INTRODUCTION
Pomalidomide chemically 4-amino-2-(6-dioxopiperidin-3-yl) isoindoline-1, 3-dione, the newest immune-modulatory drugs (IMiD), was designed to be more potent and less toxic than thalidomide and lenalidomide [1-2]. It is used for the treatment of relapsed and refractory multiple myeloma. Dr. Rober D'Amato's labs led to the first report [3-4] stating that 3-amino-thalidomide was able to directly inhibit both the tumor cell and vascular compartments of myeloma cancers. An HPLC-UV method was reported for inversion of pomalidomide in phosphate-buffered saline and human plasma in μg/ml range [5]. The LC-MS methods were also published for the Pharmacokinetic study of pomalidomide but no validation details were presented [6-7]. A validated UPLC-MS method was reported using negative ionisation mode for determination of pomalidomide from rat plasma [8]. Described here is a simple, sensitive, and selective LC-MS/MS method for pomalidomide in the human plasma concentration range of 9.998 to 1009.650 ng/ml. As there is no literature on stability data of pomalidomide in human plasma, this study performed assay validations, according to the FDA guidelines [9]. While this method with validation details were economical and applied for pharmacokinetic studies of pomalidomide.

MATERIALS AND METHODS [8]

Apparatus and software
The HPLC pump (Agilent 1200 series Binary SL) with an autosampler (Agilent 1200 series HPLC-ALSSL) was coupled with Agilent 6460 Triple Quad Tandem mass spectrometer. The column oven was Agilent 1200 series TCC SL. The chromatographic integration was performed by Agilent mass hunter software.

Chemicals and reagents
Pomalidomide and Fluconazole (IS) were procured from NATCO Pharma Ltd., Hyderabad, Formic acid, Methanol and ethyl acetate was procured from Merck Specialities Pvt. Ltd, Mumbai, India. Water used was collected from water purification systems (Milli Q, Milli Pore, USA) installed in the laboratory. Pooled drug-free expired human plasma (K2-EDTA as anticoagulant) was obtained from Blood Bank, Hyderabad, was used during validation and study sample analysis. The plasma was stored into-70±5 °C.

Standards and working solutions
Calibration standard solutions
Stock solutions of pomalidomide and Fluconazole internal standard (IS) were prepared in methanol. Further dilutions were carried out in 50% methanol. Calibration standards of nine concentration levels were prepared freshly by spiking drug-free plasma with pomalidomide stock solution to give the concentrations of 9.998, 25.241, 50.281, 150.438, 301.885, 503.815, 705.745, 906.666 and 1009.650 ng/ml.

Quality control standards
Lowest quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug-free plasma with pomalidomide to give a solution containing 26.248, 323.056 and 807.640 ng/ml respectively. They were stored at-20 °C till the time analysed.

Chromatographic conditions
Chromatographic separation was performed on Xterra, RP18, 5 μ (50 x 4.6 mm), analytical column and the mobile phase was a mixture of 0.1% (v/v) formic acid in water to methanol at a ratio of 12:88, v/v as the mobile phase. The flow rate was 0.50 ml/min. The LC eluent was split, and approximately 0.1 ml/min was introduced into the m/z 148.8 for pomalidomide and m/z 307.1/238.0 for Fluconazole.

RESULTS
The concentrations of nine working standards showed linearity between 9.998 to 1009.650 ng/ml (r² ≥ 0.9968). Chromatographic separation was achieved within 2 min. The average extraction recoveries of three quality control concentrations were 53.86% for pomalidomide and 99.01% and 98.49% respectively.

CONCLUSION
The results obtained for specificity, linearity, accuracy, precision, ruggedness and stability studies were within limits. Thus the validated economical method was applied for pharmacokinetic studies of pomalidomide.

Keywords: Pomalidomide, LC-MS/MS, Human plasma, Liquid-liquid extraction

REFERENCE
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ABSTRACT
Objective: The present work aimed to develop a simple, rapid, specific and precise liquid chromatography-tandem mass spectrophotometric (LC-MS/MS) validated method for quantification of pomalidomide and internal standard (ISTD) Fluconazole in human plasma.

