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RP-HPLC Method for Development and Validation of Dasatinib in Bulk, Tablets Applied in Nano suspension, Drug Entrapment Efficiency and Serum

Alekhya CH* and Krishna VS

Department of Pharmaceutics, Malla Reddy College of Pharmacy, India

***Corresponding author:** Alekhya CH, Department of Pharmaceutics, Malla Reddy College of Pharmacy, India; E-mail: alekhyachintanippu [at] gmail [dot] com

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Abstract

A simple, precise, accurate and robust RP-HPLC method was developed and validated for the determination of dasatinib in bulk and its Tablets, Nano-suspension drug entrapment efficiency and serum. The software used is EZ Chrome and the column employed is Syncronis C18 (250 mm × 4.6 mm, 5 µm particle size). Acetonitrile: Methanol: water 45:35:20 (v/v) was used as mobile phase at a flow rate of 1 mL min^{-1} with UV detection at 325 nm. Linearity was observed in the concentration range of 1–6 µg mL^{-1} with regression equation y =40966x-6534(R^2 = 0.999). The Nano-suspension prepared by novel precipitation-combined ultra-sonic homogenization technique and was then characterized by using particle size analysis, zeta potential measurement. Liquid- liquid extraction is performed for isolation of the drug and elimination of serum interferences samples of serum was extracted with 50µL of ortho phosphoric acid and 3ml of methanol was added and spiked with dasatinib. The method was validated as per ICH guidelines. The RSD for intra-day (99.2) and inter-day (98.3) precision were found to be less than 2 %.The developed method is simple, precise and robust for the determination of dasatinib in bulk and applied to tablets, Nano-suspension, drug entrapment efficiency and serum.

Keywords: Dasatinib; RP-HPLC; Method validation; Tablets; Nano-suspension; Drug entrapment efficiency; Serum

Introduction

Dasatinib (DAS), a small molecule tyrosine kinase inhibitor, can effectively fight against chronic myelogenous leukemic (CML) and acute lymphoblastic leukaemia (ALL) by inhibiting the activity of both Src and BCR-ABL tyrosine kinases in leukaemia cells [1-5]. However, DAS treatment has been reported to cause serious hematologic and non-hematologic adverse effects due to its interaction with non-disease-related processes and cells, which often leads to a dose reduction or treatment discontinuation in clinic [6]. Peripheral edema and pleural effusion are the common non-hematologic side effects occurred during DAS treatment, which is likely caused by endothelial hyper permeability [7-9]. Dasatinib is official in I.P and B.P. A thorough literature survey has revealed that UV spectroscopy [4-6], HPLC [7-9] method for Dasatinib with combination of other drugs, UPLC [10], LC-MS [11,12], GC-MS [13] for its estimation in bulk, pharmaceutical dosage forms and biological samples 14. Till now there is no reported method for dasatinib preparation of Nano-suspension and estimation drug entrapment.

Experimental

Materials and reagents

Dasatinib was gift sample from NATCO Pharm. (Hyderabad, A.P, INDIA). Acetonitrile and methanol was purchased from Rankem Chemical Company (India). The 0.45 µm pump Nylon filter was obtained from Advanced Micro Devices (Ambala Cantt, India) & whatman no 5 filter paper was obtained from Modern Science lab, (Nashik, India). Other chemicals used were analytical or HPLC-grade and glassware's used were Class A grade.

Instrumentation

Reverse phase - High performance liquid chromatography (Agilent) equipped with UV detector. The software used is EZ

Chrome and the column employed is Syncronis C_{18} (250 mm × 4.6 mm, 5 µm particle size).

Selection of wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. A wavelength which gives good response for the drugs to be detected is to be selected. From the UV spectra obtained for drugs, 325 nm was selected as the wavelength for study.

Selection of mobile phase

Solvent selectivity (solvent type), solvent strength (percentage of organic solvent in the mobile phase), strength and pH of buffer, flow rate etc. were varied to determine the chromatographic conditions that gave the best separation.

HPLC method validation

As per the International Conference on Harmonization (ICH) guidelines, the method validation parameters like linearity, precision, accuracy, limit of detection, limit of quantification, specificity and robustness were experimentally determined and the method validated.

Specificity

Specificity for an assay ensures that the signal measured comes from the substance of interest and there is no interference from excipient and/or degradation products and/or impurities. Specificity of the method was done by comparing the chromatogram of drug with the chromatogram of blank (mobile phase).

