

Estimation of Abiraterone in Human Plasma by Liquid Chromatography Tandem Mass Spectrometry

Srinivasa Reddy¹, Licto Thomas¹, Lokesh Ramanna¹, Arindam Mukhopadhyay^{1*}, Saral Thangam¹

¹Norwich Clinical Services Pvt. Ltd., BA & MW, Bangalore, Karnataka, India

ABSTRACT

A LCMS/MS method for the determination of abiraterone in human plasma was described. After extraction of sample from plasma by LLE method it was dried and reconstituted in mobile phase. 20 µL of sample was injected to a C₁₈ column and eluted with a mobile phase (2 mM Ammonium formate, pH 3.5: Acetonitrile: 30:70, v/v) at a flow rate of 1.2 mL/min. MRM transitions were monitored as m/z 350.3 → 156.1 (abiraterone) and m/z 354.3 → 160.1 (abiraterone D4). Sample concentrations were calculated by linear regression analysis using the analyst software 1.5.1. An excellent linear response was obtained over the concentration ranges 0.20 ng/mL to 79.50 ng/mL. The intra-day and inter-day precision were within 14.4%. The assay accuracy was 91.35–105.05%. Mean recovery was 60.20% (2.84%) for abiraterone. The limit of detection was 0.052 ng/mL. The stability issue of abiraterone in plasma was also addressed. This method can be used for bio-equivalence studies.

Keywords: abiraterone, prostate cancer, LLE, LCMS/MS, validation

INTRODUCTION

Prostate cancer is a slow growing, testosterone dependent cancer which affects approximately 1 out of 6 men in their lifetime. It causes significant morbidity and mortality in elderly males. Although it can be efficiently managed with hormonal therapy in the initial stages the cancer becomes resistant to conventional therapy as the duration increases and is termed as castration-resistant prostate cancer (CRPC)¹.

*Corresponding author: Dr. Arindam Mukhopadhyay, e-mail: arindam.mukhopadhyay@norwichclinical.com
(Received 26 September 2017, accepted 29 November 2017)

The backbone of first line treatment for metastatic prostate cancer is androgen deprivation therapy (ADT) which can be either medical or surgical². Androgen receptor signaling is crucial in the progression from primary to metastatic prostate cancer³. The enzyme, CYP17A1 (17 alpha-hydroxylase/C17, 20 lyase), catalyzes the androgen biosynthesis and is expressed at higher amount in testicular, adrenal, and prostatic tumor tissue. Abiraterone inhibits CYP17A1 in a selective and irreversible manner via covalent binding mechanism. More specifically, it inhibits the conversion of 17-hydroxyprogesterone to dehydroepiandrosterone (DHEA) by the enzyme CYP17A1 to lower serum levels of testosterone and other androgens⁴.

Abiraterone acetate is a pro-drug of its active metabolite, abiraterone. Abiraterone is poorly absorbed but abiraterone acetate is well and rapidly absorbed orally⁵. Abiraterone acetate is approved in the European Union and the US, in combination with prednisone or prednisolone, for the treatment of men with metastatic castration-resistant prostate cancer (CRPC)³.

Several chromatographic methods have been reported for determination of abiraterone in plasma⁶⁻¹¹. However, these methods had their own limitations in respect to the sample preparation, gradient elution, run time, etc. A liquid chromatographic method with fluorescence detection for estimation of abiraterone in plasma described by Tiphaine et al.⁶ involves both protein precipitation performed with acetonitrile followed by liquid-liquid extraction using diethyl ether for sample preparation. Moreover, the run time was quite long (11-min). The LCMS/MS method described by Martins et al.⁸ used SPE method for sample extraction and the linearity of this assay was in the range of 5 to 500 nM. Wani et al.⁹ used protein precipitation during sample extraction and linearity in this LCMS/MS method was in the range of 0.1–50ng/mL. The linearity range was extended from 0.20 to 201ng/mL in another LCMS/MS method described by Gurav et al.¹⁰. They used Phenacetin as an internal standard and protein precipitation method for sample extraction. In a recently published article, the abiraterone was estimated by LCMS/MS using a gradient method¹¹. In this method, sample extraction was done by protein precipitation and deuterated abiraterone was used as an internal standard.

We developed a new, sensitive and relatively simple LC–MS/MS method for estimation of abiraterone in human plasma. In this method, the stability issue of abiraterone in human plasma has also been addressed. This method is validated as per FDA regulations¹² and can be used for pharmacokinetic study.

