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Development and validation of a bioanalytical method to quantitate enzalutamide and its active metabolite N-desmethylenzalutamide in human plasma: application to clinical management of metastatic castration-resistant prostate cancer patients

Guillemette E Benoist, MSc.¹, Eric van der Meulen¹, Inge M van Oort, MD, PhD², Jan Hendrik Beumer, PharmD, PhD³, Diederik M Somford, MD, PhD⁴, Jack A Schalken, PhD², David M Burger, PharmD, PhD¹, and Nielka P van Erp, PharmD, PhD¹

¹Department of Pharmacy, Radboud university medical center, Radboud Institute for Health Sciences Nijmegen, Netherlands ²Department of Urology, Radboud university medical center, Nijmegen, Netherlands ³Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA, United States of America ⁴Department of Urology, Canisius Wilhelmina Hospital, Nijmegen, Netherlands

Abstract

Background—Enzalutamide is a potent androgen-signaling receptor inhibitor and is licensed for the treatment of metastatic castration-resistant prostate cancer. N-desmethylenzalutamide is the active metabolite of enzalutamide. A method to quantitate enzalutamide and its active metabolite was developed and validated according to the European Medicine Agency (EMA) guidelines.

Methods—Enzalutamide and N-desmethylenzalutamide were extracted by protein precipitation, separated on a C18 column with gradient elution and analyzed with tandem quadrupole mass spectrometry in positive ion mode. A stable deuterated isotope (D₆-enzalutamide) was used as an internal standard. The method was tested and stability was studied in real life patients with metastatic castration-resistant prostate cancer patients treated with enzalutamide.

Results—The calibration curve covered the range of 500–50000 ng/mL. Within- and between-day precisions were <8% and accuracies were within 108% for both enzalutamide and N-desmethylenzalutamide. Precisions for lower-limit-of-quantification level were <10% and accuracies within 116% for enzalutamide and N-desmethylenzalutamide. Enzalutamide and N-desmethylenzalutamide stability was proven for 24 hours for whole blood at ambient temperature, and 23 days for plasma at both ambient temperature and 2–8 °C. Long-term patient plasma stability was shown for 14 months at –40 °C.

Conclusions—This bioanalytical method was successfully validated and applied to determine plasma concentrations of enzalutamide and N-desmethylenzalutamide in clinical studies and in routine patient care.

Keywords

LC-MS/MS; enzalutamide; N-desmethylenzalutamide

1. Introduction

Enzalutamide (Xtandi®) is a potent androgen-signaling receptor inhibitor and is licensed for the treatment of metastatic castration-resistant prostate cancer (mCRPC). Enzalutamide improved progression-free survival and overall survival in phase III trials in the pre- and postchemotherapy setting [1, 2]. One of the two major metabolites identified in human plasma is active: N-desmethylenzalutamide is equipotent *in vitro* and is present at similar plasma concentrations as the parent enzalutamide [3]. Enzalutamide is administered at a fixed oral dose of 160 mg once daily (OD) regardless of body surface area, age, and clinical condition [3]. Enzalutamide is highly protein bound (97–98%) and has a mean half-life of ~5.8 days. Enzalutamide is metabolized by CYP2C8 and CYP3A4. The active metabolite, N-desmethylenzalutamide, is formed via CYP2C8 metabolism. Enzalutamide is a strong inducer of CYP3A4 and moderate inducer of CYP2C19 and CYP2C9 [3, 4].

For enzalutamide, no exposure–response relationship was identified for overall survival when dosed at 160 mg, and this is expected since the interpatient variability in enzalutamide and N-desmethylenzalutamide exposure (AUC, $C_{\min}$, and $C_{\max}$) is low (< 30%). In a retrospective analysis, efficacy was similar in the four different exposure quartiles based on steady-state C_{trough} [5]. This may imply that patients in the lower quartile are treated equally effectively. Therapeutic drug monitoring (TDM) might therefore be useful for patients with unexpected toxicity, inefficacy, or drug interactions. The earlier described LC-MS/MS assay for the quantification of enzalutamide and its metabolites in human plasma uses liquid–liquid extraction as the sample pre-treatment method [6]. In the current assay, the sample pre-treatment was simplified by using protein precipitation. The bioanalytical method has been developed to support clinical studies, as well as to support patient care in the case of unexpected toxicity, inefficacy, or expected drug interactions. We describe the successfully developed and validated assay.