Linearity

Linearity was performed by taking from stock solution (100µg/ml) aliquots of 1, 2, 3, 4, 5 and 6µg/ mL were taken in 10mL volumetric flasks and diluted up to the mark with diluents such that the final concentrations are in the range of 1-6µg/ml. Each of these drug solutions (10µL) was injected into the chromatographic system for three times.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ= the standard deviation of the response and S= slope of the calibration curve

Assay

Twenty tablets were weighed accurately and finely powdered. A powder equivalent to 100 mg of Dasatinib was transferred carefully to 100 mL volumetric flask and about 15mL diluent was added. The mixture was sonicated for 10minutes. The volume was made up to 100mL with diluent, filtered through Whatman no. 5 filter paper. The final solution was injected in HPLC, chromatogram was recorded and area was measured.

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the pre-analysed formulation. The analysis was conducted in triplicate. Percentage recovery was calculated.

Robustness

It is carried by calculated by comparing the area before and after the addition of working standard. Changing the flow rate of mobile phase from 0.95-1.05 mL/min, and wavelength from 323 to 327 nm. Dasatinib made in triplicates and were analysed.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method.

Repeatability

Repeatability was determined by preparing six replicates of 20 µg/ml dasatinib separately inject equal volumes (20 µl) of each solution. Record the chromatograms and measure the peak response of drug. The results were reported as %RSD.

Intermediate precision

Interday precision: Interday precision study was carried out by preparing drug solution of concentration (20 µg/ml) and analysing it at three different days to determine interday precision. Record the chromatograms and measure the peak response of Dasatinib. It is shown in Table 4. The results were reported as %RSD.

Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is carried by changing the flow rate of mobile phase from 0.95 to 1.05 mL/min and by changing in wavelength from 323 to327 nm. 2 µg/ml dasatinib was analysed and the %RSD is determined.

System suitability parameters

System suitability tests are an integral part of chromatographic method. To ascertain its effectiveness, system suitability tests were carried out by injecting freshly prepared standard stock solution of 10µg/ml dasatinib six replications and the parameters like retention time, peak area, plate number (N), and peak asymmetry of samples were calculated.

Formulation of dasatinib nano-suspension

Dasatinib Nano-suspensions were prepared by combination method of precipitation –ultrasonic homogenization.40mg of dasatinib is dissolved in 10ml of DMSO. Eduragit RS 100 and poloxamer188 and sonicated for about 10min. 1ml of tween80 is added to the above solution. Small amount of HPMC K100 is dissolved in 10ml of water. With the help of a syringe the polymer and surfactant mixture is added drop by drop to the drug and stirred vigorously. The final suspension is kept in ultrasonic homogenizer where the suspension was homogenized at 40 pulse and 50 power for about 30 min. The temperature should be maintained at 8oC in refrigerator in order to avoid particle aggregation. 0.1ml of Nano-suspension was dissolved in 100ml of volumetric flask and make up the volume with mobile phase.

Particle size

Particle size growth is mainly responsible for agglomeration. Precise sizing techniques can give useful information about the particle size distribution in Nano-suspension.18 The particle size was measured using particle size analyser HORIBA scientific Nano particle SZ-10 appropriate scattering intensity at 25˚C and sample was placed in disposable sizing cuvette at a count rate of 372.0 (kcps) for 20 s.

The proposed method applied to dasatinib nano-suspension entrapment efficiency

Entrapment efficiency is the % of drug that is successfully entrapped or absorbed into the Nano-suspension. Samples from each dasatinib Nano-suspensions were centrifuged at 10,000 rpm for 30 min using centrifuge. The amount of untrapped drug in the supernatant obtained after centrifugation was determined using HPLC. The encapsulation efficiency was determined after the reading of the filtered samples in the HPLC, performed in triplicate and calculated [14,15].

The percentage entrapment efficiency was calculated according to the following equation.

$$\%\text{Entrapment Efficiency} = \frac{Actual\ drug\ loading}{Theoritical\ drug\ loading}$$

Methods

Dasatinib spiked in serum extraction process

Trail 1: 0.2ml of serum samples, 50µL of ortho phosphoric acid and 3ml of n-hexane was added. The sample were mixed in a mechanical shaker for 20 min and centrifuged at 1000 rpm for 10 min. After centrifuged, supernant layer was separated and make up to 10ml with mobile phase. To the above solution, 2 ug/ml concentration of drug solution was spiked and solution was injected.

