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Development and validation of bexarote by bioanalytical methods using liquid chromatography-tandem mass spectroscopy (LC-MS/MS)

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Abstract

Background: The aim of this study was to develop and validate accurate and precise UPLC method with tandem mass spectrometry (Waters) for the determination of bexarotene in human plasma using bexarotene D4 as internal standard (IS).

Results: The retention time of bexarotene was 2.75 ± 0.30 min. The method was validated with respect to system suitability, linearity, accuracy, precision, matrix effect, auto sampler carryover test, and recovery. Linearity was found to be 1.04 to 351.93 µg/mL. LOQQC, LQC, INTQC, MQC, and HQC were found to be 1.0550, 2.7800, 25.2700, 131.61, and 263.23 respectively. The mean percentage recovery was found to be 95.72%

Conclusion: The bioanalytical method, a selective and sensitive liquid chromatography-mass spectrometry method to quantitate bexarotene in K_2EDTA human plasma over the concentration range 1.0440 to 351.9320 ng/mL, was successfully validated. This method is suitable for sample analysis to support bioequivalence/bioavailability and/or pharmacokinetic studies involving formulations of bexarotene.

Keywords: Bexarotene, Validation, Liquid chromatography, Mass spectrometer

Background

Bexarotene (brand name: Targretin) [1] is an antineoplastic (anticancer) agent approved by the US Food and Drug Administration (FDA) (in late 1999) and the European Medicines Agency (EMA) (early 2001) for use as a treatment for cutaneous T cell lymphoma (CTCL) (Fig. 1) [2, 3]. It is a third-generation retinoid. The retinoic acid receptors (RARs) regulate cell differentiation and proliferation whereas RXRs regulate apoptosis [4]. LC-MS-based method that utilized both RPLC and HILIC separations was carried out [1–4], followed by multivariate data analysis to discriminate the global urine profiles of BC patients and healthy controls [1, 5]. The purpose of this study was to identify a potential biomarker pattern in urine using metabonomics to aid non-invasive BC detection using complementary chromatographic techniques [6, 7].

Methods

A few methods are available in literature [3, 8–16]. A new bioanalytical LC-MS/MS method was performed on the LC-MS/MS (API 4000) [6, 7, 17], consisting of binary gradient pump UV detector (LC-20 AD) employed for analysis, and rheodyne injector with 20 µl fixed loop was used for the present study. Bexarotene was eluted with a flow rate of 1 ml/min using a mobile phase of acetonitrile: buffer 1(90:10, v/v). The retention time of bexarotene analyte is 2.75 ± 0.3 min.

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Extraction procedure: acetone-M: 10 mM ammonium format as extraction solvent

Spiked plasma samples were vortexed to ensure complete mixing of contents; 50 µl of internal standard (1 µl/ml of bexarotene D4) solution was added into all respectively labeled empty RIA vials except blank. Five hundred microliters of plasma samples was added to the respective labeled RIA vials containing internal standard solution and vortexed. Two hundred microliters of buffer 1 was added to all the samples and vortexed. 2.5 ml of extraction solvent was added to all the samples and capped. Samples were vibramaxed at 2000 rpm for 10 min. Then, the samples were centrifuged at 3500 rpm for 5 min in a refrigerated centrifuge between 2 and 8 °C. Two milliliters of supernatant was transferred into respective labeled RIA vials. All the samples were dried at 40 °C and 15 psi using LV evaporator. The dried residues get reconstituted with 300 µl of mobile phase and vortexed. The phospholipid removal cartridges get conditioned with 1 ml of acetone-M followed by 1 ml of conditioning solution. The samples were loaded into cartridges and eluted into RIA vials. The samples were transferred into respective labeled auto injector vials and loaded into LC-MS/MS [3].

Results

Method validation

The method was validated according to ICH Guidelines Q2 (R1) with respect to system suitability, linearity, accuracy, precision, matrix effect, auto sampler carryover test, and recovery.

