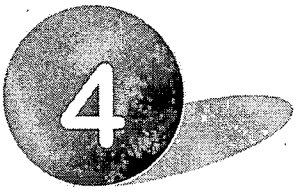

Development and Validation of Analytical Methods

Analytical Methods for Methotrexate

4.1 Development and Validation of Analytical Methods for Methotrexate

Literature survey revealed different analytical methods for the estimation of methotrexate (MTX) in bulk, pharmaceutical formulations and in biological samples like amperometric[1], voltametric[2], paper chromatography[3], spectrofluorimetric[4], HPLC[5,6], etc. For the present work simple spectrophotometric was developed whereas, reported high performance liquid chromatographic[5] method was also used for the study. Both the methods are described below.

4.1.1 Simple UV spectrophotometric method

4.1.1.1 Materials and methods

MTX was kindly donated by Biochem Pharma A.G. (Mumbai, India). HCl was purchased from E Merck (India). Commercial tablet formulation, Imutrex®, containing 10 mg of MTX was purchased from commercial source. Double distilled water was used, prepared by double distillation glass assembly. All the reagents and solvents were of reagent grade and were used without further purification. The instruments used for the estimation of drugs include pH meter (Picco+, Lab india, India), Double beam UV Visible Spectrophotometer (UV-1700-Shimadzu), Digital weighing balance (Ax 120, Shimadzu, Japan).

Standard stock solution of MTX

A stock solution was prepared by dissolving 50 mg of MTX in required quantity of 0.1 M HCl and diluting to 50 ml with methanol. From the above solution 2.5 ml was again diluted to 25 ml with same solvent to get 100 µg/ml solution of MTX.

Procedure for calibration curve

Suitable aliquots of the standard stock solution (0.2 to 1.6 ml) were taken in 10 ml volumetric flasks and the volume was made up to the mark with 0.1 M HCl to prepare a series of standard solution containing, 2 to 16 µg/ml MTX. The absorbance of all the solutions were measured at 306 nm and the calibration curve was plotted. The UV spectra and calibration curve are shown in Fig.4.1 and Fig. 4.2 respectively.

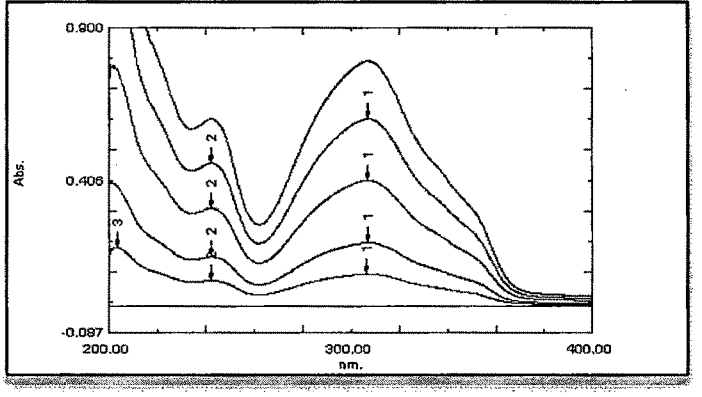

Figure 4.1: Overlain UV spectra of methotrexate in 0.1 M HCl in the concentration range of 2 to 16 µg/ml

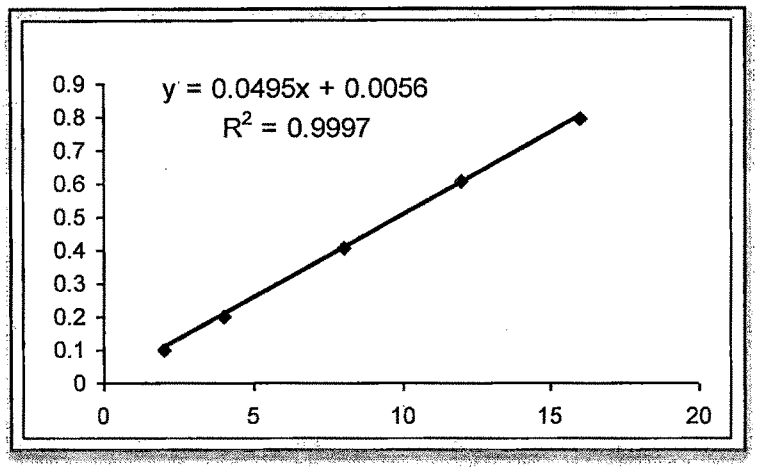

Figure 4.2: Calibration curve of methotrexate in 0.1 M HCl in the concentration range of 2 to 16 µg/ml

4.1.1.2 Results and discussion

Conformity with Beer's law

The calibration curve was linear in the concentration range of 2 to 16 µg/ml for MTX. The slope, intercept, and correlation coefficient were obtained by linear least square treatment of the results. The optical characteristics are summarized in Table 4.4.

