Evaluating bioanalytical capabilities of paper spray ionization for abiraterone drug quantification in patient plasma

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Abbreviations: AI, ambient ionization; MS, mass spectrometer; PSI, paper spray ionization; LC, liquid chromatography; TDM, therapeutic drug monitoring; mCRPC, metastatic, castration-resistant prostate cancer; CC, calibration curve; QC, quality control; IS, internal standard; PRM, parallel reaction monitoring; LOD, limit of detection, LLOQ, lower limit of quantification; AUC, area under the curve;

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Abstract

Paper spray ionization (PSI) is a direct, fast and low-cost ambient ionization technique which may have clinical utility for qualitative and quantitative analysis of therapeutic drugs and metabolites from patient specimens. We developed and validated a PSI-mass spectrometry (PSI-MS/MS) method according to the US-FDA guidelines for bioanalytical studies to measure the prostate cancer drug abiraterone directly from patient plasma. The established linearity range was 3.1 – 156.8 ng/mL with a precision (%CV) and an accuracy (%) range of 0.5 – 10.7 and 93.5 – 103.2, respectively. The mean internal standard normalized matrix factor for abiraterone was just below 1 with highest %CV of 10.2 at the low level quality control. In benchmarking the performance of this assay against a published LC-MS/MS assay we showed they were mostly equivalent, with the exception of accuracy with clinical samples. We found the quantitative values observed for abiraterone measured directly from patient plasma using PSI-MS/MS showed positive bias. Upon investigation we concluded the increased values were due to summed quantitation of isomeric abiraterone conjugates and metabolites which are separable by LC-MS/MS, but not with the current PSI-MS/MS configuration. Despite demonstrating the utility of PSI-MS/MS for rapid bioanalysis, this study also highlighted a limitation encountered with the direct analysis of abiraterone in clinical samples.

1. Introduction

Ambient ionization (AI) is defined as the generation of ions from samples in their native environmental conditions without any prior sample preparation, outside the vacuum of the mass spectrometer (MS) [1, 2]. AI offers advantages over other ionization techniques as it is direct, requires no or minimal sample processing and is compatible with high throughput analysis [3-6]. AI is also compatible with the analysis of a wide range of substances ranging from polar non-volatiles to non-polar volatile analytes in diverse matrices [4]. AI-MS was first reported in 1960 with the development of desorption electrospray ionization and direct analysis in real-time ionization of liquid samples [3, 7-9]. Since then, more than 30 AI-based techniques have been developed differing in their sampling and ionization processes [4, 10, 11].

Paper spray ionization (PSI) is an AI technique and was first reported in 2010 for the direct analysis of chemical mixtures (methylene blue and methyl violet dyes) [12] using MS. PSI works on a simple principle in which a small volume of liquid sample (approx. 10 µL) is spotted on a suitable paper substrate with the aid of a micropipette and allowed to dry. After drying, spray solvent is dispensed on the paper the substrate which is subjected to a high voltage low ampere current which facilitates the movement of ions into the MS [13-15]. PSI-MS/MS has been described for a wide variety of applications in which samples are analyzed directly, eliminating the need for extensive sample preparation steps or chromatographic separation. PSI has been reported for the analysis of various small and large molecule classes and offers the potential to be developed for pharmacokinetic drug quantitative studies [16]. PSI has been tested for the direct analysis of numerous biological samples including urine [13], dried blood spots [16], whole blood [12] and tissue samples [17],

each of which are of great interest for clinical applications. PSI-MS/MS has been employed previously for the analysis of imatinib [12, 18], and fifteen other therapeutic drugs for monitoring purposes [19]. PSI-MS/MS has also been reported for the quantification of various drugs of abuse [20] in whole blood, opioids in urine and analgesic slurry samples [21, 22] and other xenobiotics [23]. Previous PSI-MS/MS studies highlight the importance of the assessment of matrix effect, ionization suppression and recovery of the quantitative assay during direct biofluid analysis [16, 24]. One study also investigated the drug-protein interaction by employing propranolol and atenolol, dissimilar drugs to study the protein binding characteristics and reported negligible effect on analyte detection due to protein binding [16]. A study evaluating PSI for tacrolimus drug monitoring cross validated the results with an immunoassay and a conventional liquid chromatography LC-MS/MS method. They reported similar quantitative performance between the assays with rapid turnaround time for the PSI assay [25]. PSI-MS/MS has also been employed for the analysis of biofluids and different classes of biomolecules especially for the study of amyloidogenic peptides, soluble intact proteins and N-glycans [26].