Trail 2: 0.2ml of serum samples, 50µL of ortho phosphoric acid and 3ml of methanol was added. The sample were mixed in a mechanical shaker for 20 min and centrifuged at 1000 rpm for 10 min. After centrifuged, supernant layer was separated and make up to 10ml with mobile phase. To the above solution, 2 ug/ml concentration of drug solution was spiked and solution was injected.

Results and Discussion

Different mobile phase systems in different proportions were tried. From this a mixture of methanol: acetonitrile: water (4.5:3.5:2.0 v/v) produced symmetric peak shape for the drugs. A wavelength of 325nm was selected based on UV studies. Chromatographic conditions used are stationary phase Systronis C_{18} (250mm x 4.6 mm), 5m. The flow rate was maintained at 1ml/min, column temperature was set to 30ºC and diluents were mobile phase conditions were finalized as optimized method. Out of 6 trials performed, the 6th trail was selected because when compared to other trails, the 6th trial had the least in retention time, with good peak symmetry as mention in (Table 1 and Figure 1).

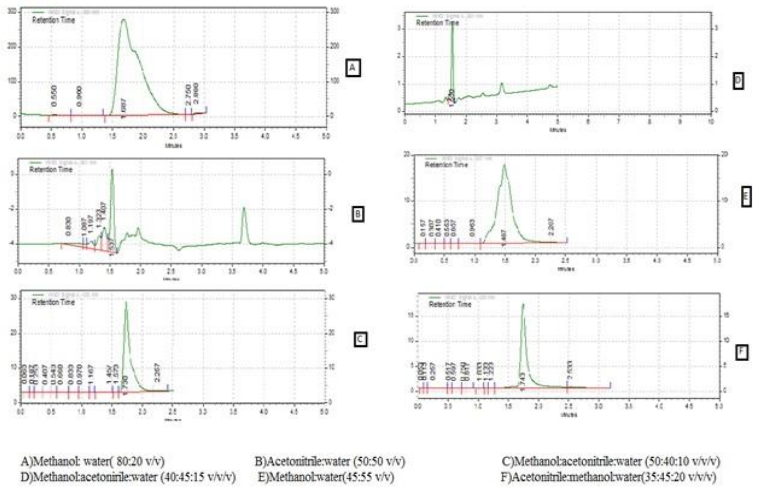

Figure 1: *Different trails of mobile phases.*

Specificity

Specificity of the method was done by comparing the chromatogram of drug with the chromatogram of blank (mobile phase) (Figure 2).

Table 1: *Selection of mobile phase.*

Trials	Mobile phase	Observation	Remarks
1	Methanol: Water (80:20) v/v	Broad peak appearance	Not satisfactory
2	Acetonitrile : Water (50:50 v/v)	Negative Extra peak with tailing	Not satisfactory
3	Methanol : Acetonitrile: water (50:40: 10 v/v/v)	Sharp peak with tailing	Not satisfactory
4	Methanol : Acetonitrile: water (40:45: 15 v/v/v)	Sharp peak with small fronting	Not satisfactory
5	Methanol:Water (45:55 v/v)	Sharp peak with extra peak	Not satisfactory
6	Acetonitrile: methanol:Water (45:35:20 v/v)	Sharp peak appear	Satisfactory

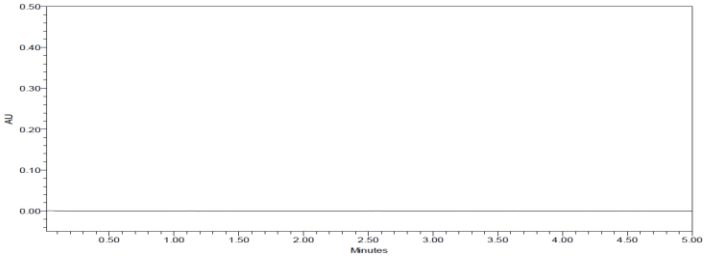

Figure 2: *Typical Chromatogram Blank (Mobile Phase).*

Linearity

Standard solutions of dasatinib in the concentration range of 1-6µg/ml were injected into chromatographic system and peak areas were measured. A graph of peak areas (on Y-axis) versus concentration (on X-axis) was plotted and calibration graph was shown in Figure 3. The regression equation was found to be y = 40966x + 6534. The correlation coefficient should not be less than 0.999 for dasatinib. The results were reported as %RSD. The precision result showed a good reproducibility shown with percentage relative standard deviation less than 2.