System suitability

Aqueous standard or extracted standard equivalent to middle level of CC standard concentration with internal standard was prepared. Six replicates from the same vial were injected into the chromatographic device. Mean, standard deviation, and percentage coefficient of variation for the retention time and area/area ratio were calculated.

Linearity

Different serial dilutions were repeated, and fresh aqueous standards (for CCs) were prepared. An appropriate regression model with minimal or no weighing (1/x or $1/x^2$) was used. The standards were run in the LC-MS/MS, and linearity was evaluated.

Selectivity/specificity

This is to check whether there is an interference in peak. Two sets of six normal lots of plasma and one hemolyzed were taken. The aqueous LLOQ dilution was prepared and was spiked in one set of six normal lots of plasma, one hemolyzed lot to achieve LLOQ concentration for analyte, and the specificity sample was processed. The internal standard dilution was prepared, and only 50 µL of internal standard dilution was added to another set of six normal lots of plasma, one hemolyzed was processed for specificity samples. Selectivity samples were prepared in the presence of both analyte and internal standard using the six normal blank plasmas and one hemolyzed.

Precision and accuracy

The precision was determined by calculating percentage %CV at each concentration level of QC sample, and the accuracy was determined by calculating the percentage of nominal value at each concentration level of QC samples.

Ruggedness

One P&A batch was performed by employing the same instrument with different analysts and alternatively performed on different instruments of same make.

Recovery

The overall mean recovery, SD, and %CV were calculated. The recovery experiment was carried out by inject the six replicates of unextracted low, medium, and high QC samples, along with freshly processed CC set and QCs (6 LOC, 6 MQC, and 6 HQ).

Stability

Evaluation of stability should be carried out to ensure that every step taken during sample preparation and sample analysis, as well as the storage conditions used, do not affect the concentration of the analyte. The stability tests conducted in method validation are as follows:

1. Stock solution stability: short-term stock solution stability, long-term stock solution stability
2. Stability in biological matrix: bench top stability, freeze-thaw stability, long-term stability, blood stability

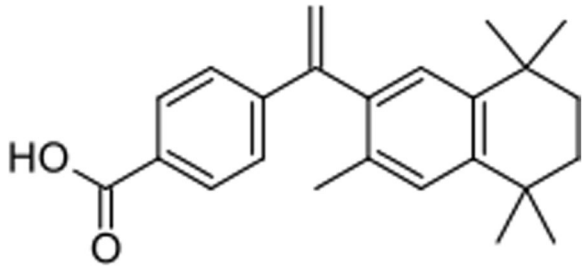

Fig. 1 Chemical structure of bexarotene. Formula: $C_{24}H_{28}O_2$. Molar mass: 348.478 g/mol

Table 1 Specificity and selectivity for bexarotene and internal standard (MTR-BA-LC-MS/MS-05)

Plasma lot ID	Specificity (blank)		Selectivity (spiked LLOQ)		% interference in blank		Area ratio	S/N ratio (≥ 5)
	Analyte	IS peak	Analyte	IS peak	Analyte (< 20%)	IS (< 5%)	Analyte/IS	Analyte
MAT-C-0293-III	1805	2581	30,895	1,932,710	5.8424	0.1335	0.0160	101.450
MAT-C-0577-III	1274	930	31,179	1,861,940	4.0861	0.0499	0.0167	118.834
MAT-C-0578-III	1070	1798	29,447	1,823,809	3.6336	0.0986	0.0161	75.245
MAT-C-0579-III	513	1694	27,120	1,813,246	1.8916	0.0934	0.0150	111.258
MAT-C-0580-III	425	506	29,375	1,868,739	1.4468	0.0271	0.0157	129.562
MAT-C-0586-III	1528	1132	25,471	1,774,320	5.9990	0.0638	0.0144	64.867
MAT-C-0544-I(H)	1147	745	28,883	1,769,483	3.9712	0.0421	0.0163	96.481
MAT-6188-I(L)	1396	2263	28,664	1,815,377	4.8702	0.1247	0.0158	95.259
MAT-6198-IX (heparin)	453	1361	27,446	1,825,500	1.6505	0.0746	0.0150	81.708
				Mean	**3.71016**	**0.07863**	**0.01567**	
						SD	**0.000731**	
						%CV	**4.67**	