METHODOLOGY

Materials

Abiraterone (purity: 99.72%) was purchased from Vivan Life Sciences, India. Abiraterone D4 (purity: 99.83%) used as an internal standard was also from Vivan Life Sciences, India.

Methanol (HPLC-grade), acetonitrile, ammonium formate, potassium fluoride, oxalic acid dihydrate and formic acid of highest purity grade were purchased locally. In this study Milli Q purified water (Millipore, Milford, MA) was used.

Plasma lots collected in house were used for the experiments.

Preparation of Analyte and Internal Standard Solutions

Stock solution of abiraterone (200 µg/ml) was prepared in methanol. This concentration was then corrected by taking into account its potency and actual amount weighed. The stock solution of abiraterone was then diluted together with 50% methanol in water to concentration ranges of 9.98 ng/ml to 4000 ng/ml.

Similarly, stock solution of abiraterone D4 (1000 µg/ml) prepared in methanol was diluted to 100 ng/ml using 50% methanol in water. The concentration of the stock solution was corrected as mentioned in case of analyte before using for dilution.

Preparation of Stability Reagent

10g of potassium fluoride was dissolved in 100 mL 5% (w/v) oxalic acid dihydrate. 100 µl of 10% (w/v) potassium fluoride in 5% w/v oxalic acid dihydrate was added to 900 µl of pooled sodium fluoride + potassium oxalate plasma and stored at -70°C.

Preparation of Calibration Standards

To prepare calibration curve standards, 20 µl of the diluted samples of abiraterone was added to 980 µl of potassium fluoride stabilized sodium fluoride + potassium oxalate plasma to obtain a concentration range about 0.20 ng/ml to 80 ng/ml. All these bulk spiked samples were stored at about -70°C in aliquot of 300 µl.

Preparation of Quality Control Samples

Stock solution of abiraterone were diluted with 50% methanol in water to obtain the concentration ranges of 10.12 ng/ml to 3000 ng/ml. 20 µl of each diluted solution was added into 980 µl of potassium fluoride stabilized sodium fluoride + potassium oxalate plasma to obtain final concentration range about 0.20 ng/ml to 60 ng/ml for abiraterone.

Yellow monochromatic light was used throughout the study because of photosensitivity of abiraterone³.

Sample Preparation

50 µl of internal standard mixture (abiraterone D4) was added to all RIA vials except blank. 200 µl of sample was then added to each labeled RIA vials. 100 µl of 0.1M Sodium dihydrogen orthophosphate dihydrate was added to respective RIA vials and mixed by vortex. 2 ml of TBME was then added to all vials, capped them and then placed on Vibramax at 2500 RPM for 10 mins. They were centrifuged at 4000 RPM for 5 mins at about 4°C. 1.6 ml of supernatant from each vial was transferred into fresh RIA vial and dried at 40°C in nitrogen evaporator. 0.3 ml of mobile phase was added and vortexed. The samples from each vial was transferred into a labeled HPLC vial and placed in the autosampler.

Chromatography

20 µL of sample was injected on a reversed phase column (BDS Hypersil C18, 100 × 4.6mm, 5µm). 2 mM Ammonium formate, pH 3.5 ± 0.2: Acetonitrile: 30:70, v/v was used as a mobile phase at a flow rate of 1.2 mL/min with splitter in Waters UPLC attached to API 4000 Mass spectrometer (Applied Biosystems, USA). The column was maintained at 40°C in the column oven. The run time was 4.0 minutes.

Mass Spectrometry

Electrospray ionization (ESI) interface operated in positive ionization mode was used for the multiple reaction monitoring (MRM). The operational conditions were optimized by infusing diluted stock solution of analyte and internal standard (Table 1).

Table 1. MS parameters optimized for analytes and internal standards

Analyte/IS	Declustering Potential (DP) (V)	Entrance Potential (EP) (V)	Collision Energy (CE) (V)	Collision Cell Exit Potential (CXP) (V)	Collision activated dissociation (CAD) (psi)	Dwell Time (ms)	Ion source voltage (V)	Curtain gas flow (CUR) (psi)
Abiraterone	90	10	65	15	8	400	5500	30
Abiraterone D4	90	10	65	15	8	400	5500	30

Source temperature was set at 500°C. Nebulizer gas (GS1) and auxiliary gas (GS2) flows were 45 and 55 psi, respectively. Quadrupoles Q1 and Q3 were set on unit resolution.