2. Materials and Methods

2.1 Chemicals and reagents

Enzalutamide, N-desmethylenzalutamide, and the deuterated stable isotope-labeled D₆-enzalutamide were obtained from Alsachim (Illkirch, France); dimethyl-sulfoxide (DMSO, Seccosolv) and acetonitrile (ACN) (Lichrosolv) were purchased from Merck (Darmstadt, Germany). Formic acid was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). High-purity Milli-Q water was produced in the laboratory using a MilliQ Gradient water purification system (Millipore, Amsterdam, The Netherlands). Cellstar tubes (5 and 50 mL) were purchased from Greiner Bio-one (Alphen a/d Rijn, The Netherlands). Polypropylene

screw vials and safe lock tubes were purchased from Agilent (Amstelveen, The Netherlands) and Eppendorf (Nijmegen, The Netherlands), respectively. Ethylenediaminetetraacetic acid (EDTA) plasma was prepared from EDTA whole blood obtained from Sanquin (Amsterdam, The Netherlands).

2.2 Preparation of stock solutions, calibration standards, quality control samples, and internal standard solution

Two independent enzalutamide and N-desmethylenzalutamide stocks were used: one for the preparation of calibration standards and one for the quality control samples (QC). Stock solutions were prepared in DMSO at a nominal concentration of 1 mg/mL enzalutamide, N-desmethylenzalutamide, and D₆-enzalutamide (used as internal standard). For a calibration curve, the stock solutions of enzalutamide and N-desmethylenzalutamide were diluted with ACN to obtain concentration levels of 1000, 2500, 5000, 10000, 25000, 35000, and 50000 ng/mL. Before each run, the calibrators were diluted ten-fold with a 200 g/L human albumin solution (Albuman®/plasma) (90:10), to obtain a calibration curve at the following concentration levels: 100, 250, 500, 1000, 2500, and 5000 ng/mL. The QC stock solution was diluted with ACN to yield QC concentration levels of 1000, 15000, and 40000 ng/mL enzalutamide and N-desmethylenzalutamide, which were also subsequently diluted ten-fold with the albuman solution/plasma (90:10). The precipitation solution was prepared by adding 75 µL IS stock solution to 250 mL ACN.

2.3 Sample preparation

Protein precipitation was used as sample pre-treatment by adding 900 µL of precipitation solution to 100 µL of albumin solution diluted plasma sample. The mixture was immediately vortexed for 5 min and centrifuged for 5 min at 18620 *g*. An aliquot of 200 µL of supernatant was transferred to a polypropylene autosampler vial.

2.4 Chromatographic conditions

The LC-MS/MS system consisted of an Acquity H-Class UPLC system, coupled to a Xevo TQ-S Micro Tandem Mass Spectrometer (Waters, Wilford, MA, USA). Chromatographic separation was performed by injecting 2 µL supernatant onto a Acquity BEH C18 Column (2.1 × 50 mm, 1.7 µm particle size, Waters). Mobile phase A consisted of 0.1% formic acid in water (Milli-Q) and mobile phase B consisted of 0.1% formic acid in ACN. The following linear gradients were used at a flow rate of 0.5 mL/min: time scale (min – min) mobile phase A (%)/mobile phase B (%): 0–2.5 95/5 → 2.5–3.5 10/90 → 3.5–4.0 95/5. The column temperature was kept at 40°C and the autosampler temperature at 4 °C. The LC eluate was directed into a tandem quadrupole, atmospheric pressure ionization (API) mass spectrometer (TQ-S detector, Acquity, Waters, Wilford, MA, USA) equipped with an electrospray ionization (ESI) source.

2.5 Mass spectrometric conditions

The mass spectrometer was operated in the positive ion mode and configured in multiple reaction monitoring (MRM) mode for detection of enzalutamide (m/z 465 → 209), N-desmethylenzalutamide (m/z 451 → 195), and D₆-enzalutamide (m/z 471 → 215) (Table

1). For both enzalutamide and N-desmethylenzalutamide, the following optimized settings on the mass spectrometer were used: capillary voltage was 4.2 kV, cone voltage was 65 V, desolvation temperature was 500 °C, desolvation gas flow was 1000 L/hour. Collision energy was 25 V, dwell time was 0.05 s, and typical retention time for enzalutamide and D₆-enzalutamide was 2.5 min and for N-desmethylenzalutamide 2.4 min.

The data were acquired and processed using MasslynxTM Software (version 4.1, Waters, Etten-Leur, The Netherlands).