The rationale of this study was to evaluate the potential of utilizing the PSI-MS/MS technique for therapeutic drug monitoring (TDM) in a clinical oncology context. TDM has evolved as a crucial patient management tool which facilitates optimizing individual dosage by monitoring drug concentration levels in patients aimed at maintaining the target therapeutic range [27, 28]. Despite being able to provide pivotal information about patient's response to therapy and benefiting clinical research, the scope of TDM is still largely limited. Some of the underlying causes behind it is the number of drug assays available are limited and the insufficiency of rapid and short turnaround time assays that can be

performed with minimal guidance to keep costs low [29, 30]. As a proof of concept, in this study we used PSI-MS/MS to measure steady-state plasma trough levels of the prostate cancer drug abiraterone [31] on a cohort of patient samples. To facilitate this evaluation we carried out a complete bioanalytical validation of the PSI-MS/MS method as per the US-FDA guidelines [32] to assess the reproducibility, precision and accuracy of the PSI-MS/MS technique and compared performance with a conventional LC-MS/MS assay recently developed and validated using a high-resolution MS instrument in our laboratory [33] . To the best of our knowledge, this is the first study investigating the PSI-MS/MS technique for quantification of an anti-cancer drug in patient plasma samples using a fully validated assay.

2. Materials and Methods

2.1. Chemicals and reagents

Abiraterone (purity 98.0%) and the internal standard (IS) abiraterone-d4 (purity 99.5%), were obtained from Tokyo Chemical Industry Co. LTD. (Tokyo, Japan) and VIVAN Life Sciences Pvt. Limited (Mumbai, India), respectively. Methanol used for spray solvent and extraction buffer was of LiChrosolv® gradient grade and purchased from Merck (Darmstadt, Germany). Glacial acetic acid (Ph. Eur. grade) used as a buffering agent was bought from VWR BDH Chemicals (Leuven, Belgium). Human K_2EDTA plasma was obtained from the Australian Red Cross Blood Service, Sydney.

2.2. Paper spray setup

PSI was conducted using a Prosolia Velox 360™ PaperSpray™ source (Zionsville, IN). The unit comprised of a stacker to hold PaperSpray™ cartridges, a carousel to move the

cartridges to the MS inlet, a binary pump to dispense spray solvent and extraction buffer solution, and a high voltage contact to supply the required voltage for ionization. Spray solvent composition and volume, sample volume and spray voltage were optimized to attain an optimum response for abiraterone. A fixed volume of sample (6 µL) was spotted onto the sample loading zone of the paper cartridge (refer Fig. S1) using a micropipette and the cartridge was left for drying for 1 hour under ambient conditions. As a precautionary measure, all the samples were vortexed immediately prior to spotting them on the paper cartridges to sustain their homogeneity. Paper cartridges were loaded into the stacker then wetted sequentially just prior to analysis with extraction buffer consisting of 10 µL of methanol (99.9%) containing acetic acid (0.1%) *v/v*. The extraction buffer was dispensed by pump A directly onto the sample in the sample loading zone. 100 µL of spray solvent (composition same as extraction buffer) was dispensed by pump B to the paper cartridges into the solvent loading zone. The spray solvent help facilitates the movement of analyte of interest into the MS inlet for analysis. Samples were analyzed for 1 minute by applying a high voltage of 4.5 kV to form the spray, which was introduced to the MS for analysis.

2.3. Mass spectrometer setup

The PSI source was coupled to the Q Exactive™ Plus mass spectrometer (Thermo Scientific™, San Jose, USA). Samples were analyzed by parallel reaction monitoring (PRM) under positive polarity conditions at 70,000 resolution (at 200 *m/z*). The compound and source parameter settings used for analysis were: spray voltage 4.5 kV, capillary temperature 350°C, S-lens RF level 50, auto gain control 2e5 with a maximum injection time of 250 ms. The chromatographic full width half maximum (FWHM) was set to 30

seconds. Quantification of abiraterone and IS was based on the fragmentation of precursor ions 350.2478 *m/z* (abiraterone) and 354.2730 *m/z* (IS) to 156.0808 *m/z* and 160.1056 *m/z* product ion respectively. The normalized collision energy applied for fragmentation of the precursor ion into product ion was 90. The Velox 360™ paper spray source was mounted to the mass spectrometer using a flange and guide rails and was operated using Velox control software (Prosolia Zionsville, IN). As a precautionary measure, the ion transfer tube of the MS was changed after every 50 runs of the plasma samples to maintain sensitivity. Quantitative analysis of PSI-MS/MS data was performed using TraceFinder (version 4.1) software.

