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Development and validation of LC–MS/MS method for quantification of bicitegravir in human plasma and its application to an intracellular uptake study

Pavan Kumar Prathipati, Subhra Mandal, Christopher J. Destache

Pharmacy Practice Department, Creighton University School of Pharmacy and Health Professions, Omaha, NE, USA

Abstract

A sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for quantification of bicitegravir in human plasma. A small volume of only 50 μ L aliquot of plasma was precipitated with acetonitrile containing an internal standard (IS). Chromatographic separation was performed on a Kinetex EVO C₁₈ column, 50 $\times$ 3.0 mm, 5 μ m using an isocratic mobile phase containing 80:20 acetonitrile–water with 0.1% formic acid. A mass spectrometer was operated in ESI positive multiple reaction monitoring mode using the m/z 450.1/289.1 for bicitegravir and 420.1/277.1 for IS. The dynamic range of the method was 1–10,000 ng/mL with a correlation coefficient of 0.9991. The precision results of calibration standards were in the range of 0.05–4.57% and accuracies were 95.07–104.70%. The mean extraction recovery was 98.64% with a precision of 2.91%. The method was validated as per US Food and Drug Administration guidelines and was found to be accurate and precise. The method was successfully applied to *in vitro* cellular uptake study.

Keywords

bicitegravir; human plasma; integrase inhibitor; liquid chromatography; mass spectrometry

1 | INTRODUCTION

Bicitegravir (BIC) is a novel HIV-1 integrase strand transfer inhibitor (INSTI). BIC inhibits the strand transfer of viral DNA into the host genome and thereby prevents HIV-1 replication. BIC developed by Gilead Sciences Inc. (Foster City, CA, USA) for the treatment of HIV-1 has been recently approved by the US Food and Drug Administration (FDA). BIC has improved resistance and safety profile compared with other INSTIs (Tsiang et al., 2016). INSTIs are recommended components of initial antiretroviral therapy with two nucleoside reverse transcriptase inhibitors (NRTI) (HHS Panel on Antiretroviral Guidelines for Adults and Adolescents, 2018). The FDA-approved fixed-dose formulation, Biktarvy, contains tenofovir alafenamide (TAF), emtricitabine (FTC) and BIC.

Two different phase III clinical trials compared the co-formulated BIC/FTC/TAF vs dolutegravir (DTG)/FTC/TAF and DTG/abacavir/lamivudine for initial treatment of HIV-1 infection. The clinical trials concluded that the BIC-containing regimen had similar viral suppression and was not inferior to the DTG regimen at 48 weeks (Gallant et al., 2017; Sax et al., 2017; Spagnuolo, Castagna, & Lazzarin, 2018). The antiviral activity, safety and pharmacokinetics of BIC as a monotherapy has been studied in HIV-1-infected adults. BIC was well tolerated and displayed rapid absorption and has a half-life supportive of once-daily therapy in HIV-infected subjects (Lazerwith et al., 2016). Overall, BIC appears to be very promising INSTI compared with other drugs in the class (Smith, Zhao, Burke Jr., & Hughes, 2018).

To study the preclinical and clinical pharmacokinetics and drug–drug interactions of BIC, a precise and validated method for quantification in biological matrix is needed. No analytical methods have been published to date to quantify BIC in the biological matrix. Here, we have developed a sensitive and precise method and validated it to support pharmacokinetic studies in animal models as well as humans. Our group has previously reported several works on nanoparticle fabrication and the characterization of antiretroviral drugs (Mandal et al., 2017; Mandal et al., 2018; Mandal, Belshan, Holec, Zhou, & Destache, 2017) and we are currently working on the fabrication of nanoparticles for sustained release of BIC. The validated analytical method was successfully applied to evaluate the *in vitro* intracellular uptake of BIC nanoparticles and free drug.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The reference standard of BIC (98.5%) was purchased from MuseChem (San Gabriel, CA, USA). Dolutegravir reference standard (98%) was purchased from Biochempartner Co. Ltd, China. LC–MS grade water, methanol, acetonitrile, dimethyl sulfoxide (DMSO) and formic acid were purchased from Fisher Scientific, USA. Dipotassium ethylenediaminetetraacetic acid (K₂EDTA) plasma was purchased from Innovative Research (Novi, MI, USA). TZM-bl (PTA-5659) cell line was purchased from ATCC (University Boulevard Manassas, Manassas, VA, USA).