MRM transitions were monitored as m/z 350.3 \rightarrow 156.1 (ABR) and m/z 354.3 \rightarrow 160.1 (ABR D4).

Sample concentrations were calculated by linear regression analysis using the analyst software 1.5.1. Data was processed by peak area ratio. The concentration of unknown was calculated from the equation ($Y = mX + c$) using regression analysis of spiked plasma calibration standards with reciprocal of the square of the drug concentration ($1/X^2$).

RESULTS AND DISCUSSION

It was reported earlier that abiraterone is unstable in blood/plasma in the absence of fluoride^{14, 15}. During the study we also noticed a gradual decrease in abiraterone concentration in plasma with time during storage (data not shown). Hence to stabilize abiraterone in plasma we added a fluoride containing stability agent as mentioned under 'Materials and Methods'.

Method Development

Specific and effective sample clean-up procedures are required for sensitive and selective LC-MS/MS assays for determination of very low concentration levels of pharmaceutical targets present in biological samples. Three methods e.g. protein precipitation (PPT), liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are generally used for preparing biological specimen. Protein precipitation method using organic solvent is the simplest one but the chances of matrix effect prevail. SPE technique for sample extraction is good but with an added cost. LLE was our method of choice for abiraterone since the extraction efficiency for highly non-polar analytes is more. This technique was shown to be robust, provided clean samples and gave good and reproducible recoveries of both analyte and IS. The extraction recovery of analyte was determined by comparing peak areas from plasma samples ($n = 6$) spiked before extraction with those from aqueous samples. The mean recoveries across QC levels (with precision) were 60.2% (2.84%) for ARB and 67.3% for ABR D4 (IS).

To make the method simpler we used isocratic mobile phase for eluting the analyte and IS. The total run time was only 4 minutes. A short run time is ideally required for being considered in high throughput analysis. The retention times for abiraterone and IS were 2.87 min and 2.83 min, respectively.

Method Validation

FDA Guidelines for specificity, linearity, intra- and inter-day precision & accuracy, and stability were followed to validate this method¹³.

Selectivity

Selectivity of the method was evaluated in eight individual human plasma lots along with one lipemic and one hemolytic lot. No interference were observed at the retention times of analyte and internal standard when peak responses in blank lots were compared against the response of spiked LLOQ containing IS mixtures. Representative chromatograms in Figures 1 (blank plasma) and 2 (blank plasma spiked with analytes/IS) demonstrate the selectivity of the method. The minimum signal to noise ratio was 140.65 (more than 5 is acceptable).

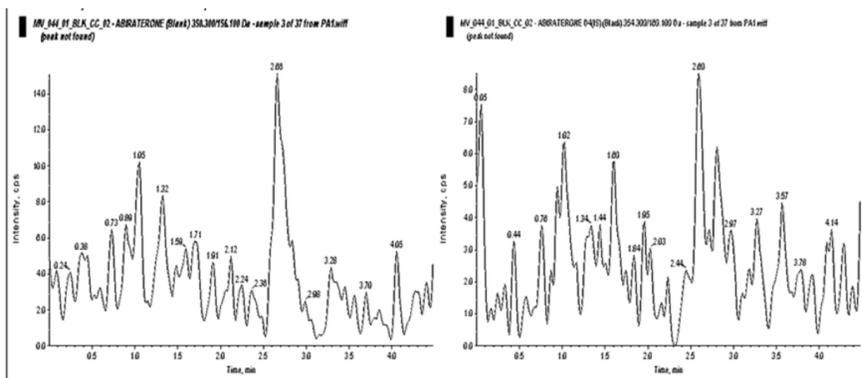


Figure 1. Chromatogram of extracted blank.