Methods: 50 μl of 0.1% formic acid was added to plasma samples prior to liquid-liquid extraction (LLE) using 2.5 ml of ethyl acetate. Chromatographic separation was achieved on Xterra, RP18, 5 μ (50 x 4.6 mm) column using a mixture of 0.1% (v/v) formic acid in water to methanol at a ratio of 12:88, v/v as the mobile phase. The flow rate was 0.50 ml/min. The LC eluent was split, and approximately 0.1 ml/min was introduced into the mass spectrometer using turbo ion spray interface at 325 °C. Quantitation was performed by transitions of m/z 260.1 precursor ion to the m/z 148.8 for pomalidomide and m/z 307.1/238.0 for fluconazole.

RESULTS: The concentrations of nine working standards showed linearity between 9.998 to 1009.650 ng/ml (r² ≥ 0.9968). Chromatographic separation was achieved within 2 min. The average extraction recoveries of three quality control concentrations were 53.86% for pomalidomide and 99.01% and 98.49% respectively.

Conclusion: The results obtained for specificity, linearity, accuracy, precision, ruggedness and stability studies were within limits. Thus the validated economical method was applied for pharmacokinetic studies of pomalidomide.

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Chromatographic separation was performed on Xterra, RP18, 5 μ (50 x 4.6 mm), analytical column and the mobile phase was a mixture of 0.1% (v/v) formic acid in water to methanol at a ratio of 12:88, v/v. Injection volume was 10 µL. The flow rate was 0.50 ml/min. Total analysis time of single injection was 20 min. Column oven temperature and autosampler temperature was set to 30 °C and 10 °C, respectively.

Mass spectrometric conditions
The LC eluent was split, and approximately 0.100 ml/min was introduced via electrospray ionisation using a Turbo Ion Spray interface set at 325 °C to generate positive ions [M+H]+. The Mass spectrometric parameters were optimised as shown in table no 1.

A UPLC-MS/MS METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF POMALIDOMIDE FROM HUMAN PLASMA

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Original Article
Sample preparation method

To 250 µl of plasma, 50 µl of ISTD (1 µg/ml) and 50 µl of 0.1% formic acid was added and vortexed. The drug was extracted with 2.5 ml of ethyl acetate, followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15 min at 4 °C. The supernatant of 2 ml was withdrawn and evaporated at 50 °C 15 psi of nitrogen until dryness at LV evaporator. The residue was reconstituted with 500 µl of mobile phase and respective samples were injected into the column.

Validation

Specificity

A solution containing 9.999 ng/ml was injected onto the column under optimised chromatographic conditions to show the separation of pomalidomide from impurities and plasma. The specificity of the method was checked for the interference from plasma.

Linearity

Spiked concentrations were plotted against peak area ratios of pomalidomide to the internal standard, and the best fit line was calculated. Wide range calibration was determined by solutions containing 9.998 to 1009.650 ng/ml.

Recovery studies

The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against un-extracted Quality control samples at HQC, MQC and LQC.

Precision and accuracy

The between-run (Inter-day) accuracy and precision evaluation were assessed by the repeated analysis of human K3 EDTA plasma samples containing different concentrations of pomalidomide on separate occasions. A single run consisted of a calibration curve plus six replicates of the lower limit of quantitation, low, medium and high-quality control samples.

Within-run (Intraday) accuracy and precision evaluations were performed by analysing replicate concentrations of pomalidomide in human K3 EDTA plasma. The run consisted of a calibration curve plus a total of 24 spiked samples, six replicates of each of the LLOQ, lower, medium and higher quality control samples.

Matrix effect

Blank plasma samples of 6 different human K3 EDTA plasma sources were processed and spiked with aqueous low-quality control and high-quality control (post extraction addition) and analysed in a single run along with diluted pure standard at each concentration level.

Ruggedness

The ruggedness of the method was assessed by analysing a precision and accuracy batch using a different column, by the different analyst in another instrument.

Stability studies

Short-term stock solution stability of pomalidomide

Solutions of pomalidomide were prepared in methanol (Stability Samples) and were kept at room temperature for 6 h 30 min. A freshly prepared solution of pomalidomide (Comparison Samples) and stability samples were diluted at approximately the same analyte concentration and analysed in a single run; analyte responses were used to determine % stability over time.

