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Bioanalytical Method Development and Validation for Determination of Sulfasalazine in Rabbit Plasma by HPLC-UV

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In present study, an advanced, simple and a rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the quantitative determination of sulfasalazine in rabbit plasma. Sulfasalazine was separated using Chromatopak C-18 basic peerless (250 mm × 4.6 mm, 5µ) column in an isocratic mode using mobile phase consisting of the mixture of 10mM Ammonium acetate pH adjusted to 4.5 and acetonitrile (70:30 v/v) with a flow rate of about 1.0 mL/min at ambient temperature. An ultra-violet detection of sulfasalazine and the internal standard was carried out at 362 nm. Both sulfasalazine and internal standard (IS, 4-hydroxy benzoate) were extracted from plasma matrices with high efficiency using a simple protein precipitation method. The method was found to be highly selective with no carryover effects. Linearity of sulfasalazine was found with the range of 2.5-100 µg/mL with the value of $r^2 > 0.995$ a correlation coefficient. At all three quality control levels, developed bioanalytical method was found as repeatable and reproducible as well. The average recoveries of sulfasalazine from plasma were in the range of 95.59-97.16%. The bioanalytical samples showed good and acceptable stability of sulfasalazine solution at different storage, packaging and handling conditions. Hence, in conclusion, the validated and developed HPLC-UV method could be effectively utilized for determination of sulfasalazine in pharmacokinetic studies involving novel formulations.

Keywords: Sulfasalazine, RP-HPLC, HPLC-UV, Plasma.

INTRODUCTION

Sulfasalazine, chemically is 2-hydroxy-5-[[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]benzoic acid, a brownish-yellow odourless crystal with a melting point of 240-245 ºC. It is a sulfonamide prodrug synthesized *via* an azo-coupling reaction formed from reacting sulfapyridine with nitrous acid and salicylic acid in an alkaline medium [1]. This work was reported by Pharmacia Inc. initially for the treatment of rheumatoid arthritis [2]. Since then, drug has been approved and prescribed for the treatment of various inflammatory bowel diseases [3], such as ulcerative colitis, Crohn′s disease and rheumatoid arthritis diseases [4-6]. The immunomodulatory, anti-inflammatory and antiproliferative properties of sulfasalazine has also been extensively exploited as a treatment alternative in various dermatological disorders [7-9].

After oral administration of sulfasalazine, only a partial amount of sulfasalazine is absorbed from the upper gastrointestinal tract, whereas most of the compound reaches the colon. It is then cleaved by bacterial azo-reductases into sulfapyridine and 5-aminosalicylic acid in colon and cecum [10]. Although the complete and exact mechanism of action (MOA) of the drug is not fully understood, it is being suggested that 5-aminosalicylic acid is effective against inflammatory bowel diseases, *i.e.* it exerts the action locally in the colon with only low amounts getting absorbed into the bloodstream, whereas sulfasalazine and sulfapyridine is known to be significantly absorbed from the large intestine, responsible for its immunomodulatory and anti-inflammatory action against rheumatoid arthritis [11]. However, on continuous use, the patient's tolerability to the drug is largely impacted due to the elevated serum total free sulfapyridine levels, leading to development of various

adverse effects [12]. The reasons are primarily attributed to the individual acetylator phenotype [13] or could be related to the dose given. Therefore, different formulation strategies have been designed to bypass some of the problems associated with the drug substance, with more emphasis towards targeted drug delivery, one example being the mucoadhesive chitosan hydrogels to treat ulcerative colitis providing local effect, preventing the major side effects [14]. Also, sulfasalazine belongs to class IV of the Biopharmaceutical classification system (BCS), with low aqueous solubility and low permeability [15]. Therefore drug delivery systems (*e.g.* non-ionic surfactant vesicle systems using the micellar/niosomal formulation for entrapment of sulfasalazine) have been developed for enhancement of the solubility and thereby the bioavailability.