2.4. Calibration curve and quality control sample preparation

The standard stock for abiraterone and IS were dissolved in methanol and calculated as 0.98 mg/mL for abiraterone and 0.1 mg/mL for IS after accounting for impurities. These stock solutions were further serially diluted in methanol to prepare working stock dilutions for abiraterone and IS. For consistency, a 2% *v/v* spiking of the stock dilution in plasma was done to yield the final concentration required in the calibration curve (CC) and quality control (QC) samples. As there was no sample processing involved in the PSI method the IS was spiked (2% *v/v*) into the plasma to yield a final concentration of 50 ng/mL. The final concentration achieved in the IS spiked human plasma for eight non-zero CC standards of the entire linearity range for abiraterone was 3.1, 6.2, 12.3, 24.6, 49.2, 70.3, 100.4 and 156.8 ng/mL. The QC samples were also prepared similarly at four different concentration levels. The final concentrations for QC samples were 3.1 ng/mL for the lower limit of quantitation QC (LLOQ QC), 6.9 ng/mL for low-level QC (LQC), 68.6 ng/mL for mid-level QC (MQC) and 137.2 ng/mL for high-level QC (HQC). CC, QC and patient

samples were all kept on ice during analysis considering the stability of abiraterone in plasma which was established previously in our lab [33].

2.5. Analytical validation of the method

2.5.1. Selectivity and sensitivity

Selectivity of the method was evaluated to examine the ability of the method to differentiate between the analytes of interest from the endogenous compounds present in the matrix. This was assessed in different lots of controlled human plasma that was used for preparing CC and QC samples. Six paper cartridges each were spotted with blank human plasma and abiraterone spiked human plasma at LLOQ level. The area response of the background interference in blank plasma was compared with the LLOQ spiked plasma samples and the response area of blank plasma ≤ 20% to that of the LLOQ spiked plasma sample was accepted for validating selectivity. For sensitivity experiments, six paper cartridges spotted with spiked plasma at LLOQ level were analyzed consecutively along with the established linearity. Acceptance criteria of ≤ 20% for the precision and accuracy of the samples was employed for establishing sensitivity.

2.5.2. Linearity, LLOQ and LOD

The linearity comprising of eight points of CC standards was established for abiraterone by plotting the peak area response ratio (response area of each analyte to response area of IS) against the nominal concentrations of the standards. The best fit for the linearity was obtained by applying a 1/x weighting factor to the plot. The CC standards were run in triplicate for analyzing the precision and accuracy in control human plasma. The acceptance criteria for linear regression (r^2) of calibration curves were set at value 0.98

or above. The back-calculated standard concentrations for each CC standard were set at ±15% variation except for LLOQ which was ±20% of the nominal concentration [32]. Due to the inherent nature of the PSI technique, the signal to noise ratio in the blank samples fluctuates when compared to LLOQ. To avoid reporting the false positive result caused due to this fluctuation we first estimated the limit of blank (LOB). The LOB was estimated by measuring replicates of the blank sample and calculating their mean. This LOB_{mean} value was used as an initial point to determine LLOQ and limit of detection (LOD) [34]. The LLOQ and LOD were determined as a reproducible signal of the intensity of the analyte ten and five times respectively that of LOB_{mean}.

2.5.3. Precision and accuracy

To evaluate the precision and accuracy of the method, three batches consisting of six replicates of each level of QC samples were analyzed along with CC standards. 6 µL of CC and QC samples (control human plasma spiked with required CC and QC concentrations) was spotted on the paper cartridges and left for drying for one hour under ambient conditions prior to analysis. For intra batch precision and accuracy analysis, six replicates at each level of QC concentration within the batch were considered, whereas for inter-batch analysis replicates at each QC level concentration from all three batches were analyzed. The acceptance criteria for each back calculated QC concentration was 85 – 115% accuracy compared to the nominal concentration except for LLOQ QC, which was 80 – 120%. The % coefficient of variation (%CV) was ± 20% for LLOQ QC samples and ± 15% for the rest of the QC samples.