2.2 | Preparation of calibration curve and quality control standards

Stock solutions of BIC and DTG were prepared in DMSO at 1 mg/mL. Working solutions for the preparation of calibration and quality control were prepared from separate stock solutions. Working solutions were prepared in water–methanol (50:50, v/v). Internal standard (IS) spiking solution (100 ng/mL DTG) was prepared in acetonitrile.

Calibration curve standards spanning the range of 1–10,000 ng/mL and quality control (QC) samples at four concentrations of 1 ng/mL (Lower limit of quantification QC, LLQC), 3 ng/mL (low QC, LQC), 3500 ng/mL (mid QC, MQC) and 8000 ng/mL (high QC, HQC) were prepared by spiking the previously screened blank K₂EDTA human plasma with a corresponding working solution. Calibration standards and quality control samples were stored at –80°C.

2.3 | Sample processing

Protein precipitation was carried out by adding 200 μ L of acetonitrile containing an internal standard (100 ng/mL DTG) to 50 μ L plasma sample. Samples were vortexed for 5 min and centrifuged at 14,000 rpm, 4°C for 5 min. A 50 μ L clear supernatant was added to 150 μ L of 50% acetonitrile in water and the samples were vortex mixed and centrifuged at 14000 rpm and 4°C for 5 min. Two microliters of the prepared sample was injected into the LC–MS/MS instrument.

2.4 | Instrumentation and LC–MS/MS operating conditions

An Exion HPLC system (Applied Biosystems, Foster City, CA, USA) equipped with a Phenomenex Kinetex EVO C₁₈ column (50 $\times$ 3.0 mm, 5 μ m) was used. An optimized flow rate of 0.250 mL/min and 20:80 v/v isocratic mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was applied for chromatographic separation and symmetrical peak shape. The autosampler and column oven temperatures were maintained at 4 and 30°C respectively. The retention times were 0.92 and 0.91 min for BIC and DTG, respectively. The total run time for each sample was 2 min.

An AB Sciex API 5500 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source was used with positive multiple reaction monitoring (MRM) mode. The m/z transitions for BIC were 450.1 $\rightarrow$ 289.1 and for DTG were 420.1 $\rightarrow$ 277.1. The ESI source parameters: source temperature, ion spray voltage, nebulizer (GS1) and drying (GS2) gas pressures were optimized through flow injection analysis by infusing mobile phase at an optimized flow rate (0.250 mL/min). The best analyte and IS signals were obtained with source temperature, ion spray voltage, GS1 and GS2 set at 550°C, 5500 V, 55 and 60 psi, respectively. The liquid chromatography–tandem mass spectrometer was controlled using Analyst 1.6.3 software. Chromatography integration and data analysis were performed by MultiQuant 3.0.2 software.

2.5 | Method validation

The developed method was successfully validated as per FDA guidelines for bioanalytical method validation (FDA, 2001).

2.5.1 | Selectivity—Six different sources of K₂EDTA human blank plasma were extracted and the peak areas at the retention times of analyte and internal standard were compared with the peak areas found in the lower limit of quantification (LLOQ, 1 ng/mL) to determine the selectivity of the assay.

2.5.2 | Effect of anticoagulant—The effect of anticoagulant on quantification of BIC was determined by comparing the extracted peak areas from LQC and HQC prepared in K₂EDTA plasma, sodium heparin plasma and serum. The percentage difference was calculated between the three matrices.

2.5.3 | Matrix effect and extraction recovery—Matrix effect was tested by comparing the peak areas of neat LQC, MQC and HQC standards prepared in 50% acetonitrile against the blanks spiked post-extraction at LQC and MQC and HQC levels. Six

replicates each of LQC, MQC and HQC were extracted and the peak areas were compared against blanks spiked post-extraction at LQC and MQC and HQC level to determine extraction recovery.

2.5.4 | Calibration curves—A blank, zero standard and eight nonzero standards in the range of 1–10,000 ng/mL in human plasma were extracted. The calibration curve (CC) was generated by plotting peak area ratio (drug peak area/IS peak area) against the nominal concentration of an analyte using a linear regression model with a $1/x^2$ weighting factor.

2.5.5 | Precision and accuracy—Six replicates at each level of quality control (LLQC, LQC, MQC, and HQC) were extracted per precision and accuracy (P&A) run along with calibration curve. Three different P&A runs were performed to evaluate method precision and accuracy. The concentrations of QC were back-calculated from CC, intra- and inter-run precision were calculated as the coefficient of variation (CV) and accuracy was calculated using the percentage ratio of observed to nominal concentration.