Discussion

Method development

The LCMS/MS procedure was optimized for the estimation of bexarotene with the mobile phase of acetonitrile: buffer 1 (90:10, v/v); the optimum flowrate was 1 ml/min with a column oven temperature and autosampler temperature of 40 °C and 10 °C respectively. Retention time of analyte is 2.75 ± 0.3 min, and IS is 2.73 ± 0.3 min.

Specificity and selectivity

Selectivity was evaluated by analyzing a total of nine lots on the instrument [17] obtained from independent sources (Table 1). No significant interferences were observed at the retention times of analyte and internal standard (see Table 3).

Signal-to-noise (S/N) ratio

The signal-to-noise ratio was determined for bexarotene at LLOQ concentrations in nine independent lots of K_2EDTA human normal plasma including one lot of hemolyzed plasma, one lot of heparin plasma, and one lot of lipemic plasma [8] demonstrating acceptable S/N intensity.

Carryover test

Carryover is calculated as the percentage peak area observed in a processed blank plasma injected immediately after a processed ULOQ calibration standard, which was used from PA-01 batch sample analysis. No significant carryover was observed for bexarotene and internal standard (see in Table 2).

Matrix effect and matrix factor

Matrix factor and matrix effect were calculated, and results are given in Tables 3 and 4.

Linearity

Linearity established [9] by preparing an eight-point standard calibration curve in K_2EDTA human plasma covering the bexarotene concentration ranges from 1.0440 to 351.9320 µg/mL using bexarotene D4 as internal standard. The calibration curve was shown to be linear for bexarotene as shown in Fig. 2; the results are seen in Table 5.

Weighting scheme

The absolute values of residuals of the back-calculated bexarotene calibration standards for the curve were tabulated, and the sum of the absolute values of the residuals was calculated for each weighting factor. The weighting factor of $1/X^2$ provided the least sum value

Table 2 Carry over test

Sample ID	Analyte peak area	IS peak area
Extracted blank	0	645
Extracted LLOQ+IS	6166	456,817
Extracted ULOQ+IS	1,955,374	413,370
Extracted blank I	0	504
Extracted blank II	0	419
Average of extracted blank	0	462
% carry over	**0.00**	**− 0.04**

Table 3 Matrix effect and matrix factor for bexarotene at LQC level

Plasma lot ID	Aqueous sample		Spiked sample		Matrix factor of analyte	Matrix factor of IS	IS normalized matrix factor	Area ratio	
	Analyte area	IS area	Analyte area	IS area				Aqueous sample	Spiked sample
MAT6223-I	48,348	1,336,922	45,987	1,317,311	1.08	1.03	1.05	0.0362	0.0349
MAT6224-I	42,001	1,271,968	42,437	1,277,296	0.99	1.00	0.99	0.033	0.0332
MAT6225-I	43,318	1,289,768	40,402	1,257,917	0.95	0.98	0.97	0.0336	0.0321
MAT6220-I	37,838	1,240,867	42,187	1,239,881	0.99	0.97	1.02	0.0305	0.0340
MAT6204-I	41,861	1,246,249	43,795	1,222,111	1.03	0.96	1.07	0.0336	0.0358
MAT6205-I	42,559	1,278,563	41,373	1,221,142	0.97	0.96	1.01	0.0333	0.0339
MATC-0544-XII(H)			43,332	1,289,828	1.02	1.01	1.01		0.0336
MAT6188-IX (L)			42,395	1,282,621	0.99	1.00	0.99		0.0331
MAT6198-(X)-Heparin Plasma			43,054	1,277,420	1.01	1.00	1.01		0.0337
Mean	**42,654.16**	**1,277,389.5**			**1.003**	**0.990**	**1.01333**	**0.033**	**0.033**
SD					**0.037**	**0.023**	**0.03082**		
%CV					**3.76**	**2.42**	**3.04**		

with residuals of calibration curve standards. Hence, $1/X^2$ was selected to use for this validation. See the results in Table 6.