2.6 Quantification

Enzalutamide and N-desmethylenzalutamide were quantified in plasma by describing the relationship between the peak area ratio to the internal standard *versus* the nominal concentration. A quadratic curve with 1/x as weighing factor was used to quantify enzalutamide and N-desmethylenzalutamide that resulted in a better fit than a more simple linear relationship. The range of the calibration curve was chosen to cover the clinically relevant concentrations that are expected in patients treated with the recommended dose. The lower limit of quantification (LLOQ) and higher limit of quantification (HLOQ) were selected at the outer range of the concentration levels measured in patients treated with enzalutamide 160 mg once daily.

2.7 Validation procedures

The validation of the assay was performed according to the European Medicines Agency (EMA) guidelines for validation of bioanalytical assays including calibration curve, carry-over, precision, accuracy, selectivity, dilution integrity, matrix factor, recovery, and stability [7].

3. Results

3.1 Assay development

Because of the high plasma levels expected in patient samples, a human albumin solution (Albuman®) was used for an initial dilution step in order to prevent saturation of the MS signal and to reduce the amount of compound (enzalutamide and N-desmethylenzalutamide) needed for preparing calibration solutions. Furthermore, because of this dilution step, a virtually equal matrix was achieved for all standards, QCs, and samples.

3.2 Assay validation

3.2.1 Calibration curve—The calibration curve was prepared and analyzed by measuring seven non-zero calibration standards (CS) in three independent analytical runs. A quadratic curve with 1/x as the weighing factor proved to be the most optimal description of the relationship between the concentration and area ratio (Figure 1). At all concentration levels (500–50000 ng/mL), the deviations of the back-calculated concentrations were within ±15% of the nominal concentrations. This is in accordance to the EMA guidelines [7].

3.2.2. Carry-over—Carry-over was tested by injecting a blank sample after the highest calibration standard, and this was repeated five times. For both enzalutamide and N-

desmethylenzalutamide, no eluting peaks with areas of >20% of the LLOQ were observed. For the internal standard, no eluting peaks with areas of >5% of the normal response of D₆-enzalutamide were observed. Carry-over was therefore considered to be acceptable.

3.2.3. Precision, accuracy—The within-run and between-run precision and accuracy were determined. To test the within-run precision, five replicates of the QC samples at all concentration levels (QC LLOQ, QC low (QCL), QC medium (QCM), QC high (QCH), and QC HLOQ) were analyzed in one analytical run. To test the between-run precision, the same QC samples were analyzed in three analytical runs on three different days. For QC low, QCM, QCH and QC HLOQ, precision, expressed as coefficients of variation (CV) values, were below 5% for enzalutamide and 8% for N-desmethylenzalutamide. Accuracy, expressed as deviations from the nominal concentrations, was within 108% for both enzalutamide and N-desmethylenzalutamide, respectively. For QC LLOQ, the precision was below 5% for enzalutamide and within $\pm 10\%$ for N-desmethylenzalutamide and accuracy was within 109% and 116% for enzalutamide and N-desmethylenzalutamide, respectively. Therefore, the precision and accuracy were within the acceptance criteria of the EMA guidelines.

3.2.4. Selectivity—The selectivity of the assay was tested in six different batches of blank control plasma. The blank controls showed no peaks co-eluting with enzalutamide, N-desmethylenzalutamide, and D₆-enzalutamide. The maximum peak area was <2% of the signal of LLOQ of enzalutamide and <3% of LLOQ of N-desmethylenzalutamide. Based on these results, we concluded that the selectivity was sufficient and that the assay was suitable for the detection of the analytes in plasma.

3.2.5. Dilution integrity—To demonstrate dilution integrity, three replicate plasma samples of enzalutamide, N-desmethylenzalutamide at 1.5*HLOQ level (75000 ng/mL) were diluted two- and four-fold with blank plasma. The mean accuracy after two- and four-fold dilution, respectively, was 101% and 98% for enzalutamide and 107% and 103% for N-desmethylenzalutamide. The mean precision after two- and four-fold dilution, respectively, was 2.8% and 2.5% for enzalutamide and 5.9% and 2.2% for N-desmethylenzalutamide.

