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Estimation of Rucaparib in Biological Matrices By LC-ESI-MS/MS

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Abstract

A simple, sensitive and specific liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of Rucaparib (RP) in human plasma using Rucaparib-d3 (RPD3) as an internal standard (IS). Chromatographic separation was performed on Zorbax SB-C18, 4.6 x 75 mm, 3.5 µm, 80 Å column with an isocratic mobile phase composed of, 5mM ammonium acetate: methanol (30:70 v/v), at a flow-rate of 0.7 mL/min. RP and RPD3 were detected with proton adducts at m/z 323.4 → 170.1 and 328.4→ 170.1 in multiple reaction monitoring (MRM) positive mode respectively. Liquid-Liquid extraction method was used to extract the drug and IS. The method was validated over a linear concentration range of 10.0 – 10000.0 pg/mL with correlation coefficient (r^2) ≥ 0.9997. Rucaparib (RP) was found to be stable throughout freeze-thawing cycles, bench top and postoperative stability studies.

Keywords
Rucaparib, Mass spectrometry; Pharmacokinetic study

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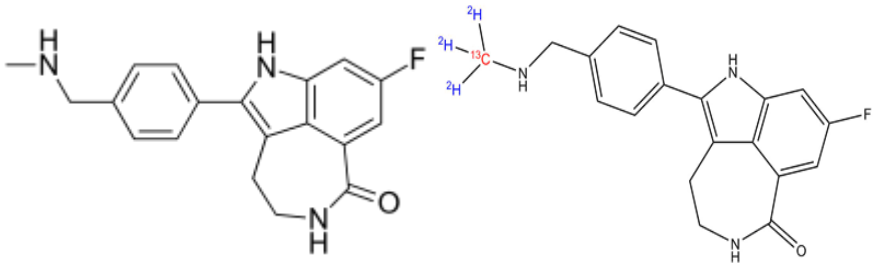
1. INTRODUCTION

Rucaparib is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes, including PARP-1, PARP-2, and PARP-3, which play a role in DNA repair. *In vitro* studies have shown that rucaparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and increased formation of PARP-DNA complexes resulting in DNA damage, apoptosis, and cell death. Increased rucaparib-induced cytotoxicity was observed in tumor cell lines with deficiencies in BRCA1/2 and other DNA repair genes. Rucaparib has been shown to decrease tumor growth in mouse xenograft models of human cancer with or without deficiencies in BRCA [1-7].

Rucaparib is an inhibitor of the mammalian polyadenosine 5'-diphosphoribose polymerase (PARP) enzyme. The chemical name is 6-fluoro-2-{4-[(methylamino)methyl] phenyl}-3,10-diazatricyclo [6.4.1.0{4,13}] trideca-1, 4, 6, 8 (13)-tetraen-9-one. The chemical formula of Rucaparib is $C_{19}H_{18}FN_3O$ and the relative molecular mass is 323.371 g/m [8-10].

Screening of the literature disclosed few published reports for the quantification of Rucaparib concentration in pharmacokinetics of Rucaparib in rat plasma by LC-MS [11].

From the literature review it was concluded that the developed methods show poor sensitivity, long retention time, lack of deuterated internal standard by using HPLC-ESI-M/MS. There are very limited methods were reported for estimation of Rucaparib using deuterated internal standard in biological samples.



A **B**

Fig.1: Chemical structures of A) Rucaparib (RP) B) Rucaparib-D3 (RPD3)

2.MATERIALS AND METHODS

2.1. Chemicals and Reagents

Rucaparib (RP) (Cadila Pharmaceuticals, India), Rucaparib-D3 (RPD3) (ALSACHIM, France), Ammonium formate and sodium hydroxide (Merck, Mumbai, India), Methanol, ethyl acetate and dichloromethane (J. T. Baker, USA), Ultra pure water (Milli-Q system, Millipore, Bedford, MA, USA), Screened human plasma (navjeevan blood bank, Hyderabad, A.P). The chemicals and solvents were used in this study analytical and HPLC grade.

2.2. Instrumentation

The 1200 Series HPLC system (Agilent Technologies, Germany). Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) using MRM. Data processing was performed on Analyst 1.4.1 software package (SCIEX).