Short-term stock solution stability of internal standard

Solutions of internal standard (Fluconazole) were prepared in methanol (Stability Samples) and were kept at room temperature for 6 h 30 min. A freshly prepared solution of internal standard (Comparison Samples) and stability samples were diluted at approximately the same analyte concentration and analysed in a single run; analyte responses were used to determine % stability over time.

Freeze-thaw stability

Samples were prepared at low and high-quality control levels, aliquoted and frozen at -70 °C. Some of the aliquots of quality control samples were subjected to five freeze-thaw cycles (Stability Samples). A calibration curve and quality control samples were freshly prepared (Comparison Samples) and processed with 6 replicates of stability samples and analysed in a single run.

Long-term stock solution stability of pomalidomide

Solutions of Pomalidomide were prepared in methanol (Stability Samples) and were kept at refrigerator (2-8 °C) for 10 D 02 H. A freshly prepared solution of internal standard (Comparison Samples) and stability samples were diluted at approximately the same analyte concentration and analysed in a single run.

Long-term stock solution stability of internal standard

Solutions of Internal standard were prepared in methanol (Stability Samples) and were kept at refrigerator (2-8 °C) for 10 D 02 H. A freshly prepared solution of internal standard (Comparison Samples) and stability samples were diluted at approximately the same analyte concentration and analysed in a single run.

RESULTS AND DISCUSSION

The chromatography observed during the course of validation was acceptable and representative chromatograms of, LLOQ, LQC, MQC, HQC, internal standard (ISTD) and standard blank samples are shown in (fig. 1).

The method developed was validated for specificity, accuracy and precision, linearity, ruggedness and stability as per USFDA guidance [10-12]. The results of validating parameters are given below.

Specificity

Nine different lots of plasma were analysed to ensure that no endogenous interferences were present at the retention time of pomalidomide and fluconazole. Nine LLOQ (9.999 ng/ml) level samples along with plasma blank from the respective plasma lots were prepared and analysed. (table 2) shows results of specificity. In all plasma blanks, the response at the retention time of pomalidomide was less than 20% of LLOQ response, and at the retention time of IS, the response was less than 5% of mean IS response in LLOQ. The typical chromatogram of plasma blank and chromatogram of LLOQ was shown in (fig. 1).
Table 2: Results of specificity for pomalidomide and fluconazole (ISTD)

| Analyte | Area of interfering peak at RT of analyte | Area observed for extracted LLOQ | % Interference at RT of analyte | Area of interfering Peak at RT of ISTD | Area observed for extracted ISTD | % Interference at RT of ISTD |
|---------|------------------------------------------|---------------------------------|---------------------------------|----------------------------------------|---------------------------------|-------------------------------|
| 01      | 0                                        | 15286                           | 0                               | 0                                       | 575244                         | 0                             |
| 02      | 0                                        | 13288                           | 0                               | 0                                       | 582214                         | 0                             |
| 03      | 0                                        | 11110                           | 0                               | 0                                       | 578922                         | 0                             |
| 04      | 0                                        | 11440                           | 0                               | 0                                       | 564562                         | 0                             |
| 05      | 0                                        | 11402                           | 0                               | 0                                       | 558925                         | 0                             |
| 06      | 0                                        | 11059                           | 0                               | 0                                       | 589001                         | 0                             |
| 07      | 0                                        | 11215                           | 0                               | 0                                       | 591285                         | 0                             |
| 08      | 0                                        | 11580                           | 0                               | 0                                       | 578862                         | 0                             |
| 09      | 0                                        | 12089                           | 0                               | 0                                       | 588638                         | 0                             |
| Mean    |                                          | 12052.111                       | MEAN                            |                                         | 180201.89                      |                               |

**Linearity**

The calibration curve (peak area ratio Vs Concentration) was linear over working a range of 9.998 to 1009.650 ng/ml with nine point calibration used for quantification by linear regression, shown in (fig 2). The regression equation for the analysis was $Y=0.0039x-0.0089$ with coefficient of correction ($r^2$) = 0.99686.

**Recovery**

The % mean recovery for pomalidomide in LQC, MQC and HQC was 50.68%, 53.43% and 57.49% respectively (table 3).
### Intraday (within run) and inter-day (between run) precision and accuracy

The within-run coefficients of variation ranged between 1.47% and 4.57% for pomalidomide. The within-run percentages of nominal concentrations ranged between 97.95% and 107.58% for pomalidomide. Results are presented in table 4.