Thus, it is essential to develop a simple, precise and rapid, advanced and sensitive analytical method for accurate estimation of drug molecules when released from various formulation systems. The literature is indicative of various chromatographic and spectrometric methods being developed for estimation of sulfasalazine either alone [16-18] or along with its metabolites [19,20] or in combination with other drugs [21-23] or in the presence of degradation products [24-26]. Recently, a green TLC-densitometric and RP-HPLC chromatographic method was developed and validated as well for the simultaneous determination of sulfasalazine and its metabolites [27]. With each method having its own set of challenges associated with it, the objective of this work is to develop a simple, rapid, accurate, advanced and sensitive bioanalytical method with easy utility in all the laboratory settings. Also, considering the therapeutic potential of sulfasalazine with novel formulations being developed against various therapies, it is worthwhile to estimate its plasma concentrations using the proposed method. Therefore, in the present work a new, simple, rapid bioanalytical method was developed as well as validated for the accurate estimation and determination of sulfasalazine in rabbit plasma with potential to be utilized in pharmacokinetic studies of novel formulations.

EXPERIMENTAL

Sulfasalazine API drugs were obtained from the Wallace Pharmaceuticals, Mumbai, India as a gift sample. 4-Hydroxy benzoate (internal standard) was procured from Sigma-Aldrich, India. Acetonitrile and methanol used in the study were obtained from Merck, India, which were of HPLC grade. Ammonium acetate buffer (purity: 96-99%) and DMSO (analytical grade) were obtained from Loba Chemie, India and acetic acid was obtained from Merck, India, respectively. A Millipore system purifier was used to prepare Milli Q water.

Animals: Male healthy New Zealand white rabbits of 3-4 months, weight ranging from 1.8-2.2 kg were procured from National Institute of Biosciences, Pune (India). The rabbits were separately housed in the cages and fed as per standard pellet diet (VRK Nutrition Ltd., Pune, India) and kept under hygienic conditions. The animals were kept on 12 h light and dark cycles with free and easy access to water *ad libitum*. The experimental protocol of this study was approved by the Institutional Animal Ethics Committee (IAEC) of Poona college of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India. The IAEC approval no. for animal studies is: IAEC/PCP/PCL10/2019-2020. The CPCSEA registration no. is 1703/PO/Re/S/01/CPCSEA, India. The blood was collected from ear vein and transferred into EDTA tubes. The blood samples of the specific group of animals were then centrifuged at 2500-3500 rpm for 10-15 min at 4 ºC and the clear plasma was removed carefully in clean and dry eppendorf tubes. The plasma was stored in deep freezer (-15 to -20 ºC) until it could be used for analysis.

Chromatographic conditions: High performance liquid chromatographic method development and validation was carried out on a Jasco Inc. (Model PU 2080 plus) intelligent LC isocratic pump with rheodyne manual injector having fixed loop of 20 µL injection volume and a single wavelength UV detector (Model UV 2075 plus), Jasco International Co. Ltd., Japan. The acquisition and data processing of chromatographic data was carried out using the Jasco ChromNav software version 1.8 LC-Net II/ADC system. The analytical column Chromatopak C-18 basic Peerless (250 mm × 4.6 mm, 5µ) was used along with a C-18 guard column (4 mm × 3 mm). The mobile phase was a mixture of 10 mM ammonium acetate, pH adjusted to 4.5 using acetic acid and acetonitrile (70:30 v/v), pumped in isocratic mode with a flow rate 1 mL/min at ambient temperature. Prior to use, the mobile phase was passed through 0.45 µm membrane filter under vacuum using water jet vacuum pump (Model AP-19-645) and degassed under sonicator (Model ATS-2-LCD). The injection volume and the run time for analysis of each sample into the HPLC system was set at 20 µL and 16 min, respectively. The chromatograms were recorded at 362 nm.

Preparation of mobile phase: Accurately weighed ammonium acetate buffer was transferred into a mobile phase solution, made up volume with water (10 mM), sonicated for 10 min. The mobile phase was then filtered using of 0.45 µ membrane filter. The pH of the mobile phase was adjusted to 4.5 by using acetic acid. Premixed of the above solution with acetonitrile in the ratio of 70:30 and degassed for about 10-15 min.

