg/mL) for Drug					
P & A (Different Column)	186139.636	106406.811	54879.992	807.645	292.068
	186921.438	118103.814	57436.891	806.731	316.011
	188207.922	126043.895*	55938.704	830.804	350.394
	209206.244*	114254.066	57780.851	853.864	323.279
	157841.628	117096.003	62204.677*	917.350	320.861
	166751.607	117507.881	60066.118*	809.532	363.081
Mean	177172.4462	114673.7150	56509.1095	837.6543	327.6157
SD	13959.84851	4852.40565	1348.76378	43.14802	25.45341
% CV	7.88	4.23	2.39	5.15	7.77
% Accuracy	103.42	111.56	109.95	96.44	104.77
P & A (Different Analyst)	206346.006*	113940.113	55435.401	832.095	340.361
	153297.969	120736.808*	58980.356	810.551	256.581
	201628.758*	119580.786*	59761.661*	889.535	369.572
	171945.400	100154.837	57196.039	898.234	809.892*
	157976.303	102689.700	56454.636	881.378	276.113
	170241.961	109612.237	59264.923*	902.243	258.780
Mean	163365.4083	106599.2218	57016.6080	869.0060	300.2814
SD	9152.39540	6318.79209	1494.93922	38.24366	51.53582
% CV	5.60	5.93	2.62	4.40	17.16
% Accuracy	95.36	103.71	110.94	100.05	96.03
P & A (Different Extractor)	206383.392*	107266.642	57412.603	776.416	208.561*
	200878.716*	119893.960*	57846.257	820.713	294.197
	160454.788	102470.248	56728.010	744.854	297.449
	165307.467	105923.967	61630.315*	794.329	331.442
	170103.434	105260.751	57073.593	765.591	236.642*
	161191.698	110439.301	56452.935	761.323	301.455
Mean	164264.3468	106272.1818	57102.6796	777.2043	306.1358
SD	4439.85540	2913.45070	550.53412	26.90914	17.12998
% CV	2.70	2.74	0.96	3.46	5.60
% Accuracy	95.88	103.39	111.11	89.48	97.90

Ruggedness

Ruggedness was performed by using three precision and accuracy batches. During all the three cases the results of

Specificity

The specificity of the intended method was established by screening the standard blank (without Spiking with Teicoplanin

of different bathes of commercially available human plasma). Six different batches of plasma were screened and were found to be free from endogenous significant interferences. Representative chromatograms of standard blank and LLOQ are shown in Figure 4 & Figure 5 respectively.

Recovery

The % mean recoveries were determined by measuring the concentrations of the extracted plasma QC samples at HQC, MQC1, MQC2 and LQC against unextracted QC samples at the same concentration. The results observed were 74.52, 75.97, 72.65 and 59.70% respectively. Recovery for IS was 48.66% as shown in the following Table 6.

Table 5. Matrix Effect showing back calculated concentration of HQC and LQC for Drug with their % accuracy in six different plasma lots

Replicate No.	Nominal Concentration (pg/mL)			
	HQC (167199.079)		LQC (912.907)	
	Back Calculated Conc. (pg/mL)	% Accuracy	Back Calculated Conc. (pg/mL)	% Accuracy
1.	165562.540	99.02	1011.155	110.76
2.	178663.743	106.86	832.579	91.20
3.	159095.543	95.15	930.103	101.88
4.	178015.211	106.47	895.089	98.05
5.	170532.455	101.99	972.254	106.50
6.	177391.945	106.10	946.707	103.70
Mean	171543.5728	102.5984	931.3145	102.0164
SD	7981.61563	4.77372	62.20921	6.81441
% CV	4.65	4.65	6.68	6.68