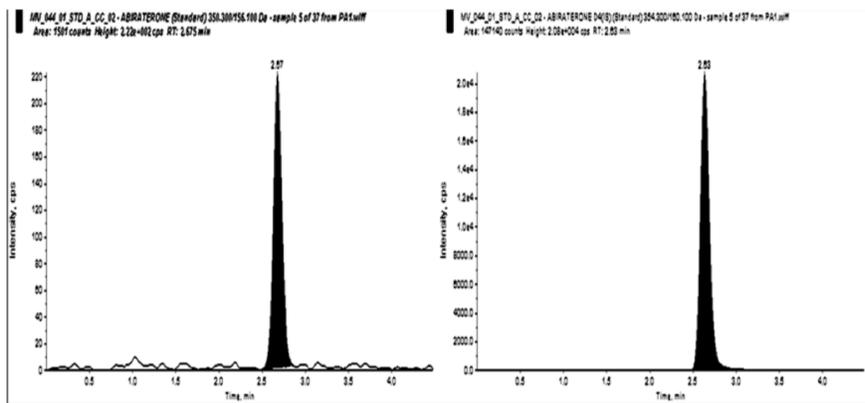


Figure 2. Chromatogram of an LLOQ calibration curve standard with IS.

Linearity and Sensitivity

Eight-point calibration curves were prepared with concentration ranging from 0.20 ng/mL to 79.5 ng/mL. The peak-area ratio (y) of analytes to internal standards was plotted against the nominal concentration ratio (x) of analyte to internal standard to determine the linearity of each calibration curve. Excellent linearity was achieved with correlation coefficients greater than 0.999 for all validation batches (Figure 3).

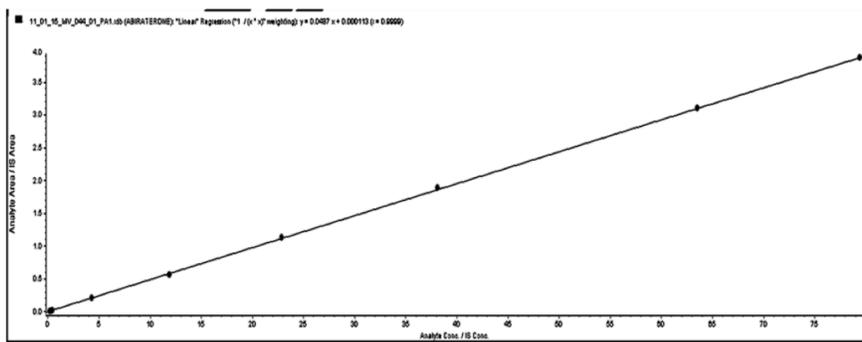


Figure 3. Calibration curve for Abiraterone.

The concentrations of calibration standards were back calculated to obtain the accuracy of each calibration point. The ranges of the calibration points' accuracy were 97.4–102%.

Six samples of LLOQ were processed and then injected along with a 'Precision and Accuracy' batch to assess the sensitivity of this method. Precision and accuracy for abiraterone at the LLOQs were 6.9% and 103.4% respectively. The LLOQ of the method is 0.20 ng/mL which is at par with the reported one [9]. Limit of detection was 0.05 ng/ml (signal to noise > 47.245). This indicates that this method is sensitive enough for a pharmacokinetic study. Moreover, a good signal-to-noise obtained at 0.05 ng/ml indicates that the LLOQ can be lowered further from 0.20 ng/mL or the volume of plasma can be decreased. This further widens the application of this method even to the pediatric patients where sample volume is always a challenge.

Precision and Accuracy

Precision and accuracy for intra- and inter-day batches for all analytes were determined by six replicate analyses of QC samples (n=6) at four different concentrations – Lower Limit Of Quantification (LLOQ), Low Quality Control (LQC), Middle Quality Control (MQC) and High Quality Control (HQC). The respective concentrations for abiraterone were 0.20ng/mL, 0.55ng/mL, 25.09ng/mL and 62.74ng/mL for LLOQ, LQC, MQC and HQC. Results of precision and accuracy were presented in Table 2. The intra-day and inter-day precision were within 14.4% for all analytes. The assay accuracy was 91.35–105.05% of the nominal values. The accuracy of the assay was expressed by [(mean observed concentration) / (spiked concentration)] x 100% and precision was evaluated by relative standard deviation (RSD).