The between-run coefficients of variation ranged between 2.88% and 4.22% for pomalidomide. The between-run percentages of nominal concentrations ranged between 99.41% and 106.97% for pomalidomide. Results are presented in table 5.

### Matrix effect

The percentage matrix effect of analyte was found to be 0.25 and 2.74 for pomalidomide for low and high-quality control samples. Results are presented in table 6.

### Ruggedness

The coefficients of variation ranged between 1.32% and 4.03% for pomalidomide. The percentages of nominal concentrations ranged between 101.06% and 110.08% for pomalidomide. Results are presented in table 7.

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### Table 3: % Mean recovery of pomalidomide for LQC, MQC and H QC

| Batch ID | CV (%) | Conc. Factor | SD (+) | Mean Recovery | Global | Recovery |
|----------|--------|--------------|--------|---------------|--------|----------|
| 01       | 0.44   | 0.02         | 0.2    | 52.86         | 53.86  |          |
| 02       | 0.38   | 0.03         | 0.3    | 52.86         | 53.86  |          |
| 03       | 0.27   | 0.04         | 0.2    | 52.86         | 53.86  |          |
| 04       | 0.25   | 0.05         | 0.2    | 52.86         | 53.86  |          |
| 05       | 0.19   | 0.06         | 0.2    | 52.86         | 53.86  |          |
| 06       | 0.09   | 0.07         | 0.2    | 52.86         | 53.86  |          |

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### Table 4: Intraday precision and accuracy of quality control standard

| S. No. | LLOQ 9.999 ng/ml | Conc. found (ng/ml) | % nominal Conc | Conc. found (ng/ml) | % nominal Conc | Conc. found (ng/ml) | % nominal Conc | Conc. found (ng/ml) | % nominal Conc |
|--------|------------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|
| 1      | 1.075            | 107.06              | 28.081         | 106.98              | 315.669        | 97.71               | 762.368        | 94.39               |
| 2      | 1.0569           | 105.70              | 27.895         | 106.27              | 355.681        | 110.10              | 782.176        | 96.05               |
| 3      | 1.0411           | 104.12              | 27.857         | 106.13              | 318.659        | 98.64               | 775.543        | 96.03               |
| 4      | 1.1487           | 114.88              | 27.942         | 106.45              | 324.372        | 100.41              | 810.934        | 100.41              |
| 5      | 1.0611           | 106.11              | 27.841         | 106.07              | 317.699        | 98.34               | 805.069        | 99.68               |
| 6      | 1.0758           | 107.60              | 28.915         | 110.16              | 327.842        | 101.48              | 810.606        | 100.37              |
| N      | 6                | 107.58              | 28.089         | 107.01              | 326.654        | 101.11              | 791.116        | 97.95               |
| SD (+) | 0.38             | 0.41                | 14.93          |                    | 20.57          |                    |                |                    |
| CV (%) | 3.51             | 4.57                | 2.60           |                    |                |                    |                |                    |

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### Table 5: Inter day precision and accuracy of quality control standard