2.3. Detection

Detection was performed by Turbo ion spray (API) positive mode with Unit Resolution using MRM positive ion mode with mass transitions of m/z (amu) 323.4→170.1 and 328.4→170.1 for RP and RPD3. The mass spectras of parent and product ions of RP and RPD3 shown in Fig- 2 and 3.

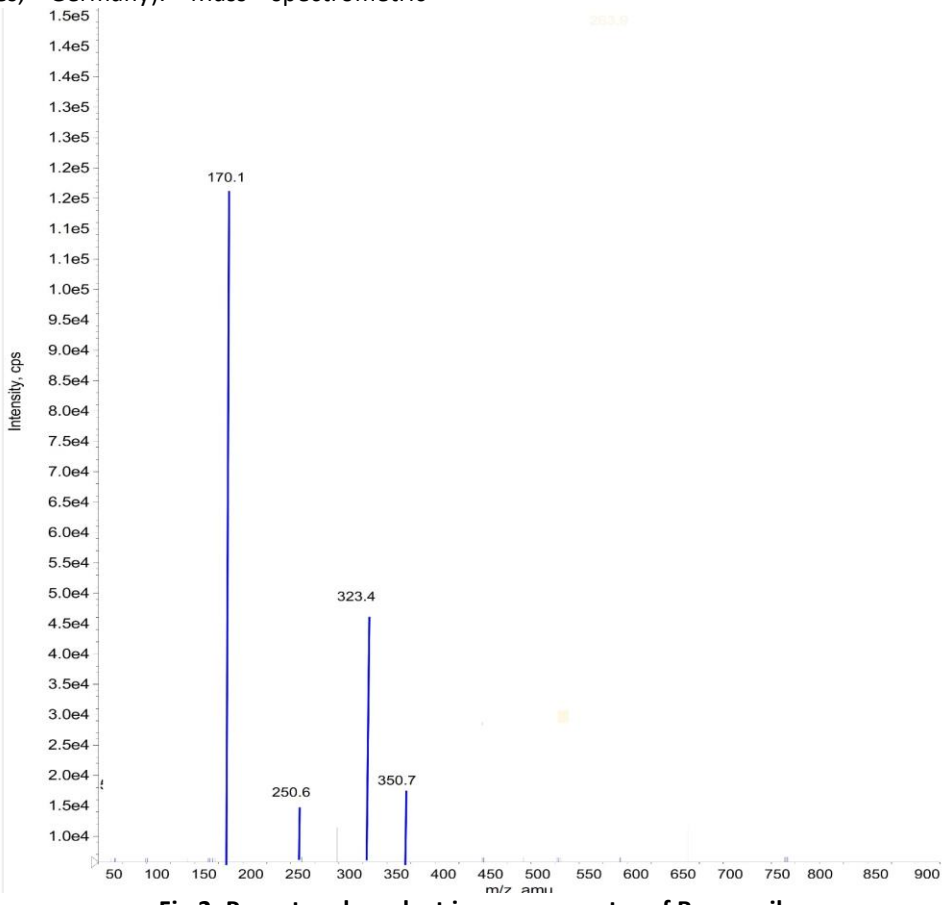


Fig.2. Parent and product ion mass spectra of Rucaparib

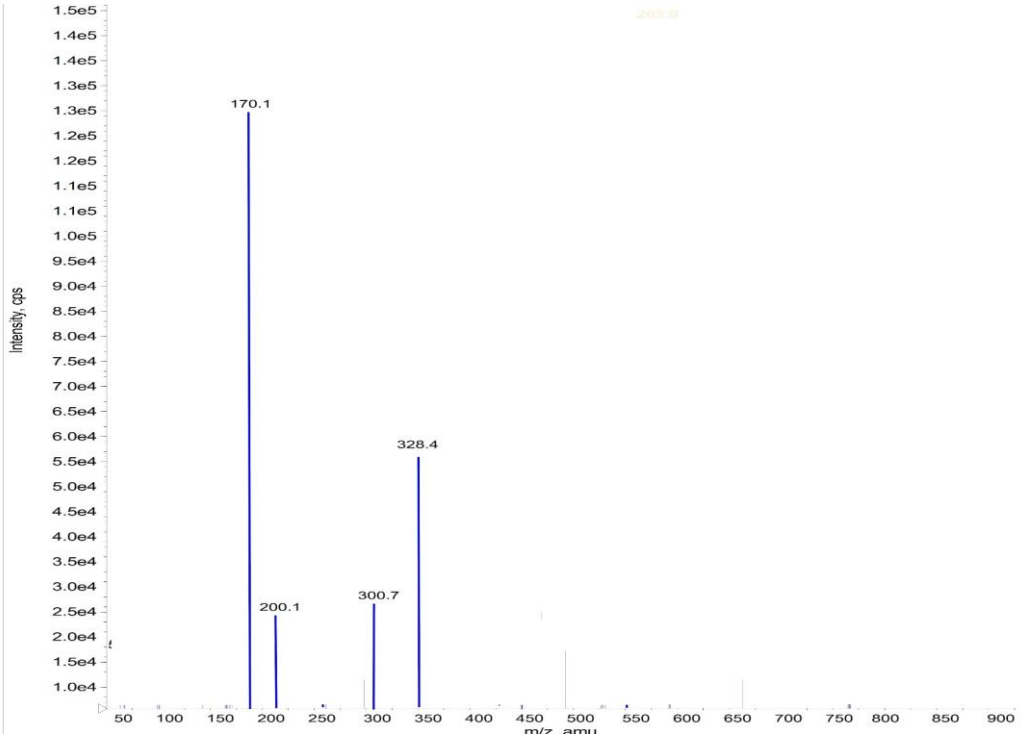


Fig.3. Parent and product ion mass spectra of Rucaparib-D3

2.4. Chromatographic conditions

Zorbax SB-C18, 4.6 x 75 mm, 3.5 µm 80 Å analytical column, mobile phase composition of 5 mM ammonium acetate in combination with methanol (30:70 v/v) with a flow-rate of 0.6 mL.min^{-1}. The column was placed at a temperature of 40°C. 20 µL of sample was injected into LC-MS/MS System. The analytes and Internal standards were eluted at 6.02 minutes (RP, RPD3) with total runtime of 13 minutes for each injection.

2.5. Preparation of standards and quality control (QC) Samples

Standard Stock solutions of RP (100.0 µg/mL) were prepared in methanol. From each stock solution 500.0 ng/mL, 25.0 ng/mL, 2.5 ng/mL intermediate dilutions were prepared in plasma. Aliquots of 500.0 ng/mL, 25.0 ng/mL and 2.5 ng/mL were used to spike blank human plasma in order to obtain calibration curve standards of 10.0, 20.0, 200.0, 800.0, 1500.0, 3000.0, 4500.0, 6000.0, 7500.0 and 10000.0 pg/mL. Four levels of QC concentrations at 10.0, 30.0, 3500.0 and 8000.0 pg/mL (LLOQ, LQC, MQC and HQC) were prepared by using the different plasma. Spiked calibration curve standards