Preparation of sulfasalazine and internal standard (IS) solutions: The stock solution of sulfasalazine (1 mg/mL) was prepared by dissolving accurately weighed samples in DMSO until it is dissolved completely, the remaining volume was adjusted using the mobile phase as diluent. Different working standard solutions were carefully prepared by serial dilution using mobile phase as diluent. The stock solution of IS (5 mg/mL) was also carefully prepared by dissolving accurately weighed samples in MeOH until it is dissolved completely, the remaining volume was adjusted using the mobile phase as the diluent. For system suitability, a premix solution of sulfasalazine and IS was prepared by dissolving appropriate quantities in the mobile phase to achieve a final and fixed concentration of 50 µg/mL and 500 µg/mL, respectively.

Sample extraction from plasma matrix: Sulfasalazine was extracted from the plasma samples by protein precipitation method. To 100 µL of rabbit plasma, 140 µL of sulfasalazine (250 µg/mL) and 70 µL of IS (5000 µg/mL) was added. For about 1 min, the sample mixture carefully was vortexed. To the mixture, added 390 µL of extraction solvent (MeOH). The sample mixture was again vortexed for about 1 min. The sample

mixture was carefully centrifuged at 2500-3500 rpm at 4 ºC for 10 min. The supernatant was carefully filtered through a 0.22µ filter and injected into the HPLC system. The final concentration of sulfasalazine was 50 µg/mL and IS was 500 µg/mL.

Bioanalytical method validation: The developed bioanalytical method for determination of sulfasalazine in biological matrix was validated as per the United States Food and Drug Administration Bioanalytical method validation guidance for industry [28].

Selectivity and specificity: The method selectivity was evaluated to confirm that the method is correctly targeting the desired analyte for quantification. Blank plasma sample (n = 6) was analyzed to check for interference and selectivity was evaluated against the lower limit of quantitation (LLOQ) level. There should not be any peak detected at room temperature of the analyte and IS or the response at the analyte retention time (RT) should be less than 20% of LLOQ response.

Sensitivity: The method sensitivity was determined by injecting the lowest non-zero sulfasalazine concentration where the S/N ratio for the lowest concentration that can be detected should be greater than 3. The LLOQ concentration was determined at levels where S/N is greater than 10 and it is the lowest concentration that can be quantified precisely and accurately. According to the USFDA guidelines, the area of the analyte peak at LLOQ level should be greater than 20% of the response obtained from the blank plasma peak. The acceptance limits for precision should be ± 20% of the %CV and the accuracy should be within ± 20% of theoretical concentration.

Accuracy and precision: Precision at LLOQ levels of sulfasalazine at 2.5 µg/mL was assessed by replicate injections of the same and determining the % CV of the response. The intraday and inter-day precision at LLOQ levels should be within ± 20% of coefficient of variation. Also, the precision of analytical method was estimated at three quality control levels apart from LLOQ *i.e*. LQC (5 µg/mL), MQC (30 µg/mL) and the HQC (100 µg/mL). The acceptance criteria for % CV for both intra and inter-day precision at all quality control levels should not exceed ± 15%. The accuracy levels were also computed by comparing the concentration obtained by back calculation to the true values obtained from the linearity equation and the acceptance limits for % accuracy should be ± 20% at LLOQ level and ±15% at other QC levels.

Linearity: The linearity of the method was evaluated through a calibration curve established against varying concentrations of sulfasalazine from LLOQ level to HQC level (2.5, 5, 10, 20, 30, 40, 75, 100 µg/mL). The internal standard concentration was fixed at 500 µg/mL. Calibration curve was obtained by plotting the ratio of drug/IS was evaluated against varying concentrations of sulfasalazine. The correlation coefficient for the equation should be greater than 0.995 to deem it linear.

Carryover: The impact of carryover from previous injection to the subsequent injection on the accuracy of analyte was determined by injecting blank samples after HQC injection. The acceptance criteria for the response in the post blank injection should be less than 20% of LLOQ response.

Extraction recovery and matrix effect: The extraction recovery study was carried out by comparing the results of analytical QC samples of extracted samples and corresponding true concentration of the analyte in the solvent that represents 100%. Replicate injections of samples at three QC levels were injected and the recovery of sulfasalazine was estimated. The recovery was determined using the following formula:

$$\text{Recovery (\%)} = \frac{\text{Peak area ratio of spiked sulfasalazine in plasma matrix}}{\text{Peak area ratio of sulfasalazine without the plasma matrix}} \times 100$$

Effect of matrix in the quantification of sulfasalazine at the lower limit of quantification level (LLOQ level) was carefully compared with matrix and without matrix response as well.