| Batch ID | LLOQ 9.999 ng/ml | Conc. found (ng/ml) | % nominal conc | Conc. found (ng/ml) | % nominal conc | Conc. found (ng/ml) | % nominal conc | Conc. found (ng/ml) | % nominal conc |
|----------|------------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|
| PandA-01 | 1.075            | 107.06              | 27.895         | 106.28              | 315.669        | 97.71               | 762.368        | 94.39               |
|          | 1.0569           | 105.70              | 27.895         | 106.27              | 355.681        | 110.10              | 782.176        | 96.05               |
|          | 1.0411           | 104.12              | 27.857         | 106.13              | 318.659        | 98.64               | 775.543        | 96.03               |
|          | 1.1487           | 114.88              | 27.942         | 106.45              | 324.372        | 100.41              | 810.934        | 100.41              |
|          | 1.0611           | 106.11              | 27.841         | 106.07              | 317.699        | 98.34               | 805.069        | 99.68               |
|          | 1.0758           | 107.60              | 28.915         | 110.16              | 327.842        | 101.48              | 810.606        | 100.37              |
|          | 1.069            | 106.10              | 26.569         | 101.22              | 315.344        | 97.61               | 762.157        | 94.37               |
| PandA-02 | 1.0896           | 108.97              | 27.498         | 104.76              | 312.676        | 96.79               | 798.395        | 98.86               |
|          | 1.0268           | 102.69              | 27.581         | 105.08              | 313.590        | 97.07               | 786.741        | 97.41               |
|          | 1.0307           | 103.08              | 27.235         | 103.76              | 317.332        | 98.23               | 793.101        | 98.20               |
|          | 1.0729           | 107.30              | 28.810         | 109.76              | 330.628        | 102.34              | 775.171        | 95.98               |
|          | 1.0867           | 108.69              | 28.880         | 110.03              | 318.223        | 98.50               | 838.267        | 103.79              |
| PandA-03 | 1.039            | 103.10              | 29.561         | 112.62              | 326.177        | 100.97              | 830.304        | 102.81              |
|          | 1.0321           | 103.22              | 29.126         | 110.97              | 328.816        | 101.78              | 874.482        | 102.88              |
|          | 1.0805           | 108.06              | 29.018         | 110.55              | 327.572        | 101.40              | 854.598        | 105.81              |
|          | 1.0443           | 104.44              | 27.968         | 106.55              | 327.423        | 101.35              | 846.592        | 104.82              |
| N       | 18               | 18                  | 18             | 18                  | 18             | 18                  | 18             | 18                  |
| Mean    | 1.0600           | 106.01              | 28.077         | 106.97              | 324.675        | 100.50              | 802.868        | 99.41               |
| SD (+)  | 0.31             | 0.83                | 12.76          |                    | 33.89          |                    |                |                    |
| CV (%)  | 2.88             | 2.95                | 4.22           |                    |                |                    |                |                    |
Table 6: Results of matrix effect obtained by preparing LQC and HQC with six different lots of plasma

| S. No. | AQS LQC response | PEX LQC response | AQS HQC response | PEX HQC response |
|--------|------------------|------------------|------------------|------------------|
| 1      | 195688           | 203204           | 5680630          | 5635662          |
| 2      | 200736           | 197892           | 5694436          | 5694225          |
| 3      | 200626           | 197423           | 5637040          | 6355785          |
| 4      | 198973           | 198788           | 5647571          | 5716531          |
| 5      | 200778           | 199084           | 5680208          | 5753628          |
| 6      | 201077           | 198505           | 5655245          | 5772353          |

Mean: 199646.333, SD: 2078.29, %CV: 1.04, %ME: -0.25

Table 7: Results of ruggedness

| S. No. | LLOQ 9.999 ng/ml | LQC 26.248ng/ml | MQC 323.056 ng/ml | HQC 807.640 ng/ml |
|--------|-----------------|-----------------|-------------------|-------------------|
|        | Conc. found     | % nominal conc  | Conc. found       | % nominal conc    | Conc. found       | % nominal conc    | Conc. found       | % nominal conc    |
| 1      | 10.729          | 107.30          | 28.810            | 109.76            | 330.62            | 102.34            | 775.171           | 95.98             |
| 2      | 10.867          | 108.69          | 28.880            | 110.03            | 318.22            | 98.50             | 838.267           | 103.79            |
| 3      | 10.309          | 103.10          | 29.561            | 112.62            | 326.17            | 100.97            | 846.592           | 108.28            |
| 4      | 10.321          | 103.22          | 29.126            | 110.97            | 327.57            | 101.35            | 854.598           | 104.82            |
| 5      | 10.805          | 108.06          | 29.018            | 112.62            | 327.42            | 101.35            | 836.569           | 103.58            |
| 6      | 10.443          | 104.44          | 27.968            | 106.55            | 324.71            | 101.06            | 846.592           | 104.82            |

N: 6, Mean: 10.579, SD: 0.53, %CV: 2.37

Stability studies

Short-term stock solution stability of pomalidomide

Pomalidomide is found to be stable in methanol for 6 h 30 min at room temperature with a % stability of 99.01%. Results are presented in table 8.

Short-term stock solution stability of internal standard

The internal standard is found to be stable in methanol for 6 h 30 min at room temperature with a % stability of 99.15%. Results are presented in table 9.