Method Validation

Accuracy

The excipients present in the formulation may cause interference during analysis. The recovery studies were carried out to ascertain the accuracy of the method. Recovery studies were carried out on commercially available formulation by addition of known quantities of standard drug solution to pre analyzed sample solution.

Ten tablets were weighed and ground to fine powder. An accurately weighed quantity of powder sample equivalent to 50 mg of MTX was transferred to a beaker and extracted with 15 ml of 0.1 M HCl by stirring on magnetic stirrer for about 30 min. Then it was filtered through Whatman filter paper no. 42 in to a calibrated 50 ml volumetric flask. Filter paper was rinsed twice with 2 ml each of 0.1 M HCl and the volume of the combined filtrate was made up to 50 ml with 0.1 M HCl. From the above solution 5 ml was again diluted to 50 ml, to get 100 µg/ml solutions.

Same procedure was used to prepare 100 µg/ml solution of standard drug. Then Aliquots of 0.8 ml and 1 ml were taken from the formulation stock solution and each of them was spiked with standard drug solution at three levels by addition of aliquots of 0.2, 0.3, 0.4 ml. The amount of drug was calculated from the calibration curve.

Table 4.1: Recovery study of pharmaceutical formulation

Aliquots of sample (ml)	Std. spiked (ml)	Conc. of formulation (µg/ml)	Total Conc. Taken (µg/ml)	Total conc. found (µg/ml)	%Recovery
0.8	0.2	8	10	10.14	101.40
0.8	0.3	8	11	10.91	99.18
0.8	0.4	8	12	11.96	99.66
1	0.2	10	12	11.98	99.83
1	0.3	10	13	12.94	99.53
1	0.4	10	14	14.16	101.14

*Average of three determinations.

Precision

The precision of the proposed method was checked in terms of Intraday and Interday.

i) Intraday precision

The experiment was repeated four times in a day and percentage RSD was calculated at each concentration level.

ii) Interday precision

The experiment was repeated on four different days. The percentage RSD was calculated at each concentration level. The results given in Table 4.2, showing % CV of approx. 1.0 % at each level clearly indicates that the proposed method is precise enough for the analysis of MTX.

Table 4.2: Intraday and Interday precision

Concentration of sample (µg/ml)	Intraday precision % CV*	Interday precision % CV*
2	0.982	1.006
4	1.893	1.634
6	1.097	1.298
8	0.863	0.862
10	0.756	0.674
12	1.051	0.679
14	0.666	1.474
16	0.745	0.714

*Average of three determinations.

Linearity

The proposed method obeys Beer's law in the concentration range of 2 to 16 µg/ml and correlation coefficient (R^2) was found to be 0.9997.

Limit of detection (LOD) and limit of quantification (LOQ)

Blank measurements were repeated twelve times. The standard deviation (SD) was calculated. Then LOD and LOQ were measured as follows,

LOD=3 * SD/slope of calibration curve

LOQ=10 * SD/slope of calibration curve

SD = Standard deviation of twelve blank readings.

LOD and LOQ were calculated and values were found to be 0.1266 µg/ml and 0.2218 µg/ml respectively for MTX.

Reproducibility

The method was repeated by taking reagents from three different manufacturers. It was found that % RSD value was less than 1%. Reproducibility data are given in Table 4.3

Table 4.3: Data for reproducibility

Concentration of sample (µg/ml)	% RSD
4	0.9369
8	0.3853
12	0.4060
16	0.2735

*Average of three determinations.

Table 4.4: Summarized data for the developed method

Parameters	Result
λ max (nm)	306
Beer's law limit (µg/ml)	2 to 16
Regression equation (Y=mX+c)	0.0495x + 0.0056
Slope	0.0495
Intercept	0.0056
Limit of detection (µg/ml)	0.1266
Limit of quantification (µg/ml)	0.2218
Coefficient of determination	0.9997
% RSD	< 1%
Accuracy	> 99%

4.1.2 High performance liquid chromatography (HPLC)

4.1.2.1 Materials and methods

MTX was kindly donated from Biochem Pharma A.G. (Mumbai, India). Commercial tablet formulation, Imutrex®, containing 10 mg of MTX was purchased from commercial source. Hydrochloric acid, potassium dihydrogen phosphate, sodium hydroxide and acetonitrile were purchased from E-Merck (India). Double distilled water was used, prepared by double distillation glass assembly. All the reagents and solvents were of HPLC grade and were used without further purification.