and Quality control standards were stored at -30°C. Standard stock solution of RPD3 (100.0 µg/mL) were prepared in methanol. RPD3 was further diluted to 30.0 ng/mL (Spiked concentration of internal standard) using 50 % methanol and stored in the refrigerator 2-8 ^{0}C until analysis.

2.6. Sample preparation

Liquid-liquid extraction was carried out to extract the drug and IS for this purpose 100 µL of respective concentration of plasma sample was taken into polypropylene tubes and mixed with 50µL of internal standard (30.0 ng/mL). This was followed by addition of 100 µL of 10mM KH_2PO4 solution and 2.5 mL of methyl tertiary butyl ether and vortexed for approximately 5 minutes. Then the Samples were centrifuged at 4000 rpm for 10 minutes at 20°C. Further, the supernatant was transferred into labeled polypropylene tubes and evaporated with nitrogen gas at 40°C. Then the samples were reconstituted with the reconstitution solution (5 mM ammonium acetate: methanol (30:70 v/v) and vortexed for 2 minutes. Finally, Sample was transferred into auto sampler vials to inject into the LC-MS/MS.

2.7. Selectivity and Sensitivity

Selectivity was performed by analyzing the human blank plasma samples from six different sources (donors) with an additional hemolysed group and lipedimic group to test for interference at the retention times of analytes. The sensitivity was compared with LLOQ of the analyte with its blank plasma sample. The peak area of blank samples should not be more than 20% of the mean peak area

of LOQ of Rucaparib and 5% of the mean peak area of Rucaparib-D3.