Sensitivity

The sensitivity for bexarotene at LLOQ level in K_2EDTA human plasma determined based on the analysis of six replicates of LLOQ (1.0440 ng/mL) samples was prepared and analyzed against calibration curve standards. See the results in Table 7.

Intra-batch precision and accuracy of bexarotene

See the results in Table 8.

Ruggedness

Accuracy, assay precision, and accuracy value for ruggedness batch (PA-03) were determined by

Table 4 Matrix effect and matrix factor for bexarotene at LQC level (MTR-BA-LC-MS/MS-05)

	Aqueous sample		Spiked sample		Matrix factor of analyte	Matrix factor of IS	IS normalized matrix factor	Area ratio	
	Analyte area	IS area	Analyte area	IS area				Aqueous sample	Spiked sample
MAT-6223-I	48,348	1,336,922	45,987	1,317,311	1.08	1.03	1.05	0.036	0.03
MAT-6224-I	42,001	1,271,968	42,437	1,277,296	0.99	1.00	0.99	0.033	0.03
MAT-6225-I	43,318	1,289,768	40,402	1,257,917	0.95	0.98	0.97	0.033	0.03
MAT-6220-I	37,838	1,240,867	42,187	1,239,881	0.99	0.97	1.02	0.030	0.03
MAT-6204-I	41,861	1,246,249	43,795	1,222,111	1.03	0.96	1.07	0.033	0.03
MAT-6205-I	42,559	1,278,563	41,373	1,221,142	0.97	0.96	1.01	0.033	0.03
MAT-C-0544-XII(H)			43,332	1,289,828	1.02	1.01	1.01		0.03
MAT-6188-IX (L)			42,395	1,282,621	0.99	1.00	0.99		0.03
MAT-6198-(X)-heparin plasma			43,054	1,277,420	1.01	1.00	1.01		0.03
Mean	**42,654.16**	**1,277,389**			**1.003**	**0.99**	**1.01333**	**0.033**	**0.03**
SD					**0.037**	**0.023**	**0.030822**		
%CV					**3.76**	**2.42**	**3.04**		

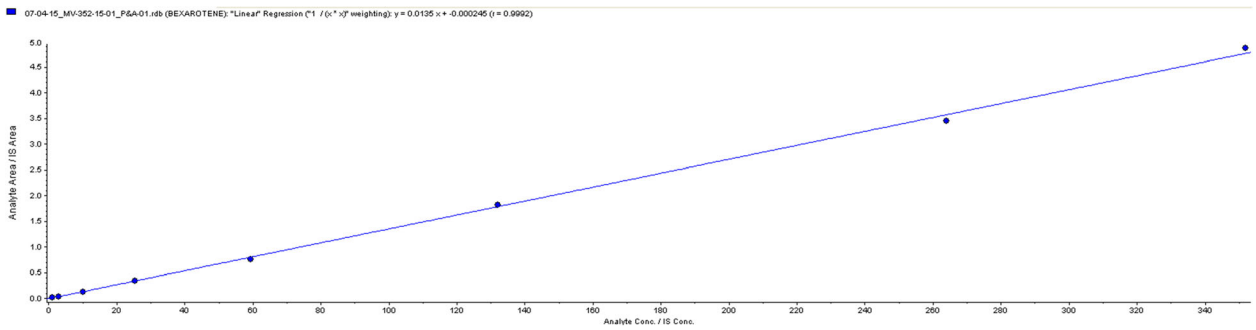

Fig. 2 Calibration curve

Table 5 Back-calculated concentrations of bexarotene for calibration curve standards