Table 2. Intra-day and inter-day accuracy and precision for the determination of abiraterone in human plasma

Sample ID	LOQQC (Nominal Conc. 0.208 ng/ml)			LQC (Nominal Conc 0.552 ng/ml)			MQC (Nominal Conc 25.096 ng/ml)			HQC (Nominal Conc 62.740 ng/ml)		
	Mean calculated Conc (ng/ml)	Mean accuracy (%)	% CV	Mean calculated Conc (ng/ml)	Mean accuracy (%)	% CV	Mean calculated Conc (ng/ml)	Mean accuracy (%)	% CV	Calculated Conc (ng/ml)	Mean accuracy (%)	% CV
PA - 1	0.20	98.5	5.3	0.52	94.4	3.8	24.14	96.2	1.1	60.57	96.5	1.1
PA - 2	0.19	92.4	5.9	0.50	91.5	2.6	24.72	98.5	2.0	60.79	96.9	1.6
PA - 3	0.19	91.3	6.9	0.51	92.1	6.2	25.12	100.1	0.9	61.49	98.0	1.8
PA - 4	0.21	100.9	5.3	0.53	95.7	2.3	24.79	98.8	0.4	59.80	95.3	0.7
PA - 5	0.22	105.0	14.4	0.53	95.4	1.7	24.92	99.3	1.4	61.16	97.5	1.0
Inter-day	0.20	97.6	9.5	0.52	93.8	3.8	24.74	98.6	1.8	60.76	96.8	1.5

Matrix Effect

Blank plasma from eight different sources was used for evaluation of matrix effect. One hemolytic and one lipemic plasma were included in these eight lots. 200 µL of blank plasma from each lot was processed as mentioned in sample preparation. Aqueous solution of analyte either at LQC or HQC level and known concentration of internal standard were added to each of the processed samples. These samples were considered as post extracted samples (presence of matrix).

Similarly, the aqueous solution of analyte either at LQC or HQC level containing same concentration of IS as above was prepared with the mobile phase solvent and was considered as aqueous samples (absence of matrix). Six replicates of each aqueous sample were injected along with post extracted samples of LQC or HQC.

Analyte and IS area responses of each post extracted sample were compared with the mean analyte area and mean IS area responses of the aqueous sample respectively. Calculation of the matrix effect was done using the formula: Matrix effect (%) = $A_2 / A_1 \times 100$ (%), Where A_1 = response of aqueous concentrations and A_2 is response of post-extracted concentrations.

Average (n=6) matrix factors were 105.19% with a CV of 5.49% at LQC level and 99.02% with a CV of 1.08% at HQC level which are within the accepted limit (% CV ≤15) (Table 3).

Table 3. Matrix effect of abiraterone in human plasma.

Matrix ID	LQC analyte area in absence of matrix	LQC analyte area in presence of matrix	LQC matrix factor for analyte	HQC analyte area in absence of matrix	HQC analyte area in presence of matrix	HQC matrix factor for analyte
PL-536	4993	5499	109.24	597558	577892	98.20
PL-537	5055	5368	106.63	586896	587961	99.91
PL-538	4925	5582	110.89	580961	586284	99.63
PL-539	5065	5116	101.63	580969	576825	98.02
PL-540	5070	4721	93.78	592048	576077	97.89
PL-541	5096	5162	102.54	592488	577367	98.11
LPL-499	-	5580	110.85	-	587693	99.87
HPL-504	-	5336	106.00	-	591694	100.55
Average	5034.00	5295.50	105.19	588486.66	582724.12	99.02
SD	63.41	290.67	5.77	6732.96	6282.38	1.07
%CV	1.26	5.49	5.49	1.14	1.08	1.08

Dilution Integrity

Dilution integrity was evaluated after spiking interference free human plasma with 2 times of HQC concentration of abiraterone (i.e. $2 \times 80 = 160$ ng/mL). These spiked plasmas was diluted either 2 fold (2T) or 4 fold (4T) with interference free human plasma. These samples (Six replicates of each dilution) were processed and then analyzed against a set of freshly spiked calibration standards. The mean accuracy and precision were 100.78% and 1.96% for 2T and 98.08% and 3.90% for 4T.

Carry – over Effect

To avoid any carry – over of injected sample in subsequent runs the cleaning ability of mobile phase used for rinsing the injection needle and port was evaluated. The order of placing samples was: LLOQ of analyte, blank plasma, upper limit of quantitation (ULOQ) of analyte and blank plasma. No carry – over was observed during the experiment. Benoist et al.¹¹ encountered with the carry – over in their recently published method which had been eliminated by using one more solvent as the mobile phase in their gradient method. However, our method is relatively simple where only isocratic elution was done without any carry – over problem.