Stability: The stability studies for the analyte were estimated at three concentration levels (LQC, MQC and HQC) as short-term stability means storage of the analyte for 24 h at room temperature. Also, the analyte samples were stored at -20°C for 24 h in the freezer. The solution is considered stable if the % CV is within ±15% in comparison to the fresh sample injected.

RESULTS AND DISCUSSION

Method development: The first step involved in the method development was to identify the maximum wavelength of absorbance of sulfasalazine. Although the λ_{max} of sulfasalazine is reported in literature *i.e*. 359 nm [1], it was important to determine the λ_{max} with respect to the solvent used. The UV chromatogram is shown in Fig. 1 and the λ_{max} when mobile phase as the diluent was used and found to be 362 nm. From a HPLC method development standpoint, the exact efficiency of the method with respect to specific separation and resolution is generally influenced with different factors including, type of stationary phase, mobile phase composition and detector. The easiest being changing the mobile phase composition [29, 30]. However, proper peak shape was not obtained, either the peak was too broad or peak-splitting or too early peak elution was observed. Based on the literature findings, it was found out that sulfasalazine existed in three ionized forms depending on the pH of medium. The deprotonation of the carboxyl group SH^- (pK_{a1} = 2.35), the sulfonamide group SH_2^{2-} (pK_{a2} = 8.0), and the hydroxyl group SH_3^{6-} (pK_{a3} = 10.89) [31,32]. So organic modifiers were used (formic acid, acetic acid) to maintain and regulate the pH of mobile phase, however there was variability in the retention of peak and this could be attributed primarily to the volatility of acids. In addition to the peak symmetry and peak shape, present objective was to have a method where the compound is sufficiently retained in the column (RT above 5 min), so as to avoid interference with blank plasma peaks. Therefore, for retaining the compound, it was essential to have a buffered system as a mobile phase with an ability to control the pH of the system. Although phosphate buffers are commonly used buffer systems in HPLC mobile phase system as it has three specific pK_a values that give its three specific buffering ranges which are: 1.1 < pH < 3.1, 6.2 < pH < 8.2 and 11.3 < pH < 13.3 (allowing for the buffering of pK_a ± 1 pH specific units). Additionally, it has a low UV cut off with very low baseline

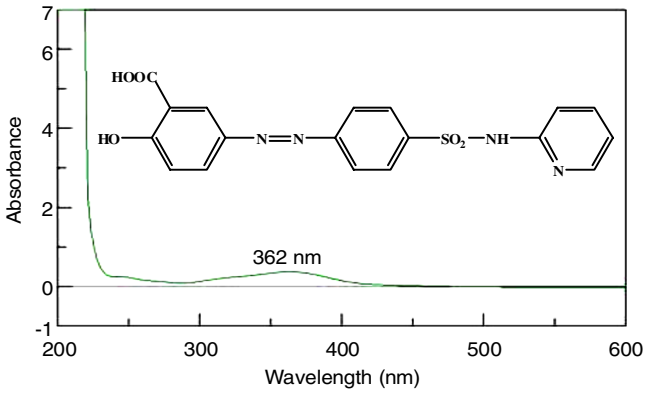

Fig. 1. UV spectra of sulfasalazine

noise, useful for detecting compounds even below 220 nm [33]. However, in present study, we intended to operate at pH ~ 4.5, which was ± 2 units away from the pK_a (2.35). Although the pH can be adjusted to 4.5 using phosphoric acid and its salts, it has negligible buffering capacity at this pH. Hence, acetate buffers were a preferable choice of buffer to function at this pH, which has a specific buffering range between 3.8 < pH < 5.8. They also have a low UV cut off and most importantly due to its volatile properties is also MS compatible. So, we initiated trials using 10 mM of ammonium acetate, adjusting pH to 4.5 with acetic acid as the aqueous phase and acetonitrile as organic phase. The composition with the ratio of 70:30 of aqueous buffer to organic phase gave a good symmetrical peak (As~1.3) and (NTP > 5000) with RT at 6.7 min, when the stationary phase used was Chromatopak C-18 basic peerless (250 mm × 4.6 mm, 5µ). The column was end-capped suitable for basic compounds that are susceptible to peak tailing. Also, an internal standard was incorporated to aid in the quantification of analyte, especially important while extracting analytes from biological matrix, negating the volumetric or extraction loss [34]. 4-Hydroxy benzoate was used as an internal standard, as it met most of the desired characteristics expected from an internal standard *i.e.* high purity, readily available, cheap, stable, possessing functional group similarity with sulfasalazine. From the method perspective it should give a sharp symmetric peak, well resolved from the main analyte peak without interfering with the matrix peaks. However, for good response at the λ_{max} of sulfasalazine (362 nm), higher concentration of IS was required to be injected (500 µg/mL). So, a mixture of both sulfasalazine and IS gave good sharp symmetrical well resolved peaks at 6.7 and 12.5 min, respectively as shown in Fig. 2.