2.5.4. Matrix factor and matrix effect

This evaluation assesses the possible ion suppression or enhancement caused to the analyte ions by the matrix constituents. Matrix factor and matrix effect was investigated for both analyte and IS at LQC and HQC levels of concentration for abiraterone. Blank plasma from six different pooled plasma lots was processed in duplicate. These samples were then reconstituted with aqueous LQC and HQC concentrations. The reconstituted samples were run along with the aqueous LQC and HQC samples used for reconstituting the blank plasma samples. Peak area response of abiraterone was compared between the blank plasma samples with the aqueous samples to calculate the matrix factor and matrix effect. Calculation of matrix factor and matrix effect was performed as such:

Matrix factor = Response area of the analyte in the presence of matrix ions/response area of the analyte in the absence of matrix ions

% Matrix effect = (1 – mean of matrix factor) * 100

IS normalized matrix factor = Analyte matrix factor/ IS matrix factor

2.6. Clinical validation in patient plasma samples

The validated PSI method was employed to quantify steady-state plasma trough levels of abiraterone in 21 mCRPC patient samples from two different time points. Patients provided informed consent and the study was approved by Macquarie University Human Research Ethics Committee (HREC, Medical Sciences Ref No. 5201600267). This study was for research purposes only, and did not inform clinical treatment decisions.

3. Results and discussion

3.1. PSI optimized conditions

The PSI conditions were optimized to develop a robust and reproducible assay for the analysis of abiraterone in human plasma, with performance assessed using established bioanalytical guidelines. The parameters optimized included spray voltage, spray solvent composition, the modifier used in the spray solvent, the optimum volume of plasma sample for PSI analysis and appropriate dispensing volume and rate of the spray solvent.

Spray voltage of the MS was optimized to achieve high signal intensity for both abiraterone and IS. Organic samples spiked with analytes were spotted on the paper cartridge and the signal intensity was analyzed after applying different voltages in the range of 3.5 kV – 5.0 kV for a period of 30 seconds (Fig. 1). The minimum voltage required to maintain a stable spray and high analyte signal was 4.5 kV. Increasing voltage above 4.5 kV provided no improvements in signal intensity.

Analyte signal intensity was tested using several organic spray solvents and modifier combinations including acetonitrile and methanol solutions with either formic acid (0.1%) or acetic acid (0.1%) Acetonitrile with acetic acid had the highest response but the signal to noise ratio of the blank samples compared to LLOQ showed high variance. Methanol (99.9%) with acetic acid (0.1%) provided adequate signal intensity and more consistent signal to noise ratio, so was chosen as the spray solvent (refer Fig. S2). Methanol with acetic acid (99.9:0.1% *v/v*) had the highest solubility of abiraterone and IS, so it was also used as an extraction solution directly dispensed on the paper cartridge. The reason for optimizing the rate and dispense volume of extraction solution and spray solvent was to extract the drug from the paper substrate and maintain a stable spray for each sample analysis. The volume of spray solvent and extraction solution dispensed was optimized to 100 µL and 10 µL respectively. The spray solvent was dispensed from pump B of the

PSI unit as 10 µL aliquot volume each with no time delay between aliquot dispense into the solvent reservoir provided in the cartridges; whereas the buffer was dispensed from pump A as a single 10 µL volume directly on the paper substrate spotted with the samples. Refer Fig. S1 for the details on paper cartridge used for analysis and its various sections.

Varying volumes of plasma samples were analyzed at LLOQ concentration. They were spotted on the paper cartridges ranging from 2 – 10 µL to evaluate the optimum volume required for analysis. A significant increase in the signal intensity was observed on increasing the volume from 2 to 6 µL. Increasing the volume above 6 µL did not result in a notable rise in the signal intensity. The 10 µL volume sample was found to be unsuitable as it was beyond the holding capacity of the paper substrate. Therefore, 6 µL sample volume was chosen for further analysis (refer Fig. S3).

3.2. Mass spectrometry

Compared to LC separation, PSI showed higher background noise so analysis using a higher resolving MS was beneficial for analysis. The analysis was carried out in PRM mode at 70,000 resolution (200 *m/z*) setting which was the minimum requirement for isolating the peak of abiraterone and IS from the background. The mass tolerance window employed for signal extraction was 5 ppm. A typical chronogram was obtained by extracting the product ion mass of abiraterone and IS with 5 ppm mass accuracy. This extracted ion chronogram was then integrated between 0.05 – 0.95 min to calculate the area under the curve (AUC) of the peak (Fig. 2).