Stability

Stability evaluations were performed in both aqueous and matrix based samples. For aqueous solution, both short-term and long-term stabilities were determined as follows:

a) Stability in aqueous solution

i) Short – Term stock solution stability (STSS)

Stock solutions of both analyte and IS were prepared separately and kept at 25°C for 26 h and named as stability stock. MQC concentration of analyte was prepared from the stability stock solution and stored at 25°C for 24h and marked as stability working solution. Just before injection, stock analyte solution and stock IS were diluted to MQC concentration of analyte and intended concentration of IS. Six replicate injections were given for MQC sample (both stock and working solutions) and diluted IS solutions. No significant differences were noticed when these results were compared with those obtained from the freshly prepared MQC solution indicating that analyte were stable at 25°C (Table 4). For IS, stability was 96.6% after 26h (data not shown). Accepted criteria for the ratio of mean response for stability samples should be between 90-110%.

ii) Long term stock solution stability (LTSS)

Aqueous MQC sample of analyte and solution of internal standard with known concentration were prepared by dilution from respective stock solutions and stored at 2-8 °C for 36 days. Mean area response of MQC of stored stock solution was then compared against MQC from freshly prepared stock solution. Similarly, mean area response for internal standard was also compared. Mean percent stabilities for abiraterone was 102.39 and 96.38 for abiraterone D4 (data not shown). These were well within accepted limit (90 – 110%). This indicated the stability of analyte and internal standard solutions for 36 days at 2-8 °C (Table 4).

Table 4. Short and long –term stability of Abiraterone aqueous solution

Short-term stability of stock solution at 25°C for 26h			Short-term stability of working solution at 25°C for 24h			Long-term stability of stock solution at 2-8°C for 36 days		
Average area of stock solution	Average area of fresh stock solution	% Stability	Average area of working solution	Average area of fresh working solution	% Stability	Average area of stock solution	Average area of fresh stock solution	% Stability
275952.3	284118.8	94.30	283617.2	284118.8	96.92	286243.8	278410.0	102.39

b) Stability in human plasma

i) Bench-top stability

Six aliquots of each analyte in human plasma (at LQC and HQC concentrations) from the -70°C were allowed to thaw unassisted at room temperature (25°C) for 6 h and processed along with a set of freshly prepared calibration standards as well as LQC and HQC samples. The stability for LQC and HQC samples were 104.74% and 100.96% respectively.

ii) Freeze thaw stability

After 4 freeze thaw cycles, the stability of abiraterone were 100.6% for LQC and 99.4% for HQC.

iii) In-injector stability

The stability for LQC and HQC samples kept in auto-sampler at 10°C for 46 h were 96.08 % and 101.05% respectively.

iv) Wet extract stability

The stability of abiraterone after 3 h at 25°C was 101.68% for LQC and 100.86% for HQC. As per FDA, accepted range for all the stability studies mentioned above is that the mean concentration for stability samples should be 85-115% of the mean concentration of freshly prepared samples. Thus, all the analytes were stable during the analysis process. Results of stability studies were provided in Table 5.

Table 5. Stability studies of Abiraterone in plasma

Parameters	Bench-top stability for 6h		Freeze-thaw stability after 4 cycles		In-injector stability for 46h		Wet extract stability for 3h	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
Nominal concentration (ng/ml)	0.55	62.74	0.55	62.74	0.55	62.74	0.55	62.74
Mean Calculated (ng/ml) gg(ngconcentration (ng/ml)	0.53	58.49	0.55	60.14	0.49	58.55	0.51	58.44
SD	0.02	0.30	0.02	0.58	0.01	0.91	0.01	0.84
%CV	4.75	0.52	3.26	0.96	2.24	1.56	2.46	1.43
% Stability	104.74	100.96	100.60	99.40	96.08	101.05	101.68	100.86

Extended precision and accuracy run

One set of CC and 50 sets of LQC and HQC as a batch (total 110 samples) were processed and then analyzed. Results of precision and accuracy were presented in Table 6. The precisions were 2.82% for LQC and 1.27% for HQC. The accuracies were 96.68% for LQC and 94.62% for HQC.