The instruments used for the estimation of drugs include pH meter (Picco+, Lab India, India), High performance liquid chromatography with UV detector (HPLC Isocratic system with LC-20AT pump, Software-LC solution, Shimadzu), Digital weighing balance (Ax 120, Shimadzu, Japan).

Table 4.5: Chromatographic conditions

Drug:	Methotrexate
Column:	Hypersil BDS
Mobile Phase:	Phosphate buffer pH 6: CAN (92:8)
Needle wash:	Methanol
Flow rate:	1 ml/ min
Detector:	UV-Visible detector (SPD-20A)
Detection Wavelength:	303 nm
Temperature:	Room temperature

Preparation of standard and sample solutions

A stock solution of MTX was prepared by dissolving 25 mg in 250 ml of mobile phase. The standard solutions were prepared by dilution of the stock solution with mobile phase to obtain a concentration range of 10-100 µg/ml. Triplicate 20 µl injections were made six times for each concentration and chromatogram was recorded. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

4.1.2.2 Results and discussion

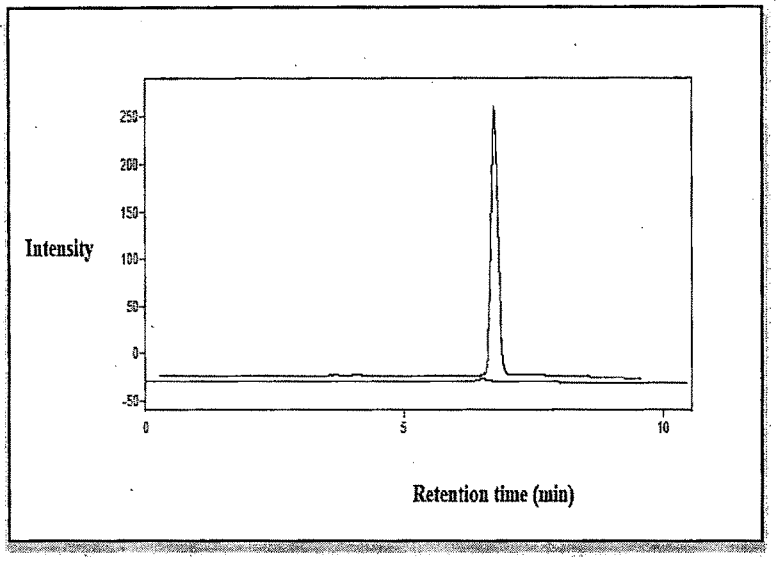

Figure 4.3: Chromatogram of MTX

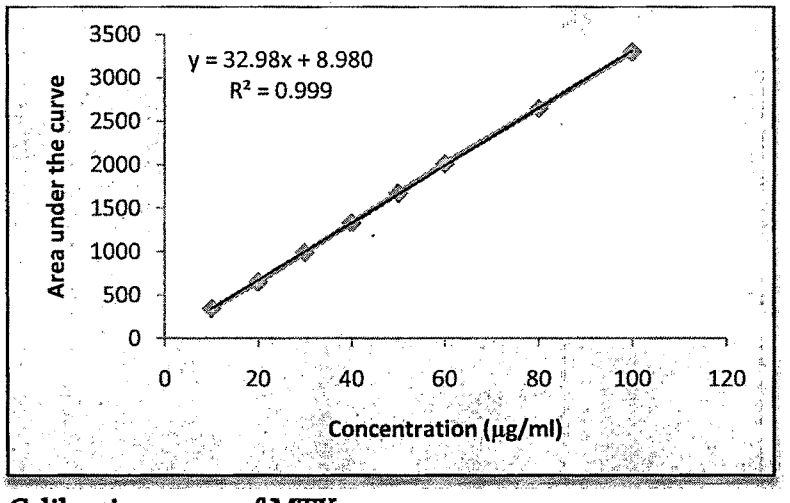

Figure 4.4: Calibration curve of MTX

Analytical Method Validation

Linearity

The calibration curve was found to follow Beer's law in the concentration range of 10 to 100 µg/ml. The linearity equation was Y= 32.98x+8.9801 with slope (a) = 32.98, intercept (b) = 8.9801 and coefficient of determination (R^2) = 0.9998.