Freeze-thaw stability

Pomalidomide is found to be stable in human K3 EDTA plasma after five freeze-thaw cycles at-70 °C with coefficients of variation of 3.27% (LQC) and 3.86% (HQC) for pomalidomide, and the percentages of nominal concentrations for pomalidomide were found to be 103.17% (LQC) and 101.23% (HQC). Results are presented in table 10.

Long-term stock solution stability of pomalidomide

Pomalidomide is found to be stable in methanol 10 D 02 H at refrigerator (2-8 °C) with a % stability of 98.49% for pomalidomide. Results are presented in table 11.

Long-term stock solution stability of internal standard

The internal standard is found to be stable in methanol 10 D 02 H at refrigerator (2-8 °C) with a % stability of 98.49% for internal standard. Results are presented in table 12.

Table 8: Short-term stock solution stability of analyte

| S. No. | Analyte | SS       | CS       |
|--------|---------|----------|----------|
| 1      |         | 256444   | 2606664  |
| 2      |         | 259748   | 261699   |
| 3      |         | 2606795  | 263079   |
| 4      |         | 2611068  | 2627041  |
| 5      |         | 2598633  | 2630048  |
| 6      |         | 2608998  | 2629280  |
| Mean   |         | 2597903.333 | 2.37 |
| SD     |         | 1.82     | 0.37     |
| %CV    |         | 99.01    | 99.15    |

Table 9: Short-term stock solution stability of internal standard

| S. No. | ISTD    | Stability solution | Comparison solution |
|--------|---------|--------------------|---------------------|
| 1      |         | 2504196            | 252152              |
| 2      |         | 2510082            | 2524682             |
| 3      |         | 2496215            | 2537401             |
| 4      |         | 2487925            | 2519696             |
| 5      |         | 2512367            | 2504040             |
| 6      |         | 2491004            | 2526432             |
| Mean   |         | 2500631.883        | 2522796.000         |
| SD     |         | 9997.37            | 10877.47            |
| %CV    |         | 0.40               | 0.43                |
| % stability | | 99.15              | 99.15               |
Table 10: Freeze-thaw stability at -70 °C

| S. No. | Freshly spiked | Freeze-thaw |
|--------|----------------|-------------|
|        | LQC            | HQC         | LQC        | HQC         |
|        | Nominal Con (ng/ml) | Nominal Con (ng/ml) |
| 1      | 26.249         | 791.749     | 26.248     | 807.648     |
| 2      | 27.816         | 767.862     | 30.051     | 839.017     |
| 3      | 27.327         | 789.495     | 28.055     | 824.962     |
| 4      | 27.830         | 821.654     | 28.198     | 791.864     |
| 5      | 27.819         | 842.104     | 29.380     | 807.64      |
| 6      | 27.272         | 770.159     | 28.557     | 801.458     |
| Mean   | 27.680         | 797.204     | 28.557     | 807.031     |
| SD     | 0.31           | 29.34       | 0.93       | 31.17       |
| %CV    | 1.10           | 3.68        | 3.27       | 3.86        |
| % stability | 103.17       | 101.23      |            |             |

CONCLUSION
Chromatographic separation was achieved on Xterra, RP<sub>18</sub>, 5 µ (50 x 4.6 mm) column using a mixture of 0.1% (v/v) formic acid in water to methanol at a ratio of 12:88, v/v as the mobile phase. The drug was extracted with 2.5 ml of ethyl acetate. The specificity of the method was checked for the interference from plasma. The calibration curve (peak area ratio Vs Concentration) was linear over working a range of 9.998 to 1009.650 ng/ml with nine point calibration used for quantification by linear regression. The % mean recovery for pomalidomide in LQC, MQC and HQC was 50.68%, 53.43% and 57.49% respectively. The within-run coefficients of variation ranged between 1.47% and 4.57% for pomalidomide. The between-run coefficients of variation ranged between 2.88% and 4.22% for pomalidomide. The percentage matrix effect of analyte was found to be -0.25 and 2.74 for pomalidomide for low and high-quality control samples. The stability test was performed to assess the long term and short term stability of sofosbuvir sample solutions, internal standard solutions. The developed method was validated for the quantitative determination of sofosbuvir from plasma was simple, rapid, specific, sensitive, accurate and precise. Hence, the method is quite suitable to detect the drug from plasma samples of human volunteers.

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CONFLICT OF INTERESTS
Declared none

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