Table 6. Extended precision and accuracy of abiraterone

LQC				HQC			
Nominal conc. (ng/mL)	Mean calculated conc. (ng/mL)	Accuracy (%)	% CV	Nominal conc. (ng/mL)	Mean calculated conc. (ng/mL)	Accuracy (%)	% CV
0.55	0.53	96.68	2.82	62.74	59.36	94.62	1.27

This LC–MS/MS method for determination of abiraterone in human plasma is relatively simple, fast, sensitive and specific. It utilizes liquid-liquid extraction technique for this relatively non-polar molecule which offers consistent and reproducible recoveries with insignificant interference and matrix effect. Moreover, this method does not have any carry – over problem as reported earlier¹¹. Stability issue of abiraterone in plasma has been resolved by using potassium fluoride^{14, 15}. FDA guideline¹² mentions that internal standard should preferably be identical to the analyte and hence this method was developed using deuterated abiraterone. This method is also validated as per this guideline¹². By using 200 µL plasma samples, the lower limits of quantification were achieved. It demonstrates that the method is reproducible, sensitive and suitable for high-throughput sample analysis. Moreover, as the sensitivity of this method is quite high this can be used even for analysis of pediatric samples where sample volume is always a challenge. This method has the potential to be useful for bio-equivalence studies and routine therapeutic drug monitoring.

COMPETING INTERESTS

All authors hereby declare that no competing of interests is associated with the publication of this manuscript.

AUTHORS' CONTRIBUTIONS

All authors have equal contribution in this work.

ACKNOWLEDGEMENT

The authors thank the management of Norwich Clinical Services for providing the opportunity to complete the project.

REFERENCES

1. Mehta, K. D.; Barkate, H. Abiraterone: A Review of its Pharmacokinetic, Pharmacodynamic Profile and Clinical Efficacy. *Urol. Nephrol. Open Access J.* **2016**, *3*, 00079. DOI: 10.15406/unoaj.2016.03.00079.
2. Schroder, F.; Crawford, E. D.; Axcrone, K.; Payne, H.; Keane, T. E. Androgen deprivation therapy: past, present and future. *BJU Int.* **2012**, *109*, 1–12.
3. Hoy, S. M. Abiraterone Acetate: A Review of Its Use in Patients with Metastatic Castration-Resistant Prostate Cancer. *Drugs.* **2013**, *73*, 2077–2091.
4. <http://www.drugbank.ca/drugs/DB05812>.
5. Pushpa, L. E.; Syeda, B. Y.; Navya, S. O.; Juveriya N.; Anusha, M.; Mani, S. G.; Sameul, S. S. A review on analytical methods for estimation of abiraterone acetate. *WJPMR.* **2016**, *2*, 23-25.
6. Tiphaine, B.; Gaëlle, N.; Olivier, H.; Audrey, T. S.; Michel, V. A. HPLC-fluorescence method

for the quantification of abiraterone in plasma from patients with metastatic castration-resistant prostate cancer. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.* **2015**, 989, 86-90.

7. Gong, A.; Zhu, X. β -cyclodextrin sensitized spectrofluorimetry for the determination of abiraterone acetate and abiraterone. *J. Fluor.* **2013**, 23, 1279-1286.

8. Martins, V.; Asad, Y.; Wilsher, N.; Raynaud, F. A validated liquid chromatographic–tandem mass spectroscopy method for the quantification of abiraterone acetate and abiraterone in human plasma. *J. Chromatogr. B.* **2006**, 843, 262–267.

9. Wani, T. A. Highly sensitive ultraperformance liquid chromatography–tandem mass spectrometry method for the determination of abiraterone in human plasma. *Anal. Methods.* **2013**, 5, 3693-3699.

10. Gurav, S. D.; Punde, R.; Farooqui, J.; Zainuddin, M.; Rajagopal, S.; Mullangi, R. Development and validation of a highly sensitive method for the determination of abiraterone in rat and human plasma by LC-MS/MSESI: application to a pharmacokinetic study. *Biomed. Chromatogr.* **2012**, 26, 761-768.

11. Benoist, G. E.; Meulen, E. V. D.; Lubberman, F. J. E.; Gerritsen, W. R.; Smilde, T. J.; Schalken, J. A.; Beumer, J. H.; Burger, D. M.; van Erp, N. P. Analytical challenges in quantifying abiraterone with LC–MS/ MS in human plasma. *Biomed. Chromatogr.* **2017**, e3986.

12. <https://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf>

13. Khedra, A.; Darwishb, I.; Bameana, F. Analysis of abiraterone stress degradation behavior using liquid chromatography coupled to ultraviolet detection and electrospray ionization mass spectrometry. *J. Pharm. Biomed. Anal.* **2013**, 74, 77– 82.

14. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202379Orig1s000ClinPharmR.pdf

15. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202379Orig1s000PharmR.pdf