Accuracy and Precision

Table 4.6 and 4.7 summarizes the accuracy and precision data of the method. Study was performed within the day and at different day, at three different concentration levels. The result was recorded as % RSD and was found within the limit; illustrate the reliability of the method.

Table 4.6: Intraday precision and accuracy

Actual conc. (µg/ml)	Observed mean* (µg/ml)	Accuracy ± RSD
15	15.08	100.53 ± 0.90
18	18.16	100.88 ± 0.56
20	19.79	98.95 ± 0.13

*Average of three determinations.

Table 4.7: Interday precision and accuracy

Actual conc. (µg/ml)	Observed mean* (µg/ml)	Accuracy± RSD
15	14.92	99.45 ± 0.23
18	17.88	99.33 ± 0.19
20	20.10	100.50 ± 0.15

*Average of three determinations.

Estimation of Formulation

Solutions containing 100 µg/ml of methotrexate of the claimed amount of marketed formulations were prepared in mobile phase. Tablet powder containing 5 mg/ml methotrexate was taken into beaker and 15 ml mobile phase was added followed by stirring on magnetic stirrer for about 15 min. The solution was filtered from Whatman filter paper no.42 into a volumetric flask. Filer paper was rinsed thrice with 2 ml of mobile phase and volume was made up to 50 ml with mobile phase. Suitable aliquots were made in mobile phase. Area under curve was noted at 303 nm for estimation of formulation (Table 4.8).

Table 4.8: Analysis of pharmaceutical formulation

Conc. of sample (µg/ml)	Conc. of sample obtained* (µg/ml)	% of Labeled claim
20	20.11	100.55
40	39.97	99.94
60	59.69	99.48

*Average of three determinations

Recovery study

Stock solution of 100 μg/ml in mobile phase was prepared from standard drug. Solutions containing 100 μg/ml of methotrexate of claimed amount of marketed formulations were prepared in mobile phase. Suitable aliquot were taken from 100 μg/ml stock solution (formulation) in 10 ml volumetric flask, and in same flask 1 ml of 100 μg/ml stock solution was added, and volumes were made up with mobile phase to prepare series of solution for recovery study. Absorbance was noted at 303 nm. Concentration of sample solutions was obtained by calculating back from the equation of the calibration curve. Results of analysis of pharmaceutical formulations and recovery study are shown in Table 4.9.

Table 4.9: Recovery study of pharmaceutical formulation by spiking method

Aliquots of sample (μg/ml)	Std. spiked (μg/ml)	Final Conc. (μg/ml)	Amount of MTX found* (μg)	% Recovery
20	10	30	29.882	99.60
40	10	50	49.883	99.76
60	10	70	71.421	101.03

*Average of three determinations

Table 4.10: Critical parameters for the HPLC method

Data	Result
λ_{max}	303 nm
Mobile phase	Phosphate buffer pH 6: ACN (92:8)
Needle wash	Methanol
Detector	U.V. Detector
Linearity range	10 to 100 μg/ml
Column	Hypersil BDS
Regression equation	32.98x + 8.980
Slope	32.98
Intercept	8.980
Coefficient of Determination(r^2)	0.999
Correlation Coefficient (r)	0.999

Analytical Methods for Dasatinib

4.2 Development and Validation of Analytical Methods for Dasatinib

Literature survey revealed chromatographic method like LC-MS/MS[7], HPLC-MS[8,9] and HPTLC[10] for the estimation of dasatinib (DTB) in biological samples and in pharmaceutical formulation. For the present work two simple spectrophotometric methods were developed. The first method was a simple UV method whereas the second was a colorimetric method. Both the methods were developed for the estimation of DTB in bulk and in pharmaceutical formulation.

4.2.1 Simple UV spectrophotometric method

4.2.1.1 Materials and methods

DTB was purchased from M/s Hwasun Biotechnology Co. Ltd, Shanghai, China. Double distilled water was used. Lactose monohydrate, microcrystalline cellulose, cascarmellose sodium, hydroxypropyl cellulose, magnesium stearate and HCl were purchased from E Merck (India). All the reagents and solvents were of reagent grade and were used without further purification. The instruments used for the estimation of drugs include pH meter (Picco+, Lab india, India), Double beam UV Visible Spectrophotometer (UV-1700-Shimadzu), Digital weighing balance (Ax 120, Shimadzu, Japan). Dasatinib tablets, each containing 10 mg of DTB were punched in laboratory.