3.2.6. Matrix factor—The matrix factor (MF) was determined in six different plasma samples at QCL and QCH concentration levels. The matrix factor in albuman/plasma (90/10) for enzalutamide ranged from 0.99 to 1.03 and for N-desmethylenzalutamide from 1.3 to 1.4. The matrix factor of 1.4 indicated enhancement of the response due to matrix effects. The maximum CV of the IS-normalized MF calculated from the six different plasma samples for the low and the high concentration was, respectively, 1.0% and 0.9% for enzalutamide and 1.0% and 2.7% for N-desmethylenzalutamide, fulfilling the acceptance criteria of <15% [7]. The assay performance data are summarized in Table 2.

3.2.7. Recovery—The recovery, covering sample preparation, and stability, were determined at three concentration levels (1000, 15000, and 40000 ng/mL) in duplicate, for enzalutamide and N-desmethylenzalutamide and for the internal standard D₆-enzalutamide. In plasma, the mean recovery of enzalutamide was 100% with a CV of 2%; for N-desmethylenzalutamide, it was 141% with a CV of 2%; and for the internal standard, the

mean recovery was 102% with a CV of 2%. The high recovery of N-desmethylenzalutamide is likely to be caused by enhancement of the response due to matrix effect. Differences in recovery are corrected for by the internal standard used.

3.2.8. Stability—Enzalutamide and N-desmethylenzalutamide and the internal standard (D₆-enzalutamide) were stable for at least 11 months in stock solution at –40 °C.

The autosampler stability (at 2–8 °C) for enzalutamide and N-desmethylenzalutamide was demonstrated to deviate no more than 4% and 11% after 7 days. Freeze–thaw stability experiments did not demonstrate degradation of enzalutamide and N-desmethylenzalutamide over three freeze–thaw cycles. The long-term stability, as tested in QC samples, was at least 11 months at –40 °C. The short-term patient sample stability was demonstrated for enzalutamide and N-desmethylenzalutamide for at least 23 days in plasma at ambient temperature and at 4 °C. The whole blood patient sample stability was shown for 24 hours at ambient temperature. The long-term patient sample stability was demonstrated for enzalutamide and N-desmethylenzalutamide for 14 months at –40 °C. (Table 3).

3.2.9. External validation—For the external validation, duplicate sets of blinded QCs at the three concentration levels were exchanged with the University of Pittsburgh Cancer Institute where a validated enzalutamide assay is in use [8]. The nominal enzalutamide and N-desmethylenzalutamide concentrations of 15000 and 50000 ng/mL (QCM and QCH) were within the calibration curve of the assay used in Pittsburgh, and the results were $\pm 5.5\%$ of the actual concentration. The exchanged QCL was below the limit of detection of the assay used in Pittsburgh and could therefore not be interpreted.

4. Clinical applicability

To test the applicability of this assay, enzalutamide and N-desmethylenzalutamide concentrations were quantified in samples collected from a patient participating in a clinical trial who was treated with 160 mg enzalutamide once daily and had reached steady-state pharmacokinetics. Figures 2 and 3 show chromatograms and a pharmacokinetic curve of a patient in this trial. Patient demographic data and specimens were used in accordance with the principles of the Declaration of Helsinki. The protocol of this study was approved by the ethics committee of Medical ethical committee of Arnhem-Nijmegen, The Netherlands.

The applicability of this assay was investigated in samples of a patient treated with enzalutamide (160 mg once daily) due to clinical problems that were encountered in his treatment.

Patient A

A 57-year-old male patient with mCRPC started enzalutamide therapy in January 2015. From March 2015, he suffered long-term fatigue, malaise, and decreased appetite as well as joint pain. In July 2015, the treating physician decided, in consultation with the clinical pharmacologist, to decrease the dose to 120 mg and to measure plasma concentrations before and after dose reduction. Since no target concentration for efficacy has been identified, the population average concentration of enzalutamide and N-

desmethylenzalutamide was used [9]. The average concentrations were divided in quartiles (median Q1–Q4 [19.35–29.65 ug/mL]). No difference in overall survival was demonstrated between the quartiles. Plasma concentrations before dose reduction were within the third concentration quartile (Figure 4)[9]. Tiredness and especially complaints regarding malaise improved 1 month after dose reduction. The plasma concentrations were within the second quartile after dose reduction and were therefore adequate. The patient continued treatment with 120 mg enzalutamide until October 2016. In Figure 4, plasma concentrations and prostate-specific-antigen (PSA) levels are shown. PSA levels remained stable during therapy and plasma levels remained within the median quartiles reported by Gibbons et al[9].