2.5.6 | Dilution integrity—A spiked plasma sample at 20,000 ng/mL was extracted after diluting with an appropriate volume of blank sample. The integrity of the dilution was tested at 2× and 4× dilution. Six replicates at each dilution were extracted and dilution factors were applied to back-calculate concentrations.

2.5.7 | Stability of BIC—The stability of BIC in the stock solution, plasma and other conditions anticipated to be encountered during sample handling and analysis was tested. The stock solution stability at 4°C for 7 days was determined by comparing the peak areas of the stability stock against the fresh stock solution diluted at MQC level with 50% acetonitrile in water. The percentage difference was calculated between stability and fresh stocks.

Stability of BIC in plasma was determined under different conditions like on the bench-top (48 h at room temperature), long-term (62 days at –80°C) and freeze–thaw (three cycles). Six replicates of LQC and HQC that had undergone the above stability conditions were processed along with freshly prepared CC, LQC, and HQC. For processed sample stability, aliquots of extracted LQC and HQC samples were stored at room temperature and in the refrigerator (4°C) for 49 h. The concentrations of QC were back-calculated from freshly prepared CC and compared against freshly prepared QCs. Reinjection reproducibility was evaluated by reinjecting the previously accepted P&A run.

2.5.8 | Effect of concomitant drugs—The presence of other antiretroviral drugs, especially tenofovir (TFV) as TAF and FTC in the plasma samples of patients taking Biktarvy is usual. To estimate the possible interference from these drugs, blank human plasma was spiked at 5000 ng/mL level with tenofovir and emtricitabine along with BIC at LQC and HQC levels. These samples were extracted and BIC concentrations were back-calculated from a calibration curve containing BIC only.

2.6 | Partial validation and application to *in vitro* intracellular uptake study

As per FDA guidelines, a partial method validation needs to be performed for a change in matrix (FDA, 2001). Apart from full method validation in plasma, a partial validation in cellular matrix was carried out with three precision and accuracy runs as described in Sections 2.5.4 and 2.5.5 with TZM-bl cells (10^5 cells/mL) spiked with CC and QCs.

TZM-bl cells (10^4 cells/well) were seeded in Nunc 96-well plates and were treated with 10 $\mu\text{g/mL}$ of BIC as BIC nanoparticles (NPs) and BIC solutions for 1, 4, 8, 16, 24, 96 and 168 h ($n = 3$). At the respective time period, cell numbers were counted, and each well was washed three times with phosphate buffered saline and air dried under a cell culture hood. After drying, 96-well plates were stored at -80°C until analysis. The dried cells were detached from the 96-well plate surface by adding 30 μL 0.5% ethylenediaminetetraacetic acid (EDTA) and vortexed for 30 min. An aliquot of 70 μL methanol was added to each well to lyse the cells. The cell lysate was processed as per the procedure mentioned in Section 2.3. For the purpose of matrix matching, blank TZM-bl cell lysate was used for CC and QC preparation. The back-calculated concentrations for unknown samples were presented as $\mu\text{g}/10^6$ cells. The pharmacokinetic parameters, half-life ($t_{1/2}$), maximum concentration (C_{max}), time at maximum concentration (T_{max}) and area under the time–response curve (AUC_{all}) were determined using Phoenix WinNonlin (Certara L.P.).

3 | RESULTS AND DISCUSSION

3.1 | Method development

The mass spectrometer parameter optimization for BIC was initiated with a precursor ion $[\text{M} + \text{H}]^+$ scan by infusing 500 ng/mL BIC in 50% methanol into the mass spectrometer. A precursor ion peak at m/z 450.1 was found that correlated with the molecular mass (449.1 amu) of BIC. The product ion scan produced a predominant ion, m/z 289.1, and other weak product ions were found at m/z 145.0, 407.2 and 432.1. Product ion mass spectra of BIC and DTG with chemical structures and possible fragmentation are presented in Figure 1.

BIC differs from other INSTI chemical structures as it contains a unique bridged bicyclic ring and a distinct benzyl tail consisting of a trisubstituted 2,4,6-trifluorobenzyl moiety. However, the physicochemical properties, partition coefficient and protein binding (99.7%), of BIC are closely comparable with DTG (Lazerwith et al., 2016). Hence DTG was chosen as the internal standard. The optimized mass spectrometer parameters and transitions for both analyte and IS are presented in Table 1.