Table 6. Recovery studies of Drug for Quality Control samples

Replicate No.	Drug Response							
	HQC		MQC1		MQC2		LQC	
	Aqueous	Extracted	Aqueous	Extracted	Aqueous	Extracted	Aqueous	Extracted
1.	3898564	3432523	2492585	2230597	1332269	1036334	24986	13179
2.	3965107	3461743	2458818	2044466	1287280	948891	25607	14609
3.	3960419	2719282	2547351	1820736	1303577	972085	24575	14697
4.	4072897	2762766	2510892	1716746	1406398	942884	24954	16302
5.	3844312	2653221	2414879	1782457	1368893	927448	26370	17215
6.	4005834	2665931	2443805	1700570	1254648	949992	25866	14955
Mean	3957855.5	2949244.3	2478055.0	1882595.3	1325510.8	962939.0	25393.0	15159.5
SD	80004.06	387754.11	48219.62	210618.50	55560.15	38722.76	671.05	1415.03
%CV	2.02	13.15	1.95	11.19	4.19	4.02	2.64	9.33
% Mean Recovery	74.52		75.97		72.65		59.70	

Table 7. Recovery studies of Imipramine

S. No.	ISTD Response	ISTD Response
	Aqueous Samples	Extracted Samples
1.	1882293	1023152
2.	1916319	1057024
3.	2009208	1066742
4.	1961889	920665
5.	2039667	1043446
6.	2032652	953801
7.	2058221	997866
8.	2067197	918854
9.	2084447	1022974
10.	2136191	966669
11.	2070214	965400
12.	2059829	990061
13.	2088405	1063097
14.	2042042	978174
15.	2080139	1018726
16.	2162292	1049587
17.	2102427	1064758
18.	2042068	993180
19.	2117142	998781
20.	2178690	1084297
21.	2129228	1041119
22.	2159091	1011290
23.	2177622	1020831
24.	2216750	986740
Mean	2075584.3	1009884.8
SD	80976.07	45098.85
% CV	3.90	4.47
% Recovery	48.66	