Standard ID	A	B	C	D	E	F	G	H	Slope	Intercept	r^2
Actual concentration (ng/mL)	**1.044**	**2.7760**	**10.096**	**25.240**	**59.390**	**131.97**	**263.950**	**351.93**			
PA–01	1.019	2.9590	9.9554	25.592	56.512	134.04	255.361	359.85	0.013	− 0.0002	0.998
PA–02	1.052	2.7224	9.9902	25.479	57.677	132.92	272.270	352.68	0.013	0.0011	0.999
Mean	1.0362	2.8407	9.9728	25.535	57.095	133.48	263.81	356.27			
SD	0.02319	0.167301	0.024607	0.079974	0.823921	0.787929	11.956327	5.072289			
%CV	2.24	5.89	0.25	0.31	1.44	0.59	4.53	1.42			
%Nominal	99.25	102.33	98.78	101.17	96.14	101.14	99.95	101.23			

Table 6 Weighting scheme

	Weighting—$1/X^2$	Weighting—$1/X$
	Absolute values of residuals	**Absolute values of residuals**
	1.83	3.01
	5.23	4.99
	0.10	0.03
	1.67	1.45
	4.85	4.60
	4.66	4.94
	0.17	0.44
	1.61	1.34
Sum	**20.12**	**20.80**

Table 7 Sensitivity

Parameters	LLOQ
Actual concentration (ng/mL)	**1.0440**
PA-01 (MTR-BA-LC-MS/MS-19)	1.0198
PA-02 (MTR-BA-LC-MS/MS-19)	1.0526
PA-03 (MTR-BA-LC-MS/MS-05)	1.0710
Mean	**1.04780**
SD	**0.025935**

Table 8 Intra-batch precision and accuracy of bexarotene

QC ID	LOQQC	LQC	INTQC	MQC	HQC
Actual concentration (ng/mL)	**1.0500**	**2.7800**	**25.2700**	**131.6160**	**263.2320**
Calculated concentration (ng/mL) (MTR-BA-LC-MS/MS-19) PA–01 (06 Apr 2015)	1.1639	2.9471	25.8272	135.3234	277.1292
	1.1622	2.8307	26.0325	128.5281	276.5622
	1.0975	2.9013	25.3157	131.3295	272.8455
	1.0866	2.8823	25.3351	132.7338	267.9314
	1.1758	3.0348	26.1218	136.8898	264.0381
	1.1043	2.9858	26.5157	133.2429	277.9904
Mean	**1.13172**	**2.93033**	**25.85800**	**133.00792**	**272.74947**
SD	**0.039664**	**0.073957**	**0.469270**	**2.947768**	**5.662107**
%CV	**3.50**	**2.52**	**1.81**	**2.22**	**2.08**
%nominal	**107.78**	**105.41**	**102.33**	**101.06**	**103.62**
Calculated concentration (ng/mL) (MTR-BA-LC-MS/MS-19) PA–02 (07 Apr 2015)	1.0709	2.7864	26.1883	134.5485	270.4602
	1.0005	2.8122	25.8356	134.5475	274.3989
	0.8813	2.8064	25.3888	138.3150	266.9063
	1.0456	2.8642	25.3942	133.0021	272.3938
	1.1056	2.9071	25.5199	132.3109	266.4461
	1.0615	2.8626	25.6117	136.5209	273.7517
Mean	**1.02757**	**2.83982**	**25.65642**	**134.87415**	**270.72617**
SD	**0.079461**	**0.045549**	**0.308551**	**2.228847**	**3.417745**
%CV	**7.73**	**1.60**	**1.20**	**1.65**	**1.26**
%Nominal	**97.86**	**102.15**	**101.53**	**102.48**	**102.85**

Table 9 Ruggedness (MTR-BA-LC-MS/MS-05)

Standard	A	B	C	D	E	F	G	H
Actual conc (ng/mL)	1.0440	2.7760	10.0960	25.2400	59.3900	131.9760	263.9500	351.9320
Calculated conc (ng/mL)	1.0710	2.5793	10.0606	25.4973	62.1811	131.0732	264.5648	350.4726
%nominal	102.59	92.91	99.65	101.02	104.70	99.32	100.23	99.59