Optimization of sample extraction procedure: While developing a bioanalytical method, the complete extraction of the specific analyte and IS from the biological matrix is essential. Different methods are used for precipitating proteins from biological matrices [35]. The simplest being the use of organic solvents like acetonitrile, methanol, acetone, *etc*. or mixture of two solvents. Methanol when used as an extraction solvent gave better recovery of analyte and IS (~100%) in plasma in comparison to acetonitrile, which gave a recovery of ~67%. Hence methanol was used as an extraction solvent in the method developed and the following protocol was fixed for all other validation parameters.

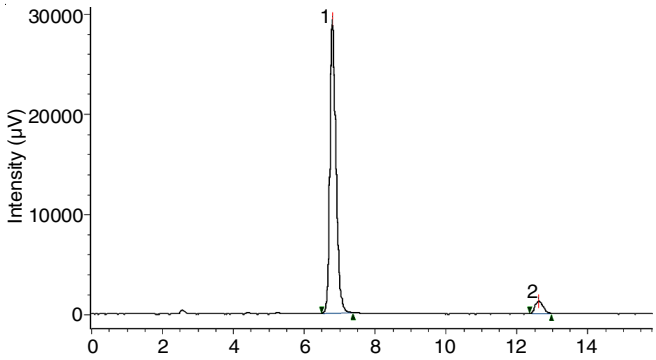

Fig. 2. Representative chromatogram of sulfasalazine and IS mixed with sulfasalazine peak at 6.7 min and IS peak at 12.5 min

Selectivity and specificity: The specificity of any method is the capacity, which specifically to unequivocally separate the analyte in any specific biological fluids. Hence, the chromatogram of the blank plasma samples is generally specific matched with chromatogram of the plasma spiked *via* analytes. Any specific developed method can be considered specific in that condition when there is no interference and crossing of endogenous components peaks with the drug peaks or otherwise. In the current study, the specificity of the developed new method was carefully evaluated by assessing the specific chromatograms of blank plasma with the chromatograms of plasma containing sulfasalazine and IS. On comparing the chromatograms as shown in Fig. 3, no peak was detected at the analyte (6.7 min) and IS (12.5 min) retention time. Therefore, it can be summarized and concluded that interference is not present of analytes with plasma matrices and hence the developed method is specific.

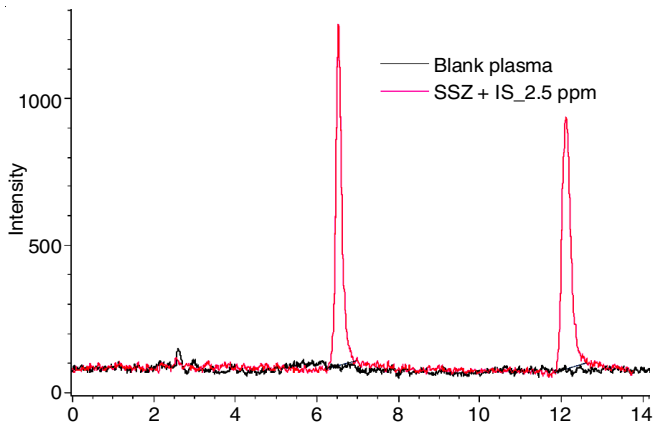

Fig. 3. Overlay chromatogram of blank plasma peak and plasma sample spiked with sulfasalazine & IS with sulfasalazine peak at 6.7 min and IS peak at 12.5 min