2.8. Precision and Accuracy

It was determined by replicate analysis of quality control samples (*n*=6) at LLOQ (lower limit of quantification), LQC (low quality control), MQC (medium quality control), HQC (high quality control) and ULOQ (upper limit of quantification) levels. The % CV should be less than 15%, and accuracy should be within 15% except LLOQ where it should be within 20 %.

2.9. Matrix effect

The matrix effect due to the plasma matrix was used to evaluate the ion suppression/enhancement in a signal when comparing the absolute response of QC samples after pretreatment (LLE) with the reconstitution samples extracted blank plasma sample spiking with analyte. Experiments were performed at MQC levels in triplicate with six different plasma lots with the acceptable precision (%CV) of ≤ 15%.

2.10. Recovery

The extraction recovery of Analyte and IS from human plasma was determined by analyzing quality control samples. Recovery at three concentrations (15.0, 2500.0, and 3500.0 pg/mL) was determined by comparing peak areas obtained from the plasma sample, and the standard solution spiked with the blank plasma residue. A recovery of more than 50 % was considered adequate to obtain required sensitivity.

2.11. Stability (Freeze - thaw, Auto sampler, Bench top, Long term)

Stock solution stability: Stability in stock solution was performed by comparing the area response of analyte and internal standard in the stability sample, with the area response of sample prepared from fresh stock solution.

Stability studies in plasma: Stability in plasma samples were performed at the LQC and HQC concentration level using six replicates at each level. Analyte was considered stable if the % Change is less than 15% as per US-FDA guidelines. The stability of spiked human plasma samples stored at room temperature (bench top stability) was evaluated for 48 h. The stability of spiked human plasma samples stored at -30 °C in autosampler (autosampler stability) was evaluated for 55.5 h. The autosampler sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0 h), with the samples that were reinjected after storing in the autosampler at 20°C for 55.5 h. The reinjection reproducibility was evaluated by comparing the extracted plasma samples that were injected immediately (time 0 h), with the samples that were re-injected after storing in the autosampler at 20°C for 27 h. The freeze-thaw stability was conducted by comparing the stability samples that had been frozen at –30 °C and thawed three times, with freshly spiked quality control samples. Six aliquots each of LQC and HQC concentration levels were used for the freeze-thaw stability evaluation. For long term stability evaluation, the concentrations obtained after 71 days were compared with initial concentrations.

3. RESULTS AND DISCUSSION

3.1. Method development

During method development, different options were evaluated to optimize mass spectrometry detection parameters, chromatography and sample extraction.

3.1.1. Mass spectrometry detection parameters optimization

Electro spray ionization (ESI) provided a maximum response over atmospheric pressure chemical ionization (APCI) mode and was chosen for this method. The instrument was optimized to obtain sensitivity and signal stability during infusion of the analyte in the continuous flow of mobile phase to electrospray ion source operated at both polarities at a flow rate of 5 µL/min. Rucaparib gave more response in positive ion mode as compare to the negative ion mode. The predominant peaks in the primary ESI spectra of Rucaparib and Rucaparib-D3 corresponds to the $[M+H]^+$ ions at m/z 323.4 and 328.4 respectively [Fig.2 and 3]. Product ions of Rucaparib and Rucaparib-D3 scanned in quadrupole-3 after a collision with nitrogen in quadrupole-2 had an m/z of 170.1 and 170.1 respectively [Fig.2 and 3]. Mass parameters were optimised as Source temperature 500 °C, Heater gas 45 (nitrogen) psi, nebulizer gas 30 (nitrogen) psi, Curtain gas 20 (nitrogen) psi, CAD gas 5 (nitrogen) psi, Ion Spray (IS) voltage 5500 volts, Source flow rate 600 µL/min without split, Entrance potential 10 V, Declustering potential 70 V ,Collision energy 30 V, Collision cell exit potential 15 V for both Analyte and I.S .