Table 10 Precision

QC ID	LOQQC	LQC	INTQC	MQC	HQC
Actual concentration (ng/mL)	**1.0500**	**2.7800**	**25.2700**	**131.6160**	**263.2320**
Calculated concentration (ng/mL)	1.2696	2.6583	28.1908	140.8541	270.9386
	0.9477	2.6411	26.3654	142.1994	276.2971
	0.9881	2.6797	27.1780	140.3503	277.4336
	1.0373	2.7942	27.6385	138.9731	275.7356
	1.0366	2.6154	27.3704	141.6173	269.9140
	1.0515	2.8529	27.9700	141.5019	269.4408
Mean	**1.05513**	**2.70693**	**27.45218**	**140.91602**	**273.29328**
SD	**0.111988**	**0.094595**	**0.649825**	**1.147251**	**3.575913**
CV	**10.61**	**3.49**	**2.37**	**0.81**	**1.31**
Nominal	**100.49**	**97.37**	**108.64**	**107.07**	**103.82**

analyzing six replicates each of LOQQC, LQC, INTQ C, MQC, and HQC samples using different instrument (MTR-BA-LC-MS/MS-05) of the same make and model (UPLC with Triple Quad API 4000), different analytical column (BAC-0644), and different analyst (Table 9).

Intercept = 0.0019, Slope = 0.0118, r^2 = 0.9996

Precision

The precision of the assay was measured by the percentage co-efficient of variation over the concentration range of LOQQC, LQC, INTQC, MQC, and HQC samples of bexarotene during the course of partial validation. See the results in Table 10.

Table 11 Recovery of bexarotene

Quality control sample ID	Aqueous analyte area	Extracted analyte area
LQC	16,201	17,052
	16,045	16,462
	20,592	16,952
	20,215	16,941
	20,098	20,900
	19,423	17,689
Mean	**18,762**	**17,666**
% recovery	**94.16**	
MQC	766,900	764,709
	766,878	748,447
	756,525	743,886
	769,926	744,175
	766,882	741,445
	757,276	753,506
Mean	**764,065**	**749,361**
% recovery	**98.08**	
HQC	1,548,386	1,398,325
	1,543,021	1,373,844
	1,562,977	1,421,433
	1,573,624	1,379,191
	1,557,258	1,372,197
	1,542,212	1,352,389
Mean	**1,554,580**	**1,382,897**
% recovery	**88.96**	
Recovery result		
LQC	94.16	
MQC	98.08	
HQC	88.96	
Mean	**93.73**	
SD	**4.57**	
%CV	**4.88**	

Recovery of bexarotene and IS

The recovery of bexarotene was determined by comparing the detector response of analyte at three distinct levels of extracted low-, medium-, and high-quality control samples of PA-01 with detector response obtained from unextracted aqueous quality control samples at low, medium, and high level respectively. See the results in Tables 11 and 12.

IS recovery = 95.72%

Stability

Freeze-thaw stability

Six replicates of bexarotene samples at LQC and HQC concentration in K_2EDTA human plasma were analyzed after four freeze-thaw (FT4) cycles. See the results in Table 13.

Bench top stability

Bench top stability of bexarotene in K_2EDTA human plasma was evaluated at room temperature. Six replicates of LQC and HQC samples were processed after keeping the samples on bench for about 12.30 h. See the results in Table 14.

Table 12 Recovery of internal standard

Quality control Sample ID	Aqueous IS area	Extracted IS area
LQC	412,999	430,053
	412,341	432,371
	406,330	434,330
	409,620	436,925
	412,767	511,773
	405,852	440,319
MQC	463,632	417,498
	456,101	430,227
	465,696	418,482
	465,204	414,215
	465,835	400,164
	462,232	417,807
HQC	421,616	372,759
	423,021	366,983
	416,624	384,868
	413,104	380,280
	427,069	383,931
	424,578	359,396
Mean	**431,367.83333**	**412,910.05560**

Table 13 Freeze-thaw stability for bexarotene (at − 70 °C ± 15 °C and − 30 °C ± 10)