Considering the hydrophobicity and $\text{p}K_{\text{a}}$ (9.81) of BIC, chromatography scouting was initiated with a combination of C_{18} column and a mobile phase containing high organic (acetonitrile) ratio with 0.1% formic acid to aid ionization. Low retention times for both analyte and IS with symmetrical peak shape and reproducibility were obtained with a combination of superficially porous Kinetex EVO C_{18} column (50×3.0 mm, $5 \mu\text{m}$) and a mobile phase composition containing 0.1% formic acid in acetonitrile and 0.1% formic in water, 80:20 v/v ratio.

BIC has high plasma protein binding capacity (Lazerwith et al., 2016), hence extraction optimization was started with simple protein precipitation. The two most widely used organic protein precipitation solvents, methanol and acetonitrile, were tested. The extraction efficiency of acetonitrile was found to be higher than that of methanol for BIC. The calibration range was selected based on the previously reported pharmacokinetic study of BIC (Lazerwith et al., 2016). As the dynamic range of this assay was wide (10,000-fold), avoiding detector saturation at higher concentrations of BIC and getting linear calibration was challenging. For the purpose of adjusting analyte intensity to mitigate detector saturation, we optimized the horizontal and vertical position of ESI probe depending on the LLOQ response not altering optimized source and compound-dependent parameters.

3.2 | Method validation

The assay was validated as per the FDA guidelines (FDA, 2001). Endogenous interference from the blank human plasma at the retention times of analyte and IS was found to be <1%, which is within the acceptance limits of 20 and 5% of the analyte and IS areas, respectively, found in the LLOQ. The precision (CV) of the peak areas of LLOQ was <4.52%. The mean signal-to-noise ratio calculated for LLOQ (1 ng/mL) was 15.6, which indicates that the method is adequately sensitive. The chromatograms of blank and LLOQ are presented in Figure 2.

There was no effect of anticoagulants (K₂EDTA and sodium heparin) on the quantification of BIC. The percentage difference observed between K₂EDTA and sodium heparin plasma vs serum was <5%, which signifies no anticoagulant effect. The overall mean percentage matrix effect was 103.52% with CV 4.23% at the three concentrations tested. The percentage mean extraction recovery was in the range of 96.01–99.99% with CV between 1.06 and 3.67%. The overall percentage mean extraction recovery was 98.64% with CV 2.91.

The correlation coefficient (r) of the calibration curves run on three different days was 0.9991. The mean percentage accuracies of back-calculated standard concentrations were in the range 95.07–104.70% with precisions (CV) of 0.05–4.57%. The intra-run precisions and accuracies were in the ranges 1.12–4.44 and 94.67–101.83%, respectively. The inter-assay precisions and accuracies were in the ranges 1.10–7.52 and 96.82–99.52%, respectively. The results of calibration and P&A demonstrate that the method is accurate. The results of the calibration curve and P&A are presented in Tables 2 and 3, respectively. The dilution integrity of the assay tested at 2- and 4-fold had mean accuracy and precision values in the ranges 102.62–103.83 and 0.45–0.48% for both dilutions.

The stability of analyte in plasma at different conditions was established by comparing the stability QCs with fresh QCs. For all of the stability sample results, accuracies were within 85–115% of the nominal and CV was <15%. Bictegravir was found to be stable for at least 48 h on the benchtop in plasma, 62 days at –80°C and 49 h post-processing at room temperature, 4°C and after three freeze–thaw cycles. The results of stability are presented in Table 4. Precision and accuracies of the reinjected reproducibility run were within acceptance limits.

There was no interference from other antiretroviral drugs as the plasma samples containing BIC at LQC and HQC level with TFV and FTC (5000 ng/mL each) were within acceptable limits—CV 15% and accuracy $100 \pm 15\%$ —at both levels when back-calculated under a calibration curve containing BIC only.

3.3 | Partial validation and application to *in vitro* intracellular uptake study

The partial method validation including three P&A runs in TZM-bl cell lysate yielded linear calibration curves with correlation coefficient (r) >0.9989 . The intra- and inter-run precision and accuracies of BIC at four QC levels were found to be in the ranges 1.08–3.91 and 95.23–102.14% and 1.19–5.70 and 95.25–101.87%, respectively.