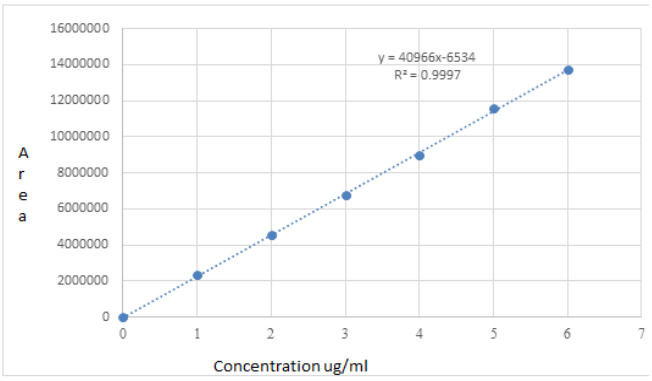

Figure 3: *Calibration curve of Dasatinib.*

Limit of detection and limit of quantification

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD = 0.046µg/ml and LOQ = 0.1400µg/ml.

Assay

Estimation of dasatinib tablet dosage forms were carried out 100.00 as assay value. The % purity for Dasatinib should be 98-102 %. The result of assay obtained was found to be in good agreement with the labelled claim, indicating the absence of interference of the excipients and results are shown in (Table 2) and chromatogram was recorded and area was measured which was shown in (Figure 4).

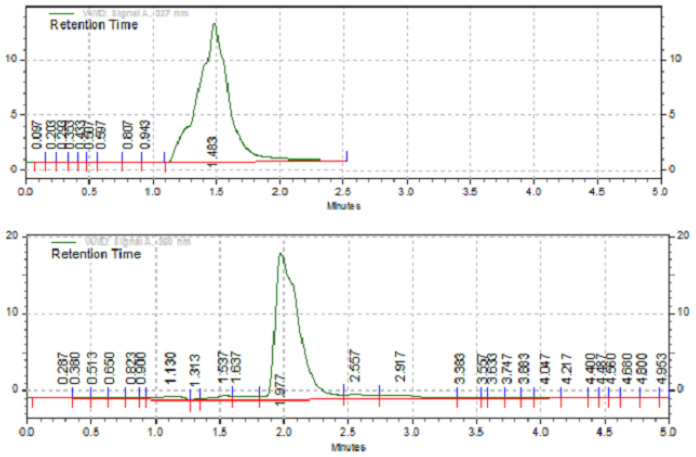

Figure 4: *Typical Chromatogram of Dasatinib flow rate a.1.1 b. 0.9 mL/min.*

Table 2: *Assay results of Dasatinib.*

Formulation	Label claim	Amount found	% Purity
SPRYCEL (100mg)	100mg	98.98mg	99.83%

Accuracy

The % RSD for the individual recoveries of each level and mean recovery should not be more than 2%. The % recovery at each level and mean recovery should be between 98.0-102% and results are shown in (Table 3).

Robustness

In the case of the robustness study, the values obtained for the statistical parameters of the chromatographic responses for all variations (detection wavelength and flow rate) at the target concentration level of 2 µg/ml. The small magnitude of % RSD obtained as a result of introducing small calculated variations in

the eluent composition, detection wavelength and flow rate suggested the robustness of the developed system. The results were presented in the (Table 4). The chromatograms of flow rate and wave length are shown in (Figure 5).

Table 3: *Accuracy studies of Dasatinib.*

Spiked level (%)	Formulation Conc (µg/ml)	Pure Drug Conc (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean recovery ±SD	%RSD
50	1	2	1.91	95.93	99.77±0.76	0.9
	1	2	2.02	101.4		
	1	2	2.05	102		
100	1	4	3.68	98.6	99.53±0.520	1.09
	1	4	3.76	99.5		
	1	4	4.05	100.5		
150	1	6	5.71	95.2	98.6±0.501	0.89
	1	6	6.01	100.2		
	1	6	6.02	100.4		