3.3. Analytical Validation

3.3.1. Selectivity and sensitivity

For the evaluation of selectivity, the AUC of abiraterone in six different blank control plasma lots were compared with LLOQ spiked control plasma. The AUC of abiraterone in blank plasma was found to be <20% of the LLOQ spiked plasma (Table S1). For sensitivity, the six consecutive runs of LLOQ samples had a %CV of 3.1 and highest %bias of -17.1 from the nominal concentration. The results were consistent with the requirements for a bioanalytical method validation according to US-FDA guidelines.

3.3.2. Linearity, LLOQ and LOD

The range of linearity validated for the PSI technique was 3.1 ng/mL – 156.8 ng/mL. To validate the linearity, an 8-point non-zero CC was run in triplicate to assess the precision and accuracy of each standard in plasma (Table 1). The linear regression (r^2) value reported was 0.998. Fig. S4 illustrates the corresponding line equation and residual plot for the accuracy of each CC standard of linearity. The range of precision (%CV) and accuracy for the standards were 0.5 – 10.7 and 93.5 – 103.2 respectively. The LLOQ and LOD were calculated as ten and five times respectively to the AUC of the LOB_{mean}. The %CV of LLOQ reported was 10.5 whereas the mean accuracy was 93.5%. All the reported values were well within the acceptance criteria as per the US-FDA guidelines for bioanalytical methods. The values of LOD and LLOQ reported for the PSI-MS/MS assay were higher due to low sensitivity when compared to the LC-MS/MS assay. The findings were consistent with previous study results [16, 19, 25]. We had a different approach to calculate the LOD and LLOQ of the assay as compared to the conventional 3 and 5 times respectively to the noise in the blank sample. Ten times the value of LOB_{mean} for LLOQ was sufficient to achieve the required precision and accuracy of the assay with the least interference of the background. The achieved LLOQ was adequate to quantify

abiraterone in the patient plasma but was insufficiently sensitive to quantify an active metabolite, Δ(4)-abiraterone, which was possible using the LC-MS/MS assay [33].

3.3.3. Precision and accuracy

The precision and accuracy of the developed method met the acceptance criteria as per the guidelines. This requires the %CV of all the quality control samples (LQC, MQC and HQC) to be ≤15% for precision and between 85 – 115 % range for accuracy except for LLOQ QC which should be ≤20% for precision and between 80 – 120 % range for accuracy. The inter-day and intra-day precision and accuracy of all the three batches consisting of QC samples also met the acceptance criteria. The range of %CV (precision) and % accuracy reported was 1.4 – 15.5 and 84.7 – 115.5 for intra-day respectively, whereas 2.1 – 13.8 and 100.1 – 94.6 for inter-day. Table 2 summarizes the results from the three precision and accuracy batches.

3.3.4. Matrix factor and matrix effect

A potential benefit of the PSI-MS/MS technique is that it involves direct analysis of the samples without prior processing. As the samples are analyzed in their native matrices the impact of endogenous ions from the matrix on the analyte ions can be determined. Matrix effect observed for the PSI-MS/MS techniques was very high, above 90% at LQC and HQC level with high %CV. This confirms abiraterone ion suppression due to the matrix ions. A similar matrix effect was seen in the IS. The calculated IS normalized matrix factor was just below 1 at LQC and HQC levels with their %CV well within the acceptance range (Table S2). This indicates that while the matrix effect still exists, it affects both analyte and IS to the same extent so it has a minimal effect on quantification. The high

results of matrix effect indicate a 10-fold suppression in the signal intensity of the analyte. In addition to analyte signal suppression due to matrix ions, lower signal can also be partly due to lower recovery of the analyte from the paper substrate as reported in other drug quantitation studies using PSI [16, 24, 25].