3.1.2. Chromatography optimization

Initially, a mobile phase consisting of ammonium acetate and acetonitrile in varying combinations was tried, but a low response was observed. The mobile phase containing 5mM ammonium formate: acetonitrile (20:80 v/v) and 5mM ammonium formate: methanol (20:80 v/v) gives the better response, but poor peak shape was observed. A mobile phase of 0.1% formic acid in water in combination with methanol and acetonitrile with varying combinations were tried. Using a mobile

phase containing 5mM ammonium formate: acetonitrile (20:80 v/v) gave the best signal along with a marked improvement in the peak shape was observed for Rucaparib and Rucaparib-D3. Short length columns, such as Symmetry Shield RP18 (50 x 2.1 mm, 3.5 µm), Inertsil ODS-2V (50 x 4.6 mm, 5µm), Hypurity C18 (50 x 4.6 mm, 5 µm) and Hypurity Advance (50 x 4.0 mm, 5 µm), Xbridge C18, 50x4.6 mm 5 µm were tried during the method development. A good separation and elution were achieved using 5 mM ammonium acetate: methanol (30:70 v/v) as the mobile phase, at a flow-rate of 0.6 mL/minutes and injection volume of 20 µL. Liquid-liquid extraction was chosen to optimize the drug and internal standard. The retention time was optimized 6.07 minutes for RP and RPD3 (Figure.2 & 3).

For an LC-MS/MS analysis, utilization of stable isotope-labeled or suitable analog drugs as an internal standard proves helpful when a significant matrix effect is possible. In our case, Rucaparib-D3 was found to be best for the present purpose. The column oven temperature was kept at a constant temperature of about 40 °C. Injection volume of 5µL sample is adjusted for better ionization and chromatography.

3.2. Method validation

A thorough and complete method validation of Rucaparib in human plasma was done following US FDA guidelines [12]. The method was validated for selectivity, sensitivity, matrix effect, linearity, precision and accuracy, recovery and stability.

3.2.1. Selectivity and specificity

The analysis of RP and RPD3 using MRM function was highly selective with no interfering compounds. (Fig: 4). Specificity was performed by using six different lots of human plasma. Here showing only one blank plasma interference. Chromatograms are shown in (Fig. 5).