Table 4: *Repeatability studies of Dasatinib.*

Concentration [µg/ml]	Area	RT	Area Mean ±SD	%RSD
2	487534	1.800	479218 ± 9025	0.5
2	468667	1.660		
2	476756	1.753		
2	486003	1.623		
2	468887	1.702		
2	487462	1.698		

Table 5: *Inter-day precision of Dasatinib.*

	Inter day				Intra day			
Concentration[µg/ml]	AUC	RT	AUC Mean ±SD	%RSD	AUC	RT	AUC Mean ±SD	%RSD
2	469387	1.643	449473± 31877	0.28	486097	1.73	487595±1879	0.38
2	410015	1.713			489743	1.80		
2	477304	1.801			488528	1.78		
2	407318	1.670			484678	1.77		

2	465382	1.5048			487754	1.79		
2	467434	1.5043			488775	1.81		

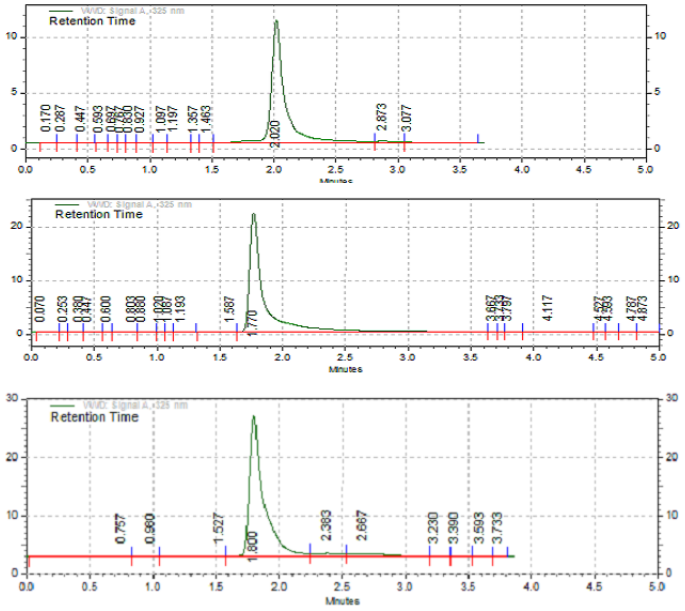

Figure 5: *Chromatogram of Accuracy at I. 50%; II. 100%; III. 150%.*

Precision

Repeatability of the method was determined by analysing six samples of same concentrations of drug i.e. 2µg/mL. Chromatographs were recorded and area of each chromatograph was measured and the values are represented in the Table IV. A method is said to be precise if the % RSD is < 2 %, the results show % RSD for repeatability studies was 1.06 which indicates the results meet the acceptance criteria and hence the method is said to be precise. The inter-day and intra-day precision study was performed at 10µg/mL concentration levels and each value is the average of six determinations. The results were reported as %RSD and the results are presented in the (Table 5).

Table 6: *System Suitability Parameters.*

Parameters	Results
Retention time (min)	1.743
Theoretical plates	4708
Asymmetry	0.23

System suitability parameters

System suitability tests are an integral part of chromatographic method. System suitability tests like theoretical plates, tailing factor and HETP results of Rilpiverine hydrochloride are shown in (Table 6).