Procedure for dasatinib tablet manufacturing

The dasatinib tablets were punched in laboratories by standard procedure[11,12]. The other ingredients used for tablet punching were lactose monohydrate, microcrystalline cellulose, cascarmellose sodium, hydroxypropyl cellulose, magnesium stearate and purified water.

Tablets were compressed using Rimek ten station rotary tablet machine using 05-mm diameter flat faced punches and die (Cadmach Machinery Private Ltd., Ahmedabad).

Standard stock solution of DTB

A stock solution was prepared by dissolving 50 mg of DTB in required quantity of 0.1 M HCl and diluting to 50 ml with the same. From the above solution 2.5 ml was again diluted to 25 ml with same solvent to get 100 µg/ml solution of DTB.

Procedure for calibration curve

Suitable aliquots of the standard stock solution (0.2 to 1 ml) were taken in 10 ml volumetric flasks and the volume was made up to the mark with 0.1 M HCl to prepare a series of standard solution containing, 2 to 10 µg/ml DTB. The absorbance of all the above solutions were measured at 329 nm and the calibration curve was plotted. The UV spectra and calibration curve are shown in Fig.4.5 and Fig. 4.6 respectively.

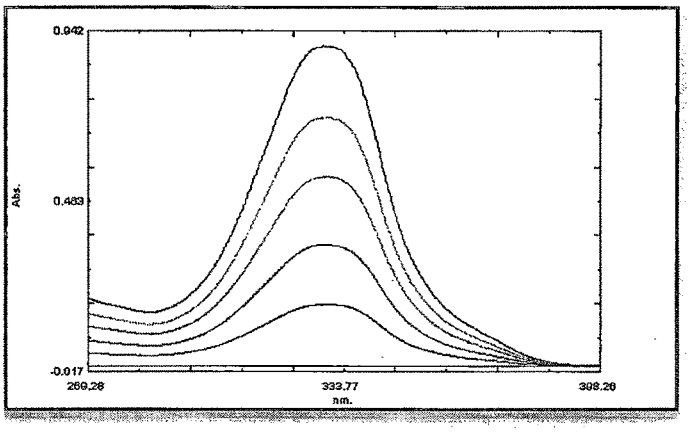

Figure 4.5: Overlain UV spectra of dasatinib in 0.1 M HCl in the concentration range of 2-10 µg/ml

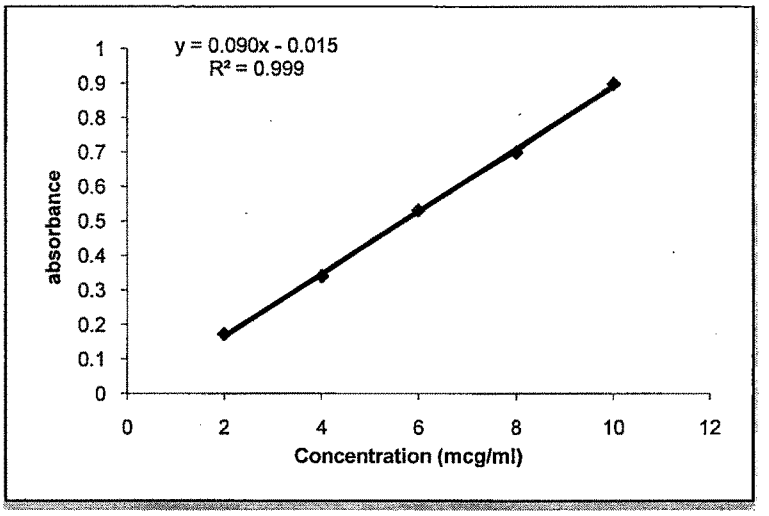

Figure 4.6: Calibration curve of dasatinib in 0.1 M HCl in the concentration range of 2-10 µg/ml

4.2.1.2 Results and discussion

Conformity with Beer's law

The calibration curve was linear in the concentration range of 2 to 10 µg/ml for DTB. The slope, intercept, and correlation coefficient were obtained by linear least square treatment of the results. The optical characteristics are summarized in Table 4.14.

Method Validation

Accuracy

The excipients present in the formulation may cause interference during analysis. The recovery studies were carried out to ascertain the accuracy of the method. Recovery studies were carried out on commercially available formulation by addition of known quantities of standard drug solution to pre analyzed sample solution.