QC ID	− 70 °C ± 15 °C		− 30 °C ± 10 °C	
	LQC FT4	HQC FT4	LQC FT4	HQC FT4
Actual concentration (ng/mL)	**2.7800**	**263.2320**	**2.7800**	**263.2320**
Calculated concentrations (ng/mL)	2.7739	277.0834	2.9870	270.1236
	2.7193	264.5538	2.8392	272.0022
	2.9684	272.1494	2.6626	263.9169
	2.6266	269.6594	2.7717	276.9206
	2.9136	268.7336	2.9150	266.3756
	2.8143	271.7938	2.7318	262.5824
Mean	**2.80268**	**270.66223**	**2.81788**	**268.65355**
SD	**0.125448**	**4.165615**	**0.120119**	**5.407447**
%CV	**4.48**	**1.54**	**4.26**	**2.01**
%nominal	**100.82**	**102.82**	**101.36**	**102.06**

Auto sampler stability for bexarotene

Six replicates of LQC and HQC samples were processed and kept stored in auto sampler at 10 °C for 96.80 h [11]. See the results in Tables 15 and 16.

Long-term stock solution stability for bexarotene

Stock solution bexarotene with concentration of 975.6417 μg/mL was kept in the refrigerator for 14 days [12]. A fresh stock of 986.1296 μg/mL was prepared on the day of analysis. Both stocks were diluted to LQC and HQC equivalent concentration of 0.1317 μg/mL and 0.1331 μg/mL and 13.1712 and 13.3127 μg/mL for stored and fresh stock respectively. The area ratios of stability stock solution at LQC and HQC level were compared against freshly prepared stock solution LQC and HQC level. See the results in Tables 17 and 18.

Limit of detection

From LLOQ sample (1.0540 ng/mL), four different lower concentrations (0.8440, 0.6340, 0.4240, and 0.2120 ng/mL) including five times the lower concentration (LOD dilution) were prepared, and six replicates of these samples were analyzed. So the selected LLOQ (approx. 1.0540 ng/mL) was more suitable to quantify bexarotene in plasma using LC-MS/MS.

Reinjection reproducibility [14–16]

CC standards, LQC, and HQC samples of PA-02 were reinjected after 08.95 h. Percentage nominal for LQC

Table 14 Bench top stability for bexarotene

Stability hours	12.30 h	
QC ID	LQC (stability)	HQC (stability)
Actual concentration (ng/mL)	**2.7800**	**263.2320**
Calculated concentration (ng/mL)	2.7480	272.6737
	2.7554	268.0237
	2.8146	268.2582
	2.8589	258.8974
	2.8595	263.2458
	2.7803	266.8280
Mean	**2.80278**	**266.32113**
SD	**0.049512**	**4.729323**
%CV	**1.77**	**1.78**
%nominal	**100.82**	**101.17**

Table 15 Auto sampler stability for bexarotene

Stability hours	96.80 h	
QC ID	LQC (stability)	HQC (stability)
Actual concentration (ng/mL)	2.7800	263.2320
Calculated concentration (ng/mL)	2.8654	274.3981
	2.8602	273.6167
	2.8930	272.6483
	2.9033	266.1408
	2.7949	270.6783
	2.7693	267.6223
Mean	**2.84768**	**270.85075**
SD	**0.053940**	**3.349675**
%CV	**1.89**	**1.24**
%nominal	**102.43**	**102.89**

Table 16 Auto sampler stability for internal standard

Stability hours	0 h	96.80 h
QC ID	CS (IS area)	Stability samples (IS area)
LQC	314,806	326,389
	371,920	288,982
	313,781	303,778
	307,364	301,846
	368,512	314,200
	289,032	327,489
HQC	296,546	311,897
	293,776	307,720
	363,070	288,166
	320,306	313,358
	315,974	351,347
	335,867	297,954
Mean	**324,246.16667**	**311,093.83333**
% stability	**95.94**	

and HQC for bexarotene was 98.99 and 100.30%, respectively. The percentage CV for LQC and HQC for bexarotene was 3.66 and 1.97%, respectively. See the results in Table 19.