Sensitivity: The LOD level of the method was found to be 0.5 µg/mL where S/N was found to be greater than 3. The LLOQ was found out to be 2.5 µg/mL, where S/N was found to be greater than 10. Also at this concentration level, the analyte response was found to be greater than 5 times the area of the blank plasma peak. In the LLOQ precision study *i.e.* intra-day precision, the % CV was within the accepted limits of ± 20% (2.96%) and for the inter-day precision study, the % CV was

TABLE-1
ACCURACY AND PRECISION DATA OF SULFASALAZINE

Quality control level	Intra-day							Inter-day						
	Run	Area response ratio	Conc. (µg/mL)	Mean	SD	CV (%)	Accuracy	Run	Area response ratio	Conc. (µg/mL)	Mean	SD	CV (%)	Accuracy
LLOQ (2.5 µg/mL)	1	0.91	2.57	2.50	0.07	2.96	102.70	1	0.84	2.37	2.43	0.14	5.93	94.80
	2	0.90	2.54				101.57	2	0.93	2.62				104.95
	3	0.85	2.40				95.92	3	0.81	2.29				91.41
LQC (5 µg/mL)	1	1.81	4.92	5.11	0.17	3.23	98.32	1	1.70	4.62	4.95	0.25	5.03	92.36
	2	1.87	5.08				101.69	2	1.85	5.03				100.51
	3	1.96	5.32				106.37	3	1.92	5.22				104.31
MQC (30 µg/mL)	1	21.25	28.97	28.63	0.82	2.86	96.55	1	21.99	29.97	28.32	1.20	4.24	99.90
	2	20.18	27.50				91.66	2	20.44	27.85				92.85
	3	21.58	29.42				98.06	3	19.92	27.14				90.48
HQC (100 µg/mL)	1	75.44	98.72	95.73	2.55	2.66	98.72	1	69.26	90.63	94.49	3.26	3.45	90.63
	2	73.35	95.99				95.99	2	75.34	98.60				98.60
	3	70.68	92.49				92.49	3	72.02	94.24				94.24

within the accepted limits of ± 20% (5.93%). The % accuracy at the LLOQ level was within the ± 20 % of the theoretical concentration (91.41-104.95%). The results are summarized in Table-1.

Accuracy and precision: The % CV for intra-day precision at the three quality control levels (5 , 30 and 100 µg/mL) were found to be within the accepted limits of ± 15% *i.e*. 3.23, 2.86 and 2.96%, respectively. The percentage accuracy of the concentration obtained in comparison to the true concentration values was also within the acceptance limits of ± 15% *i.e*. it ranged between 98.32-106.37%, 91.66-98.06%, 92.49-98.72% at the three concentration of QC levels (5, 30 and 100 µg/mL), respectively. The % CV for inter-day precision at the three quality control levels (5, 30 and 100 µg/mL) were found to be within the accepted limits of ± 15% *i.e*. 5.03, 4.24 and 3.45%, respectively. The % accuracy of the concentration obtained in comparison to the true concentration values was also within the acceptance limits of ± 15% *i.e*. it ranged between 92.36-104.31%, 90.48-99.90%, 90.63-98.60% at the three concentration of QC levels (5, 30 and 100 µg/mL), respectively. Based on the experimental results obtained, the specific method can be said to be repeatable and reproducible. The results are summarized in Table-2.

TABLE-2
RECOVERY VALUES AT DIFFERENT QC LEVELS OF SULFASALAZINE

Quality control level	Mean area response ratio	SD	Recovery (%)
LLOQ (2.5 µg/mL)	0.84	0.02	90.68
LQC (5 µg/mL)	1.83	0.03	97.16
MQC (30 µg/mL)	20.51	0.41	94.26
HQC (100 µg/mL)	72.77	1.33	95.59

Linearity: The linearity range provides the specifically direct relationship in between area responses and the drug content present in a specified defined range in biological samples. An eight point and specific calibration curve was constructed for sulfasalazine ranging from the LLOQ concentration of 2.5 µg/mL to 100 µg/mL. The linearity specific curve was drawn by the plotting concentration in the range of 2.5-100 µg/mL *vs*. drug/IS area response ratio. A linear regression equation was obtained and later calculated from the corresponding graphs as summarized in Fig. 4. In this method, the specific linearity equation which was obtained with r^2 value obtained as 0.997 and greater than the accepted limits of 0.995. The equation can be used to determine the unknown concentration of sulfasalazine when present in the linear range between 2.5-100 µg/mL.