Ten tablets were weighed and ground to fine powder. An accurately weighed quantity of powder sample equivalent to 50 mg of DTB was transferred to a beaker and extracted with 15 ml of 0.1 M HCl by stirring on magnetic stirrer for about 30 min. Then it was filtered through Whatman filter paper no. 42 in to a calibrated 50 ml volumetric flask. Filter paper was rinsed twice with 2 ml each of 0.1 M HCl and the volume of the combined filtrate was made up to 50 ml with 0.1 M HCl. From the above solution 5 ml was again diluted to 50 ml, to get 100 µg/ml solutions.

Same procedure was used to prepare 100 µg/ml solution of standard drug. Then aliquots of 0.4 ml and 0.6 ml were taken from the formulation stock solution and each of them was spiked with standard drug solution at three levels by addition of aliquots of 0.2, 0.3, 0.4 ml. The amount of drug was calculated from the calibration curve. Data are reported in Table 4.11.

Table 4.11: Recovery study of pharmaceutical formulation

Aliquots of sample (ml)	Std. spiked (ml)	Conc. of formulation (µg/ml)	Total Conc. Taken (µg/ml)	Total* conc. found (µg/ml)	% Recovery
0.4	0.2	4	6	6.14	102.33
0.4	0.3	4	7	6.98	99.71
0.4	0.4	4	8	8.10	101.25
0.6	0.2	6	8	7.99	99.87
0.6	0.3	6	9	9.12	101.33
0.6	0.4	6	10	9.97	99.70

*Average of three determinations.

Precision

The precision of the proposed method was checked in terms of Intraday and Interday.

i) Intraday precision

The experiment was repeated four times in a day and percentage RSD was calculated at each concentration level.

ii) Interday precision

The experiment was repeated on four different days. The percentage RSD was calculated at each concentration level. The results given in Table 4.12, showing % CV of approx. 1.0 % at each level clearly indicates that the proposed method is precise enough for the analysis of DTB.

Table 4.12: Intraday and Interday precision

Concentration of sample (µg/ml)	Intraday precision % CV*	Interday precision % CV*
2	0.925	0.957
4	0.998	0.898
6	0.959	0.674
8	1.011	1.021
10	1.016	0.987

*Average of three determinations.

Linearity

The proposed method obeys Beer's law in the concentration range of 2 to 10 µg/ml and correlation coefficient (R^2) was found to be 0.999.

Limit of detection (LOD) and limit of quantification (LOQ)

Blank measurements were repeated twelve times. The standard deviation (SD) was calculated. Then LOD and LOQ were measured as follows,

LOD=3 * SD/slope of calibration curve

LOQ=10 * SD/slope of calibration curve

SD = Standard deviation of twelve blank readings.

LOD and LOQ were calculated and values were found to be 0.01985 µg/ml and 0.09364 µg/ml respectively for DTB.

Reproducibility

The method was repeated by taking reagents from three different manufacturers. It was found that % RSD value was less than 1%. Reproducibility data are given in Table 4.13

Table 4.13: Data for reproducibility

Concentration of sample (µg/ml)	% RSD*
2	0.897
4	0.985
6	0.892
8	0.995

*Average of three determinations.

Table 4.14: Summarized data for the developed method

Parameters	Result
λ max (nm)	329
Beer's law limit (µg/ml)	2 to 10
Regression equation (Y=mX+c)	0.090x - 0.015
Slope	0.090
Intercept	0.015
Limit of detection (µg/ml)	0.01985
Limit of quantification (µg/ml)	0.09364
Coefficient of determination	0.9997
% RSD	< 1%
Accuracy	> 99%

4.2.2 Colorimetric spectrophotometric method

4.2.2.1 Materials and methods

DTB was purchased from M/s Hwasun Biotechnology Co. Ltd, Shanghai, China. Folin ciocalteu reagent (FCR), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from E Merk (India). Double distilled water was used, prepared by double distillation glass assembly. All the reagents and solvents were of reagent grade and were used without further purification. The instruments used for the estimation of drugs include pH meter (Picco+, Lab india, India), Double beam UV Visible Spectrophotometer (UV-1700-Shimadzu), Digital weighing balance (Ax 120, Shimadzu, Japan). Dasatinib tablets, each containing 10 mg of DTB were punched in laboratory.

4.2.2.2 Optimization of parameters

Concentration and volume of reagent

DTB was found to yield blue colored product with FCR and NaOH and having absorbance maxima at 745 nm. Therefore, investigations were carried out to establish the most favorable conditions for the formation of this colored product. The influence of the concentration as well as volume of reagent on the reaction has been studied. Different concentrations and different volumes were tried for all the reagents.