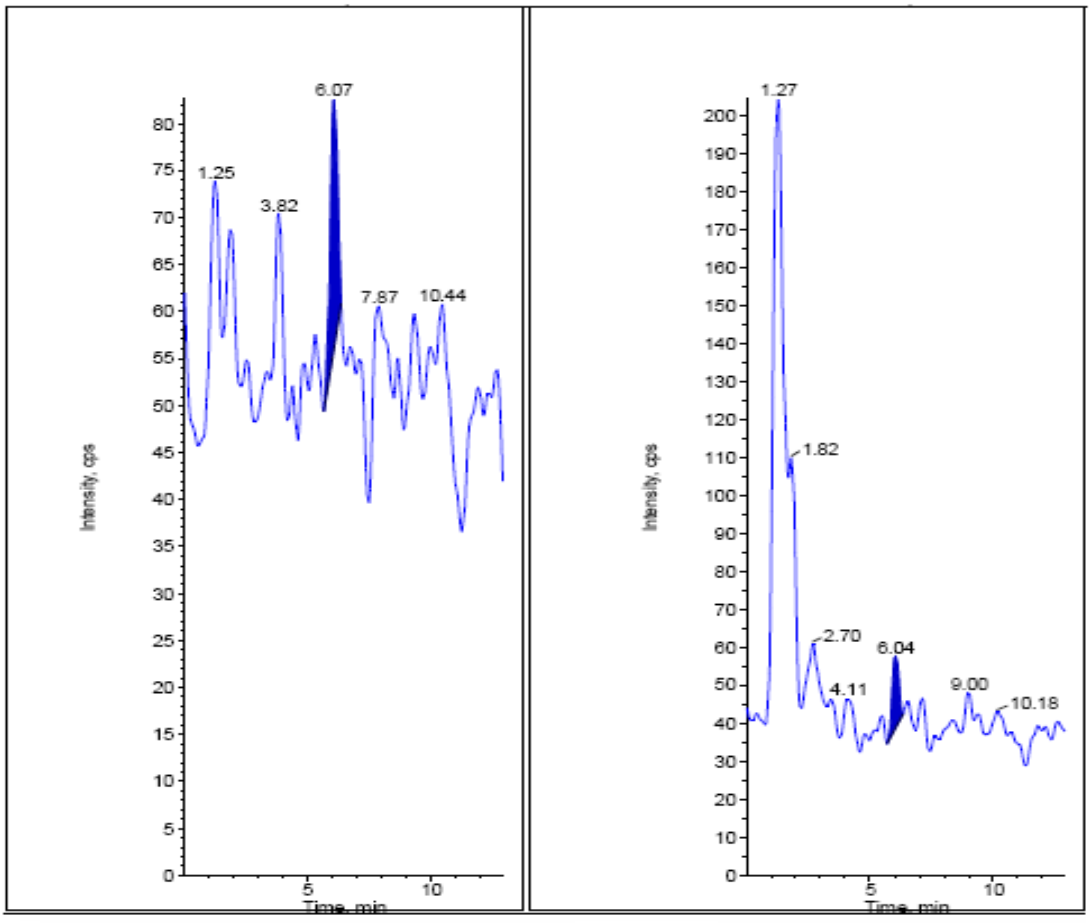


Fig:4: Chromatogram of Blank human plasma

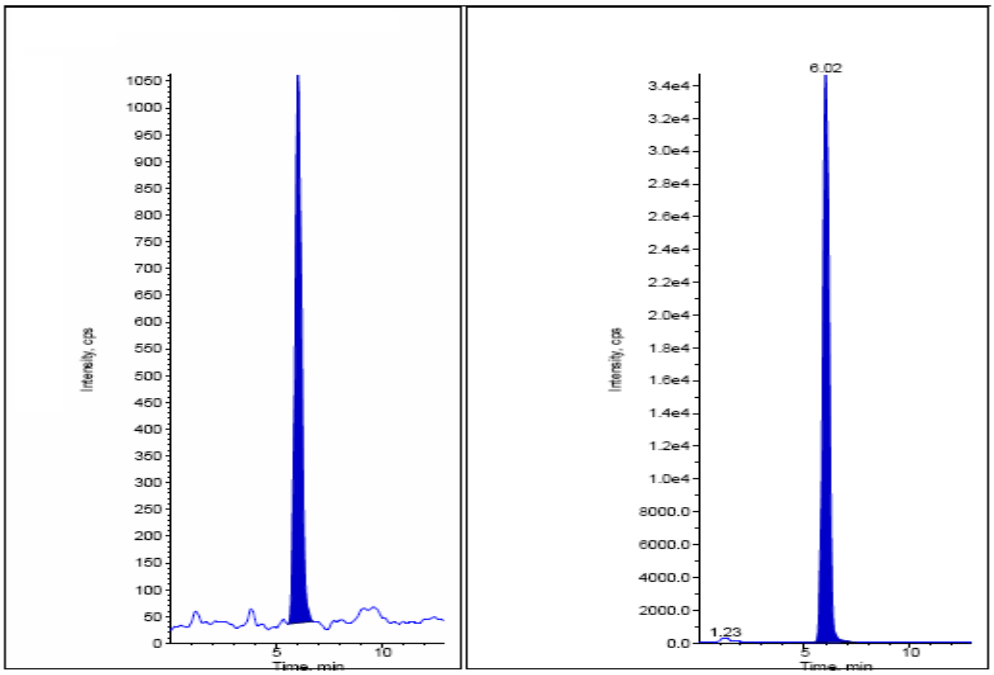


Fig:5: Chromatogram of Rucaparib and Rucaparib-D3 at LOQ level

3.2.2. Matrix effect

The matrix effect due to plasma was used to evaluate the ion suppression/enhancement in a signal when comparing the absolute response of QC samples after pretreatment (Liquid-liquid extraction with diethyl ether) with that of the reconstituted samples. Experiments were performed at MQC levels in triplicate with six different plasma lots. The acceptable precision (%CV) of ≤ 15% was maintained.

3.2.3. Linearity

Calibration curve was plotted as the peak area ratio (RP/RPD3) versus (RP) concentration. Calibration was found to be linear over the concentration range of 10.0 – 10000.0 pg/mL. The correlation coefficient (r^2) was greater than 0.9997 for all curves (Table 2).