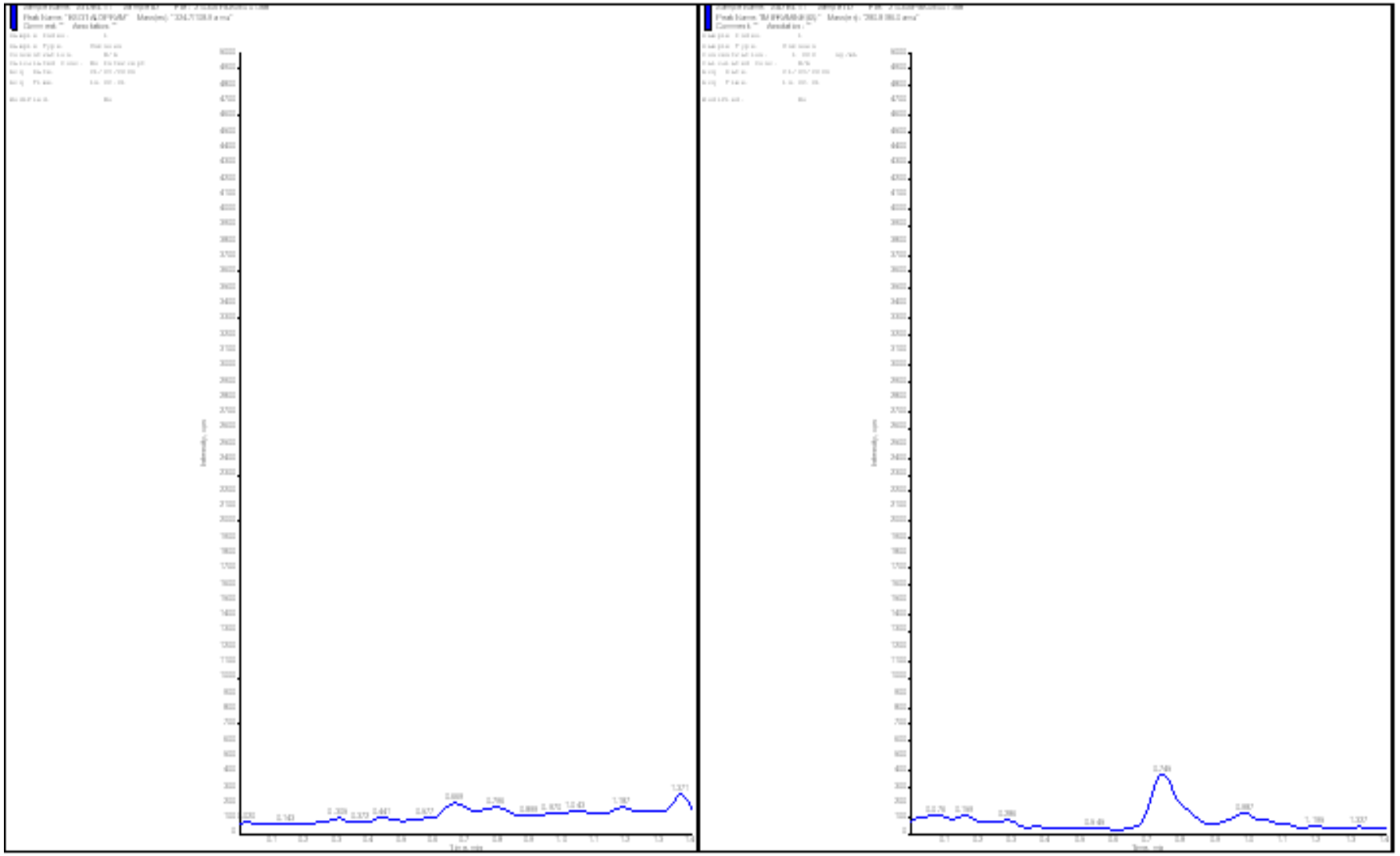