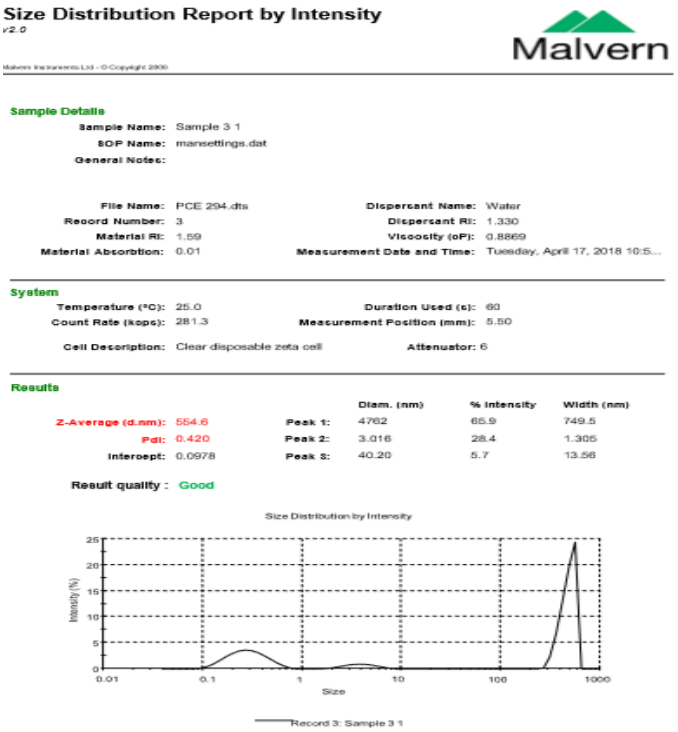

Figure 6: *Size distribution report by intensity.*

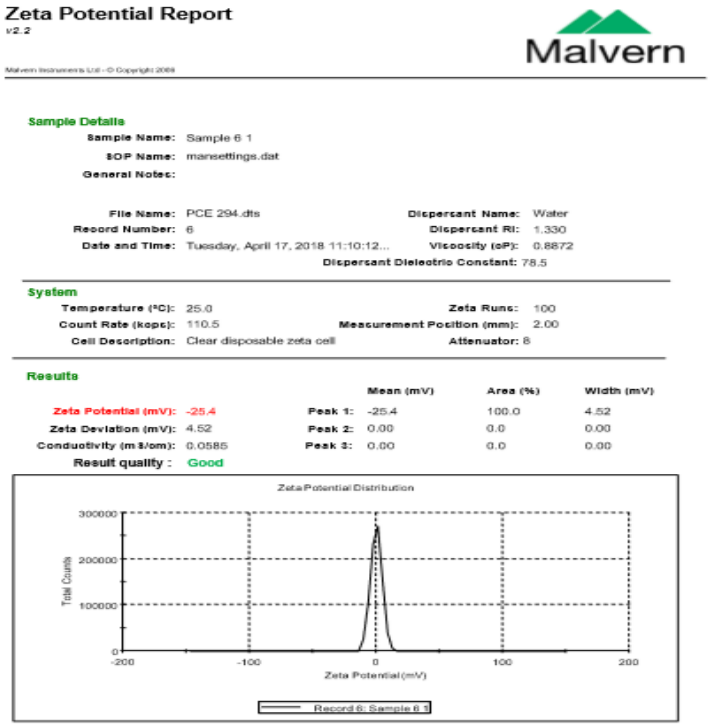

Figure 7: *Zeta potential report.*

Dasatinib nano-suspension particle size and zeta potential

The Nano-suspension prepared by precipitation-ultrasonic homogenization method are discrete, uniform, nearly spherical Nano-Meteric particle in the size of 345 nm are mentioned in (Figure 6 and 7). The zeta potential of the prepared dasatinib Nano-suspension formulations was found to be -25.4 [16-18].

Drug entrapment efficiency

Dastanib Nano-suspension formulation the drug particles were reduced to Nano sized. During the formulation process there was not any drug loss step involved, so theoretically the formulation was considered as being 100% drug content. The formulations, formulation drug loading efficiency of 84.2%.

Dasatinib spiked in serum

Out of 2 trial performed, the 2nd trial was selected for further studies because when compared to other trials 2nd trial was found good separation of saliva, with good peak symmetry. The proposed method is applied to dasatinib spiked with serum and done in high performance liquid chromatography with ultraviolet detection and the peak obtained was good and same retention time to bulk sample.

Conclusion

Calibration graphs were plotted using standard drug peak areas Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient were found to be a, b and c respectively. The results show that within the concentration range tested, there was excellent correlation between peak area and concentration. Dasatinib was found to be linear in the range of 1-6 µg/ml. The LOD and LOQ are 0.23 µg/ml and 0.72µg/ml respectively. The precision of the developed method was studied under intraday, inter day and repeatability. System suitability parameters like number of theoretical plates (N), peak asymmetry Factor (As) and Resolution (Rs) were studied by injecting the standard five times and results were well under the acceptance criteria. Developed RP-HPLC methods were simple, rapid, precise, economical, specific and reproducible for the qualitative and quantitative determination of Dasatinib. It was concluded that the developed methods offer several advantages such as rapidity, usage of simple mobile phase and sample preparation step. This method can be applied for routine analysis of quality control samples.

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Declaration of Interest

The author has no relevant affiliations or financial involvement with a financial interest in or financial with the subject matter or materials discussed in the manuscript.

Conflicts of Interest

There is no conflict of interest.

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