3.4. Quantitative comparison between LC-MS/MS and PSI-MS/MS technique and its limitation

Having demonstrated that PSI-MS/MS can be used to establish a validated bioanalytical assay for abiraterone we then compared assay performance with a LC-MS/MS method we previously reported [33] (Table 3). The linearity of the PSI-MS/MS method was over a shorter dynamic range as compared to the LC-MS/MS method; however, the precision and accuracy of the CC and QC samples for the PSI method were well within the acceptance range prescribed in the US-FDA guidelines for bioanalytical methods. The PSI-MS/MS method was less sensitive compared to the LC-MS/MS method due to the combinational result of background interference, ionization suppression and lower recovery of analyte from the paper substrate. The PSI-MS/MS method had at least 5X faster sample runtime compared to the LC-MS/MS method (one minute and one hour sample drying compared to 5 minutes runtime and additional off-line sample processing for the LC-MS/MS method).

We next conducted a clinical validation of the PSI-MS/MS method to quantify abiraterone steady state plasma trough levels from 21 patients with mCRPC. The samples were quantified in triplicate against the validated linearity. The quantified values reported by the PSI-MS/MS method were approximately 1.5 – 11 fold higher when compared to the LC-MS/MS technique [33]. We investigated the likely cause of higher AUC values

measured using the PSI-MS/MS method and predict two reasons behind it. Under this investigation, we selected five patient samples and processed them along with the CC and QC samples as per the protocol used for sample processing in the LC-MS/MS method which involved acetonitrile precipitation. The processed patient plasma samples, CC and QC were then spotted on the paper cartridges for PSI-MS/MS. The abiraterone values observed in patient plasma from samples processed in this manner were 3 – 5 fold lower as compared to unprocessed patient samples which were directly analyzed by PSI-MS/MS method. The lowering of abiraterone values in processed plasma samples was consistent with the elimination of some water-soluble drug conjugates (including sulfates, N-oxide sulfates and acyl glucuronides) during sample processing. Radio-labelled tracing of abiraterone metabolism has reported 15 plasma metabolites, with abiraterone sulfate and N-oxide abiraterone sulfate accounting for ~85% of signal [35]. Moreover, six of the biotransformed metabolites yielded ions of 350 *m/z* following in-source breakdown to yield the protonated species of the parent drug; this has also been reported with other compounds analyzed by PSI-MS/MS [35]. It should be noted that previous studies using LC-MS/MS have reported isomeric abiraterone metabolites (e.g. 3-keto-5α-abiraterone and 3-keto-5β-abiraterone) [35, 36], and we also observed isomers in our study (Fig S5). Unfortunately, the unavailability of commerical reference standards limits their confirmation. Taken together, the summed quantitation of abiraterone and isomeric metabolites present in patient plasma provides an explanation for the higher values measured using PSI-MS/MS compared to LC-MS/MS where isomeric metabolites can often be resolved from the parent compound. These findings indicate the lack of selectivity of the PSI-MS/MS technique in patient plasma samples in the current setting

involving abiraterone. This is an obvious limitation that would need to be overcome to enable drug monitoring using PSI-MS. Paper substrate modifiers have been tested for enhancing PSI signal intensity [37], and integrating a facile analyte separation mechanism may be one approach to address the issue we observed here.

4. Conclusions

In this study, we developed and validated a bioanalytical method using PSI-MS/MS to quantify the prostate cancer drug, abiraterone. This assay showed similar bioanalytical performance compared with LC-MS/MS assays. However, the quantitative accuracy of using PSI-MS/MS for direct analysis of abiraterone in patient plasma samples was problematic, and we propose this is due to the presence of isomeric metabolites and abiraterone drug conjugates that yield ions of protonated abiraterone parent mass and therefore contribute additional signal in the PRM assay. This demonstrates the lack of selectivity of PSI-MS/MS technique for measuring abiraterone in clinical samples. This study highlights the importance of performing clinical validation with patient samples prior to deeming a method suitable for drug monitoring.

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Conflict of interest

All authors declare no conflict of interest related to this work.

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Table 1. Precision and accuracy of calibration curve standards used for quantification of abiraterone spiked into control human plasma.

Abiraterone	Nominal concentration (ng/mL)	% Accuracy	%CV
STD A	3.1	93.5	10.5
STD B	6.2	101.7	10.7
STD C	12.3	100.7	3.2
STD D	24.6	100.8	2.3
STD E	49.2	103.2	2.2
STD F	70.3	101.4	3.7
STD G	100.4	101.2	3.3
STD H	156.8	97.5	0.5

N = 3 for each concentration.

Table 2. Quality control samples spiked into control human plasma to assess the inter-day and intra-day precision and accuracy of the method.