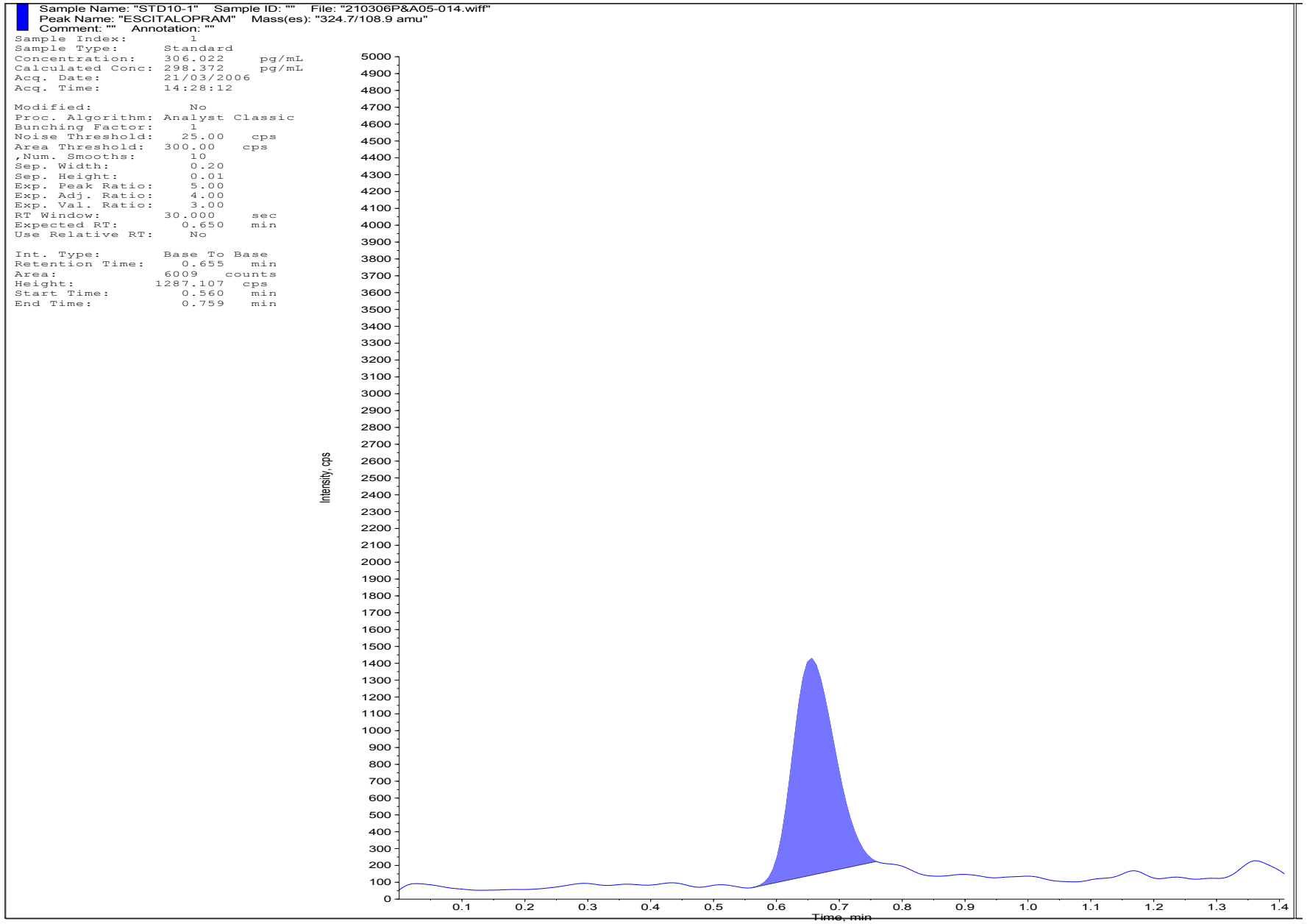