Table 1: Calibration curve

Spiked plasma concentration (pg/mL)	Concentration measured(mean) (pg/mL) (*n* = 5)	Precision (CV %) (*n* = 5)
10.0	9.9±0.1	1.5
20.0	20.4±0.4	2.2
200.0	198.7±6.2	3.1
800.0	782.5±27.4	3.5
1500.0	1486.5±41.6	2.8
3000.0	2964.8±100.8	3.4
4500.0	4478.9±107.5	2.4
6000.0	5863.4±111.4	1.9
7500.0	7469.6±283.8	3.8
10000.0	9869.6±213.8	2.16

Table 2: Precision and accuracy

Spiked plasma concentration (pg/mL)	Within-run (n=6)			Between-run (n=30)		
	Concentration measured (pg/mL) (mean ± S.D.)	Precision (CV %)	Accuracy %	Concentration measured (pg/mL) (mean ± S.D.)	Precision (CV %)	Accuracy %
300.0	28.9±0.5	1.6	96.3	29.4±0.7	2.4	98.0
3500.0	3426.7±95.9	2.8	97.9	3512.4±119.4	3.4	100.4
8000.0	7896.4±189.5	2.4	98.7	7945.6±166.9	2.1	99.3

Table 3: Stability of the samples

Stability experiments	Storage condition	Spiked plasma concentration (pg/ml)	Concentration measured (*n*=6) Mean ± SD	CV(%) (*n*=6)	Accuracy (%)
Bench top in plasma	RT 72 hr	30	29.5±0.6	2.2	98.3
		8000	7868.1±133.8	1.7	98.4
Processed (extracted sample)	Autosampler 78 hr	30	29.2±0.8	2.6	97.3
		8000	7937.7±150.8	1.9	99.2
Freeze/Thaw stability	-30^{0}C Cycle-3	30	28.9±0.9	3.1	96.3
		8000	7958.3±191.0	2.4	99.5
Long term stability in human plasma	- 30^{0}C 71 days	30	28.6±0.9	3.2	95.3
		8000	7896±213.2	2.7	98.7

3.2.4. Precision and Accuracy

Precision and accuracy for this method were controlled by calculating the Within-run and Between-run variations at three concentrations (30.0, 3500.0 and 8000.0 pg/mL) of QC samples in six replicates. As shown in Table.3 the Within-run Precision and Accuracy were between 1.6 to 2.8 and 96.3 to 103.43.7% for. Similarly, the Between-run Precision and Accuracy were between 2.1 to 3.4 and 98.0 to 100.4%. These results indicate the adequate reliability and reproducibility of this method within the analytical range.

3.2.5. Recovery

The extraction recoveries [O--L.were determined at three different concentrations 30.0, 3500.0 and 8000.0 pg/mL and 99.2 ±2.4, 94.2±1.7 and 96.9±6.1% respectively. The overall average recovery was found to be 96.0 ± 2.8 and 98.76 ± 4.47. Recoveries of the analyte and IS were consistent, precise and reproducible.

3.2.6. Limit of quantification (LOQ) and Limit of Detection (LOD)

The LOQ signal-to-noise (S/N) values found for six injections of NR at LOQ concentration was 44.12.

3.2.7. Stability (Freeze-thaw, Auto sampler, Bench top, Long term)

Stock solution stability was performed to check stability of RP and RPD3 in stock solutions prepared in methanol and stored at 2-8 °C in a refrigerator. The freshly prepared stock solutions were compared with stock solutions prepared before 26 days. The % change for RP and RPD3 were -0.02% and 0.03% indicate that stock solutions were stable at least for 26 days. Room temperature and autosampler stability was investigated at LQC and HQC levels. The results revealed that RP was stable in plasma for at least 72 h at room temperature, and 78 h in an auto sampler. It was confirmed that repeated freezing and thawing (three cycles) of plasma samples spiked with RP at LQC and HQC levels did not affect their stability. The long-term stability results also indicated that RP were stable in a matrix up to 71days at a storage temperature of -30°C. The results obtained from all these stability studies were tabulated in Table.4. Precision (%CV) is less than 5% for Room temperature, long-term, Freeze thaw, auto sampler stability.

4. CONCLUSIONS

The proposed method was five folds higher sensitive than the reported method and analyte was compared with deuterated internal standard. The method described here is fast (requires less than 2.5 min of analysis time), rugged, reproducible bioanalytical method. The developed method is simple and efficient and can be used in pharmacokinetics studies as well as in the monitoring of the investigated analyte in body fluids.

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