5. Discussion

We have developed a new method to quantitate enzalutamide and its active metabolite N-desmethylenzalutamide in human plasma. Previously, three LC-MS/MS methods were published [6, 8, 10]. During development of our method, we expected that the high concentration of enzalutamide could cause saturation of the MS signal, and therefore we introduced a dilution step with albuman®/plasma (90:10) for calibrators, quality controls, and patient samples. All previously published methods used a different approach to overcome this challenge; the method of Bennett *et al.* did not explicitly describe the issue of MS signal saturation, and the low recovery described in this method resulting in a lower MS signal might explain that this issue was not encountered. Moreover, the internal standard that was used ($^{13}\text{CD}_3$ -enzalutamide) was labeled in the N-methyl function group. This group is susceptible to metabolism and chemical hydrolysis during sample preparation, potentially giving rise to the formation of the major metabolite N-desmethylenzalutamide[6]. Therefore, this internal standard is not the preferred isotopomer. The method of van Nuland *et al.* described the issue concerning saturation of the MS signal, and therefore diluted the patient samples before analysis. This is comparable to our approach. The main difference is the dilution medium albuman®/plasma (90:10) we used in our method, which is a more reproducible matrix over time compared to purchased human blank plasma[10]. Additionally, in our method, all samples underwent similar sample pretreatment (standards, quality controls, and patient samples), while van Nuland *et al.* only diluted the patient samples, which might introduce dilution integrity issues. The bioanalytical method of Kim *et al.* used an isotopolog MRM method whereby the signal was diluted by a factor 12 in order to prevent saturation of the MS signal and to increase the linear dynamic range of the method[8]. In conclusion, all previous publications aimed for a very sensitive method with a wide linear dynamic range while avoiding saturation of the MS signal. In our study we showed that enzalutamide and N-desmethylenzalutamide are stable in whole blood for 24 hours, and that patient plasma samples are stable at ambient temperature for 23 days. This information is of value for the practical aspects of collection and shipment of patient samples. Two of the previously published methods did not describe whole blood stability of patient samples, and Kim *et al.* did describe whole blood stability, but tested this for a much shorter period (4 hours)[6, 8, 10].

Enzalutamide is widely prescribed for mCRPC patients and is generally well tolerated. As dose-dependent fatigue is the most common reported adverse event [11, 12] we routinely measure enzalutamide in routine patient care as described above for our patient A.

6. Conclusion

A selective, sensitive, and practical bioanalytical method to quantitate enzalutamide and N-desmethylenzalutamide was successfully developed and validated according to the EMA guidelines. The method will be used to support pharmacokinetic studies and for routine patient care in cases where unexpected toxicity, inefficacy, and drug interactions are suspected.

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References

1. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. *N Engl J Med*. 2014; 371:424–433. [PubMed: 24881730]
2. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012; 367:1187–1197. [PubMed: 22894553]
3. EMA. [Accessed 28 February 2017] Xtandi: EPAR - public assessment report [web site]. 2016. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002639/WC500144996.pdf
4. FDA. US Food and Drug Administration. [Accessed 22-02-2016] Prescribing information Xtandi (enzalutamide) [web site]. 2015. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/203415s007lbl.pdf
5. FDA US Food and Drug Administration. Clinical pharmacology and biopharmaceutics review. Xtandi (Enzalutamide). 2012:1–83.
6. Bennett D, Gibbons JA, Mol R, et al. Validation of a method for quantifying enzalutamide and its major metabolites in human plasma by LC-MS/MS. *Bioanalysis*. 2014; 6:737–744. [PubMed: 24328824]
7. EMA. [Accessed 01-03-2017] Guideline on bioanalytical method validation [web site]. 2011. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf
8. Kim KP, Parise RA, Holleran JL, et al. Simultaneous quantitation of abiraterone, enzalutamide, N-desmethyl enzalutamide, and bicalutamide in human plasma by LC-MS/MS. *J Pharm Biomed Anal*. 2017; 138:197–205. [PubMed: 28219796]
9. Gibbons JA, Ouatas T, Krauwinkel W, et al. Clinical Pharmacokinetic Studies of Enzalutamide. *Clin Pharmacokinet*. 2015; 54:1043–1055. [PubMed: 25917876]
10. van Nuland M, Hillebrand MJX, Rosing H, et al. Development and Validation of an LC-MS/MS Method for the Simultaneous Quantification of Abiraterone, Enzalutamide, and Their Major Metabolites in Human Plasma. *Ther Drug Monit*. 2017; 39:243–251. [PubMed: 28490047]
11. Higano CS, Beer TM, Taplin ME, et al. Long-term Safety and Antitumor Activity in the Phase 1–2 Study of Enzalutamide in Pre- and Post-docetaxel Castration-Resistant Prostate Cancer. *Eur Urol*. 2015; 68:795–801. [PubMed: 25698064]
12. Scher HI, Beer TM, Higano CS, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1–2 study. *Lancet*. 2010; 375:1437–1446. [PubMed: 20398925]

Figure 1.
A typical calibration curve of enzalutamide (Range 500–50000 ng/mL).