Conclusion

Bioanalytical method is developed and validated as per ICH guidelines for the estimation of bexarotene in human plasma by using LC-MS/MS. The mobile phase was selected after trying various combinations of polar solvents. The proportion of solvents and variation of buffers were found to be quite critical as slight variation in it adversely affected the resolution of peaks. Considering all the facts, the validation parameter was finally fixed for this method. The bioanalytical method, a selective and sensitive liquid chromatography-mass spectrometry method to quantitate bexarotene in K_2EDTA human plasma over the concentration range from 1.0440 to 351.9320 ng/mL, was successfully validated. This method is suitable for sample analysis to support bioequivalence/bioavailability and/or pharmacokinetic studies involving formulations of bexarotene.

Table 17 Long-term stock solution stability for analyte LQC

S. No.	Solution 1 (14 days)			Solution 3 (0 day)		
	Analyte area	IS area	Area ratio	Analyte area	IS area	Area ratio
1	47,688	1,336,789	0.0357	41,817	1,175,778	0.0356
2	48,589	1,346,976	0.0361	41,215	1,151,561	0.0358
3	48,301	1,361,402	0.0355	41,798	1,116,568	0.0374
4	45,066	1,366,924	0.0330	41,422	1,148,997	0.0361
5	47,950	1,385,003	0.0346	39,561	1,164,781	0.0340
6	48,638	1,388,752	0.0350	41,488	1,142,573	0.0363
		Mean	0.03498		**Mean**	0.03587

Table 18 Long-term stock solution stability for internal standard LQC

S. No.	Solution 2 (14 days)			Solution 3 (0 day)		
	Analyte area	IS area	Area ratio	Analyte area	IS area	Area ratio
1	58,926	1,650,164	28.00401	41,817	1,175,778	28.11723
2	58,933	1,642,596	27.87226	41,215	1,151,561	27.94034
3	58,486	1,695,246	28.98550	41,798	1,116,568	26.71343
4	55,830	1,678,648	30.06713	41,422	1,148,997	27.73881
5	59,683	1,697,852	28.44783	39,561	1,164,781	29.44266
6	62,675	1,732,273	27.63898	41,488	1,142,573	27.53984
		Mean	28.50262		**Mean**	27.91538

Abbreviations

FDA: US Food and Drug Administration; RXR: The retinoid X receptor; EMA: European Medicines Agency; CTCL: Cutaneous T cell lymphoma; RPLC: Reverse phase liquid chromatography; HILIC: Hydrophilic interaction liquid chromatography; LC-MS: Liquid chromatography and mass spectroscopy; RARs: Retinoic acid receptors; IS: Internal standard; HPLC: High performance liquid chromatography; RIA: Radioimmunoassay; ICH: International Council for Harmonisation; LLOQ: Lower limit of quantification; ULOQ: Upper limit of quantification

Table 19 Reinjection reproducibility for bexarotene

Batch ID	Reinjection reproducibility			
	PA-02 samples		Reinjected samples (08.95 h)	
	LQC	HQC	LQC	HQC
Actual concentrations (ng/mL)	**2.7800**	**263.2320**	**2.7800**	**263.2320**
Calculated concentration (ng/mL)	2.7864	270.4602	2.6865	266.3424
	2.8122	274.3989	2.8310	269.9017
	2.8064	266.9063	2.8128	260.9096
	2.8642	272.3938	2.7216	263.9505
	2.9071	266.4461	2.8619	267.5449
	2.8626	273.7517	2.5983	255.4501
Mean	**2.83982**	**270.72617**	**2.75202**	**264.01653**
SD	**0.045549**	**3.417745**	**0.100850**	**5.206446**
%CV	**1.60**	**1.26**	**3.66**	**1.97**
% nominal	**102.15**	**102.85**	**98.99**	**100.30**
Ratio of means			**0.97**	**0.98**

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