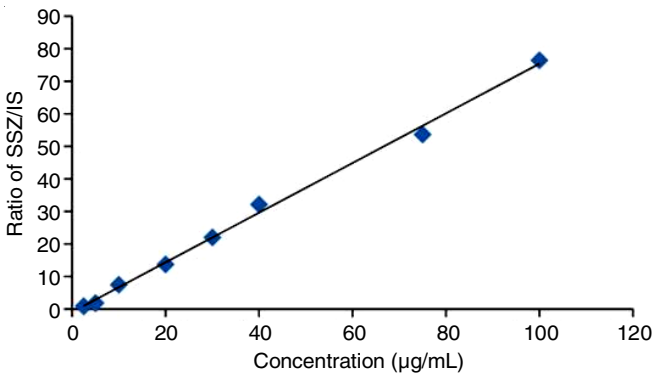

Fig. 4. Calibration curve – area ratio of sulfasalazine/IS *vs.* concentration (µg/mL)

Carryover: The response in post-blank injection after injecting sulfasalazine HQC sample was found to be within the accepted limits of carryover limits set as < 20% of LLOQ response. Therefore, the method can be stated as devoid of any carryover effect of the previous sample injected.

Recovery and matrix effect: The recovery of sulfasalazine from plasma was carried out with the standard addition and specific method at the specific three QC levels. The mean percentage (%) recovery of the SSZ from the plasma matrix was found to be 95.59-97.16%. Moreover, the obtained results of recovery percentage at LLOQ level verified that there is no more matrix interference with sulfasalazine in the rabbit plasma sample, since any matrix interference at the lowest level of quantification method can result in erroneous quantification of the sample.

TABLE-3
SHORT TERM STABILITY DATA OF SULFASALAZINE

Quality control level	Bench top stability_24 h							Refrigerator_24 h						
	Run	Area response ratio	Conc. (µg/mL)	Mean	SD	CV (%)	Accuracy	Run	Area response ratio	Conc. (µg/mL)	Mean	SD	CV (%)	Accuracy
LQC (5 µg/mL)	1	1.68	4.56	4.75	0.16	3.27	91.27	1	1.82	4.94	4.78	0.12	2.45	98.88
	2	1.75	4.75				95.08	2	1.74	4.73				94.53
	3	1.82	4.94				98.88	3	1.72	4.67				93.45
MQC (30 µg/mL)	1	21.34	29.08	28.33	1.51	5.33	96.95	1	21.87	29.81	28.71	0.81	2.84	99.36
	2	21.78	29.68				98.95	2	20.44	27.85				92.85
	3	19.24	26.22				87.41	3	20.89	28.47				94.90
HQC (100 µg/mL)	1	68.45	89.57	93.46	2.97	3.18	89.57	1	70.04	91.65	94.91	2.58	2.72	91.65
	2	73.96	96.78				96.78	2	74.87	97.97				97.97
	3	71.86	94.04				94.04	3	72.68	95.11				95.11

Stability: The results from the short term stability of sulfasalazine solution indicated from the % CV value that they are well within ± 15% of the accepted limits when sample solutions were kept at bench-top and refrigerator conditions for 24 h. The accuracy at the three QC levels ranged between 87.41-99.36%. The results for solution stability are summarized in Table-3. Based on the results, the sample solution can be considered stable at least for 24 h of preparation.

Conclusion

A simple, fast, precise, accurate, specific and sensitive RP-HPLC bioanalytical method has been established for the quantitative estimation of sulfasalazine in rabbit plasma. With a simple sample preparation by protein precipitation and a good percentage of recovery obtained in plasma, the newly developed analytical method holds good potential to be used for quantification of sulfasalazine in other biological matrices as well. Further, the proposed validated analytical method could be utilized in pharmacokinetic studies for evaluating and determine the sulfasalazine concentration in the new formulations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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