Temperature and time

The stability of developed chromogen was assessed under two different temperature conditions, i.e., at room temperature and at 40^0 C. For color stability, aliquots of concentration 10, 20, 40, 60, and 80 µg/ ml were prepared and complex was made, as per procedure given in previous section. The samples were kept in transparent and amber colored glass vials under room temperature and elevated

(40 ± 2 ºC) temperature conditions, in controlled oven for varying period of time. The samples were analyzed initially and then periodically at 10, 20, 40, 60, and 80 min. for absorbance determination.

Preparation of standard solutions and calibration curve

Standard stock solution of DTB

A stock solution was prepared by dissolving 50 mg of DTB in required quantity of 0.1 M HCl and diluting to 50 ml with same solvent. From the above solution 2.5 ml was again diluted to 25 ml with purified water to get 100 µg/ml solution of DTB.

Standard solution of FCR

A standard solution of FCR was prepared by diluting the reagent with double distilled water to get a concentration of 1 N.

Standard solution of NaOH

A standard solution of sodium hydroxide was prepared by dissolving 4 gm of reagent in sufficient quantity of double distilled water and finally diluting to 100 ml with double distilled water.

Procedure for calibration curve

Suitable aliquots of the drug solution (1 to 8 ml) were taken in 10 ml volumetric flasks. To each flask was added 0.8 ml of standard FCR solution, 6.0 ml of purified water, and all the flasks were shaken well for at least 2 to 5 min., followed by addition of 1 ml of standard NaOH solution. Finally volume was made up to the mark with purified water to prepare a series of standard solutions containing 10 to 80 µg/ ml DTB. The absorbance of blue color chromogen was measured at 745 nm against reagent blank within one hour and the calibration curve was plotted.

Method validation

Accuracy of the methods was determined by recovery studies in the tablet formulation of DTB. Recovery studies were carried out by addition of known quantities of standard drug solution to pre-analyzed sample. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The percent coefficient of variance (% CV) was calculated at each concentration level. The reproducibility was confirmed by repeating the methods, taking HCl from three different manufacturers and by three different analysts, and the percent relative standard deviation (% RSD) was calculated. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by repeating the blank measurements twelve times at 745 nm.

4.2.2.3 Result and discussion

Conformity with Beer's law

The calibration curve was linear in the concentration range of 10 to 80 µg/ml for DTB. The slope, intercept, and correlation coefficient were obtained by linear least square treatment of the results. The optical characteristics are summarized in Table 4.18.

The main object of the study was to develop an accurate, precise, sensitive and reproducible method for determination of dasatinib. For this, colored complex of dasatinib was formed with the help of FCR in presence of NaOH, and its application in analytical detection was explored. This formed complex was blue in color and showed wavelength of maximum absorbance (λmax) at 745 nm. The stability of color as well as the developed complex is a prerequisite towards such motif. Hence, the stability of this complex was assessed under different conditions of temperature, time and concentration of reagents.

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. By keeping one constant at a time and the optimum concentration of FCR was 1 N and of NaOH was 1 N. Similarly optimum volume of FCR and NaOH was found to be 0.8 ml and 1 ml respectively. Whereas it was found that the complex was stable at room temperature, and showed no change in absorbance value throughout the study (Fig. 4.7). The solution was also found to be retaining its stability for 1h (Fig. 4.8); so all the readings were taken within the specified time range. On other side, it was found that the color of complex starts fading when exposed to higher temperature 40 ± 2° C (Fig. 4.9). This suggests that the exposure of these colored solutions to high temperature should be avoided during the analysis.