The intracellular uptake profile of BIC nanoparticle and solution at 10 $\mu\text{g/mL}$ are presented in Figure 3. The maximum concentration (C_{max}) and area under the time–concentration curve (AUC_{all}) of BIC was 3.1 and 2.8 times higher with NP than solution, indicating that the nanoformulation of BIC has improved cellular uptake potential. A similar elimination half-life ($t_{1/2}$) was observed for both NP and solution, which could be due to the drug not effluxing out of cells in *in vitro* experimental conditions. The *in vitro* pharmacokinetic parameters of BIC from NP and solution at 10 $\mu\text{g/mL}$ are presented in Table 5.

4 | CONCLUSION

A precise and simple method was developed and validated for quantification of the newest antiretroviral drug, bictegravir, which was recently approved. This is the first report of the LC–MS/MS method for the quantification of BIC in plasma. The method was validated as per FDA guidelines and additionally a partial method validation in cell lysate was also performed. The method described here showed acceptable precision and accuracy and produced a significant amount of information about the stability of the molecule. In terms of turnaround time, a simple protein precipitation extraction and short run time would be beneficial for efficient use of this method for preclinical and clinical pharmacokinetic evaluation of bictegravir. This method was successfully applied to determine the BIC NP and solution intracellular uptake profile.

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Abbreviations

BIC	bictegravir
CC	calibration curve
CV	coefficient of variation

DMSO	dimethyl sulfoxide
DTG	dolutegravir
ESI	electrospray ionization
FTC	emtricitabine
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HQC	high quality control
INSTI	integrase strand transfer inhibitor
IS	internal standard
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LLOQ	lower limit of quantification
LLQC	lower limit quality control
LQC	low quality control
MQC	mid quality control
NP	nanoparticle
NRTI	nucleoside reverse transcriptase inhibitors
P&A	precision and accuracy
QC	quality control
RPV	rilpivirine
SD	standard deviation
TAF	tenofovir alafenamide
TFV	tenofovir

REFERENCES

- Gallant J, Lazzarin A, Mills A, Orkin C, Podzamczar D, Tebas P, ... Quirk E (2017). Bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir, abacavir, and lamivudine for initial treatment of HIV-1 infection (GS-US-380-1489): a double-blind, multicentre, phase 3, randomised controlled non-inferiority trial. *Lancet*, 390, 2063–2072. [PubMed: 28867497]
- HHS Panel on Antiretroviral Guidelines for Adults and Adolescents. (2018). Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Retrieved from <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.
- Lazerwith SE, Cai R, Chen X, Chin G, Desai MC, Eng S, ... Jin H (2016). Discovery of bictegravir (GS-9883), a novel, unboosted, oncedaily HIV-1 integrase strand transfer inhibitor (INSTI) with

improved pharmacokinetics and In Vitro Resist Profile. Paper presented at the ASM Microbe, Boston, MA http://www.natap.org/2016/HIV/062016_05.htm

Mandal S, Belshan M, Holec A, Zhou Y, & Destache CJ (2017). An enhanced emtricitabine-loaded long-acting nanoformulation for prevention or treatment of HIV infection. *Antimicrobial Agents and Chemotherapy*, 61, e01475–16. [PubMed: 27821449]

Mandal S, Kang G, Prathipati PK, Fan W, Li Q, & Destache CJ (2018). Long-acting parenteral combination antiretroviral loaded nano-drug delivery system to treat chronic HIV-1 infection: A humanized mouse model study. *Antiviral Research*, 156, 85–91. [PubMed: 29885378]

Mandal S, Prathipati PK, Kang G, Zhou Y, Yuan Z, Fan W, ... Destache CJ (2017). Tenofovir alafenamide and elvitegravir loaded nanoparticles for long-acting prevention of HIV-1 vaginal transmission. *AIDS*, 31, 469–476. [PubMed: 28121666]

Sax PE, Pozniak A, Montes ML, Koenig E, DeJesus E, Stellbrink HJ, ... Quirk E (2017). Coformulated bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir with emtricitabine and tenofovir alafenamide, for initial treatment of HIV-1 infection (GS-US-380-1490): a randomised, double-blind, multicentre, phase 3, noninferiority trial. *Lancet*, 390, 2073–2082. [PubMed: 28867499]

Smith SJ, Zhao XZ, Burke TR Jr., & Hughes SH (2018). Efficacies of cabotegravir and bictegravir against drug-resistant HIV-1 integrase mutants. *Retrovirology*, 15, 37. [PubMed: 29769116]

Spagnuolo V, Castagna A, & Lazzarin A (2018). Bictegravir. *Current Opinion in HIV and AIDS*, 13, 326–333. [PubMed: 29746268]

Tsiang M, Jones GS, Goldsmith J, Mulato A, Hansen D, Kan E, ... Jin H (2016). Antiviral activity of bictegravir (GS-9883), a novel potent HIV-1 integrase strand transfer inhibitor with an improved resistance profile. *Antimicrobial Agents and Chemotherapy*, 60, 7086–7097. [PubMed: 27645238]

USFDA (2001). Guidance for industry: Bioanalytical method validation. Center for Drug Evaluation and Research, Center for Veterinary Medicine Retrieved from <https://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf>.