Figure 2. Mass chromatograms of a patient sample; A: enzalutamide; B: N-desmethylenzalutamide; and C: D₆ enzalutamide. Figure 3: Pharmacokinetic curve of a patient treated with enzalutamide 160 mg once daily at steady state.

Figure 3. Pharmacokinetic curve of a patient treated with enzalutamide 160 mg once daily at steady state.

Figure 4.

Patient A: prostate specific antigen (PSA) levels and plasma concentrations of enzalutamide +N-desmethylenzalutamide during treatment. Q1–Q4 median enzalutamide + N-desmethylenzalutamide concentration levels from the analysis of Gibbons et al (n=704).

Table 1

Analytes with their selected mass transitions and proposed fragmentation pathways

Compound	Transition	Proposed fragmentation
Enzalutamide	465→209
N-desmethylenzalutamide	451→195

Table 2

Assay performance data of enzalutamide and N-desmethylenzalutamide in human plasma at validated quality control concentration levels

Nominal concentration (ng/mL)	Within-run Precision (%CV)	Within-run Accuracy (% deviation)	Between-run precision (%CV)	Between-run Accuracy (% deviation)
Enzalutamide				
500	3.9	108	5.0	102
1000	4.8	107	2.4	104
15000	1.6	105	3.5	101
40000	3.3	105	2.9	102
50000	2.0	101	0.5	101
N-desmethylenzalutamide				
500	3.5	116	9.9	104
1000	7.2	108	2.2	105
15000	2.6	104	3.2	104
40000	2.1	106	2.9	103
50000	5.0	103	1.6	100

Table 3

Stability of enzalutamide and N-desmethylenzalutamide

Nominal concentration (ng/mL)	Determined mean concentration of enzalutamide	Determined mean accuracy (% dev)	Nominal concentration of N-desmethylenzalutamide	Determined mean concentration of N-desmethylenzalutamide	Determined mean accuracy (% dev)
Stock solution (−40 °C, 11 months) 1.00 mg/mL	1.00	98.5	Stock solution (−40 °C, 11 months) 1.00 mg/mL	100	103.5
Quality control stability (−40 °C, 11 months)					
998.9	1032	103	1002	1002	100
14983.5	14838	99	15030	15418	103
39956	40358	101	40080	41696	104
Plasma stability after three freeze–thaw cycles (−40 °C/ambient temperature)					
1001	992	99.1	999	986	98.7
15015	14859	98.9	14985	15225	101.6
40040	39925	99.7	39960	39902	99.9
Quality control plasma stability (2–8 °C, 19 days)					
1001	1001	97.8	999	1002	98.6
15015	14901	98.7	14985	14485	96.7
40040	40054	98.1	39960	38350	94.9
Quality control plasma stability (Ambient temperature, 19 days)					
1001	969	94.7	999	1019	100.3
15015	14862	98.4	14985	14385	96
40040	42631	104.4	39960	40458	100.2
Autosampler stability (2–8 °C, 7 days)					
1001	1004	99.7	999	943.1	94.4
15015	15117	99.3	14985	15457.1	103.2
40040	41602	103.3	39960	44102.9	110.4

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Nominal concentration (ng/mL)	Determined mean concentration of enzalutamide	Determined mean accuracy (% dev)	Nominal concentration of N-desmethylenzalutamide	Determined mean concentration of N-desmethylenzalutamide
				Determined mean accuracy (% dev)
Long-term patient sample stability (−40 °C), (% of baseline measurement)				
Plasma, 14 months			101	107
Short-term patient sample stability (2–8 °C), (% of baseline measurement)				
Plasma, 23 days			97	98
Short-term patient sample stability (ambient temperature), (% of baseline measurement)				
Whole blood, 24 hours			105	104
Plasma, 23 days			91	98