Figure 4. A representative chromatogram of standard blank


Sample Name: "STD10-1" Sample ID: "" File: "210306P&A05-014.wiff"
Peak Name: "ESCITALOPRAM" Mass(es): "324.7/108.9 amu"
Comment: "" Annotation: ""
Sample Index: 1
Sample Type: Standard
Concentration: 306.022 pg/mL
Calculated Conc: 298.372 pg/mL
Acq. Date: 21/03/2006
Acq. Time: 14:28:12
Modified: No
Proc. Algorithm: Analyst Classic
Bunching Factor: 1
Noise Threshold: 25.00 cps
Area Threshold: 300.00 cps
,Num. Smooths: 10
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
RT Window: 30.000 sec
Expected RT: 0.650 min
Use Relative RT: No
Int. Type: Base To Base
Retention Time: 0.655 min
Area: 6009 counts
Height: 1287.107 cps
Start Time: 0.560 min
End Time: 0.759 min
Intensity, cps
Time, min

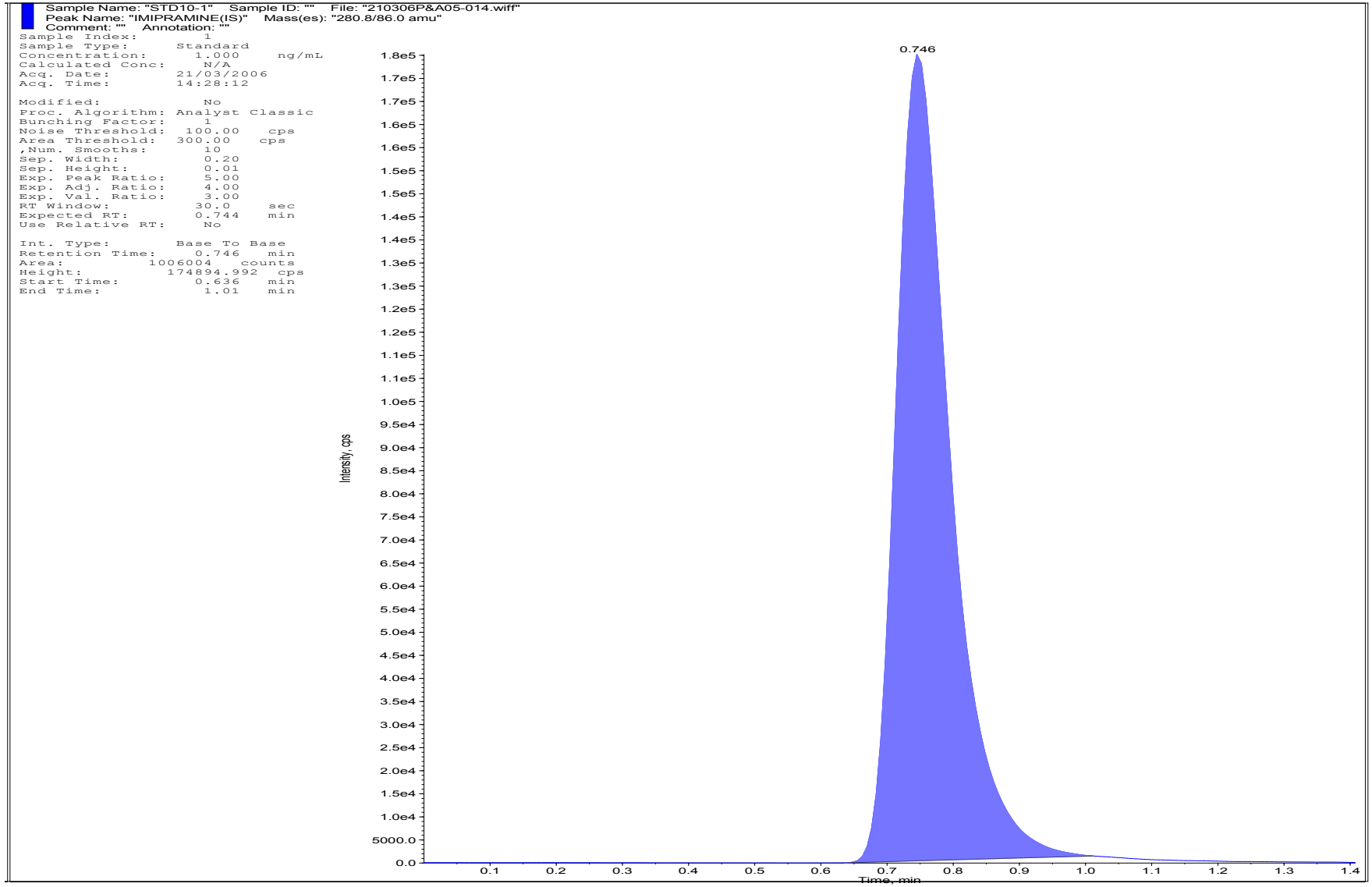


Figure 5. A representative chromatogram of LLOQ sample

Table 8. Stability data of QC samples in human plasma

S.No	Parameter	Condition	Result obtained	Acceptance criteria
1	Bench top stability	6 hrs at ambient temperature	93.43-114.14%	85-115%
2	Freeze thaw stability	Stored at below -20ºc	95.71-104.42%	85-115%
3	Autosampler stability (wet extract)	Stored in autosampler for 26hrs at 10ºc	93.59-10.42%	85-115%
4	Short term stock solution stability	Stored at ambient temperature for six and half hrs	103.20%	90-110%
5	Long termstock solution stability	After storage of 11 days at below -8ºc	102.60%	90-110%
6	Dry extract stability	Stored in deep freezer a below -20ºc for 24hrs	100.71-105.23%	85-115%
7	Long term stability of drug in plasma	Stored for about at -20ºc	92.42-100.82%	85-115%
		-50ºc	103.63-108.52%	
8	Dilution integrity	1:5 dilution	106.10%	85-115%
		1:10 dilution	108.88%	

Stability

Stability studies like bench top, freeze taw, autosampler, short term stock solution, long term stock solution, dry extract were determined by keeping the QC samples (LQC and HQC) at different conditions. The results were within the acceptance limits as shown in the following Table 8.

Conclusion

A method using LC-MS/MS for the determination of Teicoplanin I human plasma employing simple liquid- liquid extraction was developed. The present method is simple, rapid, specific and sensitive and additionally demonstrates good accuracy and precision when compared to the published methods. The method can serve as useful tool for the routine determination of Teicoplanin in human plasma.

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