FIGURE 1. Product ion mass spectra of bictegavir (BIC) (a) and dolutegavir (DTG) (b) with chemical structures and possible fragmentation

FIGURE 2.

Chromatograms of BIC in blank plasma and LLOQ, BIC (left) and corresponding internal standard (right)

FIGURE 3.
The intracellular release profiles of BIC from NP and solution at 10 µg/mL

TABLE 1

Optimized multiple reaction monitoring (MRM) parameters of bictegavir and internal standard

Parameter	BIC (450.1 → 289.1)	DTG (IS) (420.1 → 277.1)
DP (V)	150	120
EP (V)	10	10
CE (V)	40	35
CXP (V)	25	12
CUR (psi)	25	
CAD	Medium	
IS voltage (V)	5500	
Source temperature (°C)	550	
GS1 (psi)	55	
GS2 (psi)	60	

BIC, Bictegavir; CAD, collision associated dissociation; CE, collision energy; CUR, curtain gas; CXP, collision cell exit potential; DP, declustering potential; DTG, dolutegavir; EP, entrance potential; IS, internal standard.

TABLE 2Summary of calibration curve standards ($n = 3$)

Nominal concentration (ng/mL)	Mean observed concentration (ng/mL)	CV	Mean percentage accuracy	Correlation coefficient (r)
1	1.00	0.82	100.00	0.9991
2	2.02	1.76	101.00	
10	9.51	0.91	95.07	
50	52.12	3.99	104.23	
250	249.80	0.29	99.92	
1000	1047.04	1.10	104.70	
5000	5004.66	0.05	100.09	
10,000	9709.24	4.57	97.09	

CV, Coefficient of variation.

TABLE 3

Summary of intra and inter-run precision and accuracy of BIC

QC level	Nominal concentration (ng/mL)	Intra-run (<i>n</i> = 6)				Inter-run (<i>n</i> = 18)			
		Mean observed concentration (ng/mL)	Percentage accuracy	CV	observed concentration	Mean (<i>n</i> = 18) (ng/mL)	Percentage accuracy	CV	
LLQC		1	101.83	4.44	1.02		98.19	7.52	
LQC		3	96.33	2.72	2.86		97.30	2.66	
MQC		3500	94.67	1.12	3313.34		96.82	1.10	
HQC		8000	98.33	1.18	7866.32		99.52	1.17	

TABLE 4

Stability data of BIC under different conditions

	Freeze-thaw stability (three cycles)		Bench-top stability (48 h)		Long-term stability (62 days at -80°C)		Processed sample stability (49 h at 4°C)	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
Theoretical concentration	3	8000	3	8000	3	8000	3	8000
Mean $\pm$ SD (n = 6)	3.01 $\pm$ 0.06	8009.23 $\pm$ 14.10	3.24 $\pm$ 0.18	8054.03 $\pm$ 9.25	2.98 $\pm$ 0.06	7991.75 $\pm$ 14.26	3.04 $\pm$ 0.10	7970.25 $\pm$ 44.63
CV	1.9	0.18	5.42	0.11	192	0.18	3.16	0.56
Percentage difference	-4.71	0.05	288	0.51	-5.6	-0.42	-3.68	-0.54

TABLE 5

Pharmacokinetic parameters of BIC as NP and in solution on 10 µg/mL treatment concentration

Parameter	Units	NP	Solution
$t_{1/2}$	hour	60.36	58.90
$C_{\max}$	µg/10 ⁶ cells	38.86 ± 4.59	12.57 ± 7.43
$T_{\max}$	hour	1	8
AUC _{all}	hour µg/10 ⁶ cells	1817.92 ± 371.44	653.69 ± 176.70

NP, Nanoparticle; $t_{1/2}$, half-life; $C_{\max}$, maximum concentration; $T_{\max}$, time at maximum concentration; AUC_{all}, area under the time–response curve. Data represented as means ± standard error, $n = 3$.