Analyte	Nominal concentration (ng/mL)	Accuracy Intra-Day (%)	Accuracy Inter-Day (%)	Precision Intra-Day (%)	Precision Inter-Day (%)
Abiraterone	3.1 (LLOQ)	115.5 84.7 101.2	94.6	15.5 9.4 10.2	13.8
	6.9 (LQC)	94.8 94.0 95.2	94.7	5.5 10.7 2.9	6.7
	68.6 (MQC)	101.0 99.6 99.7	100.1	2.5 2.9 2.4	2.6
	137.2 (HQC)	100.9 98.7 99.8	99.8	2.4 1.9 1.4	2.1

N = 6 for each concentration level for intra-assay accuracy and precision calculation. N = 18 for each level concentration for inter-assay accuracy and precision calculation.

Table 3. Summary on the comparison between PSI and LC-MS/MS analytical performances.

	Range of linearity (ng/ml)	%CV of CC standards	%CV of QC samples	%Difference* at LLOQ
PSI	3.1 – 156.8	0.5 – 10.7	2.1 - 13.8	-17.1
LC- MS/MS[33]	0.07 – 509.6	0.7 - 2.2	2.5 - 4.1	1.4

*%Difference = [(Observed concentration – Nominal concentration) / Nominal concentration] X 100. The %Difference value reported is the highest deviation of an individual sample from nominal.

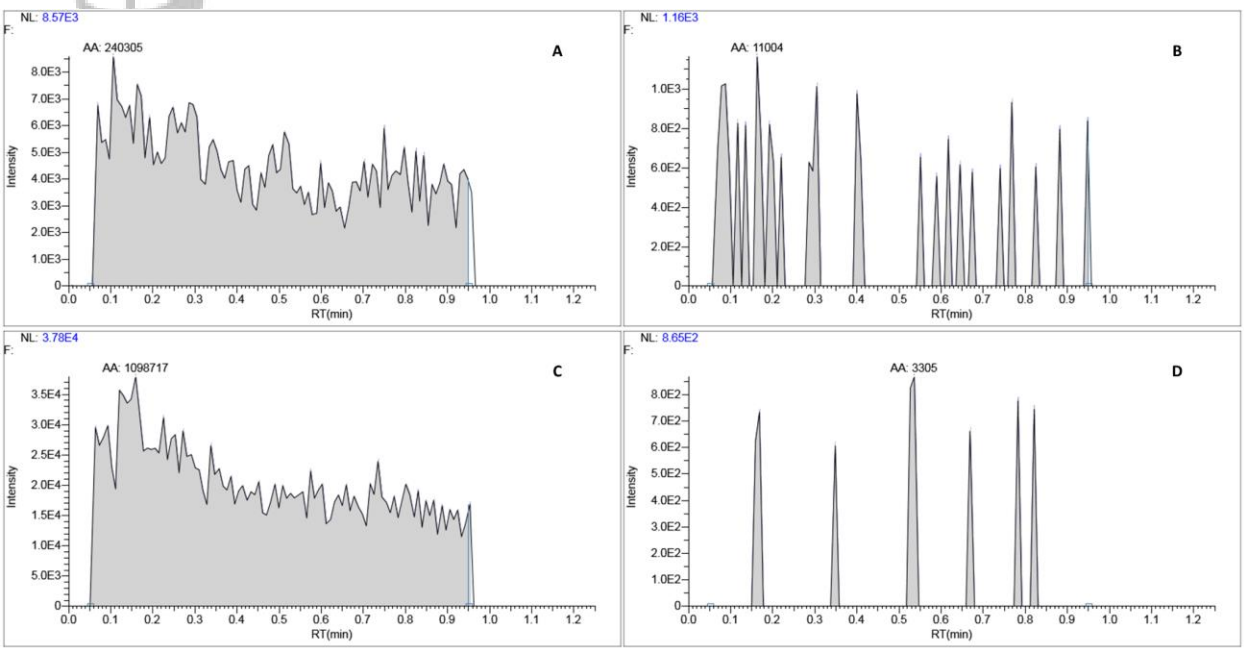

Figure 1. Extracted ion chronograms of QC samples in control plasma and their comparison with blank plasma. A) Abiraterone (m/z 350.2478→156.0808) spiked at LLOQ, B) comparative blank plasma. C) Abiraterone –d4 (IS, m/z 354.2730→160.1056) spiked at 50 ng/mL, D) comparative blank plasma.