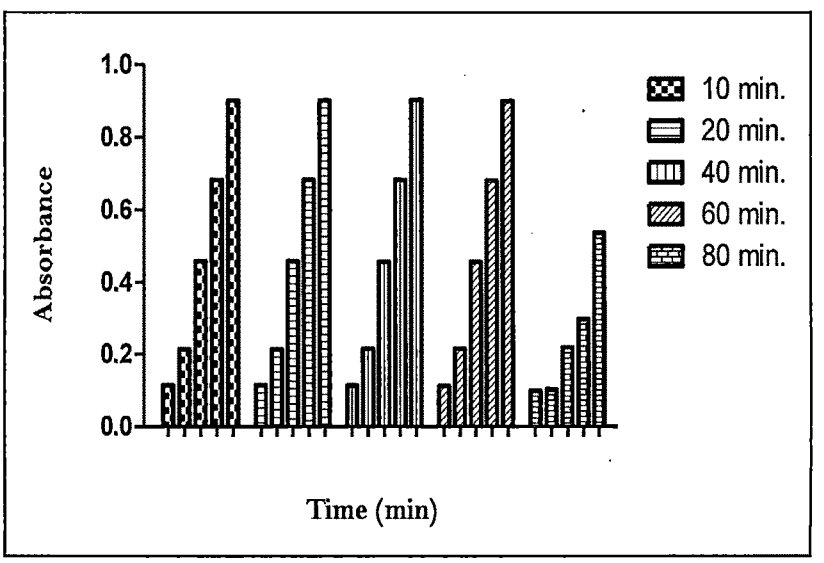

Figure 4.7: Stability of color at room temperature

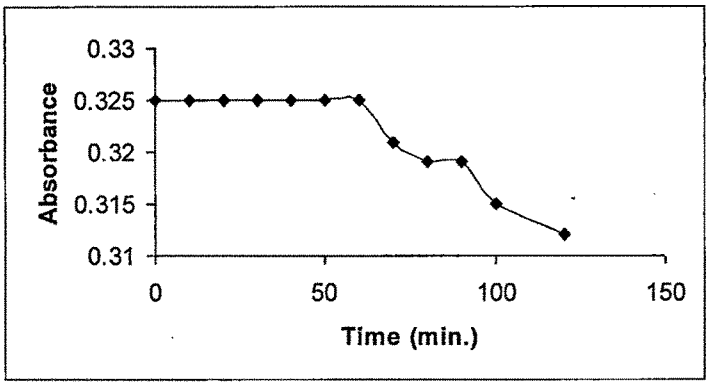

Figure 4.8: Duration of stability of color

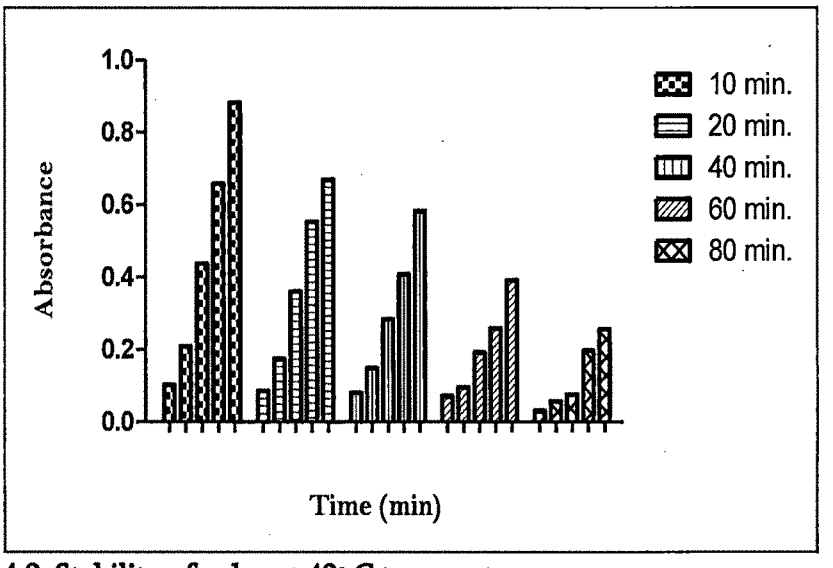

Figure 4.9: Stability of color at 40° C temperature

The proposed method is simple, rapid, precise and do not suffer from any interference due to common excipients of tablet. Beer's law is obeyed in the concentration range of 10-80 μg/ml. The method was validated in terms of accuracy, precision and reproducibility. The accuracy of the method was proved by analyzing the synthetic mixture and tablet formulations (Table 4.15). Values greater than 99.0% indicate that the proposed method is accurate for the analysis of drug.

Table 4.15: Analysis of synthetic mixture and tablet dosage form

Dosage form	Label claim (mg/tablet)	Amount found* (mg/tablet)	% Label claim
S.M.	10	9.95	99.56 ± 0.123
Tablet-1	20	20.04	100.20 ± 0.695
Tablet-1	50	49.99	99.98 ± 0.212

*Average of three determination.

The recovery studies were carried out by adding known amount of standard solution of DTB to pre-analyzed drug solutions. The resulting solutions were then analyzed by the proposed method. The results of recovery studies were found to be satisfactory and the results are presented in Table 4.16.

Table 4.16: Recovery studies

Conc. of formulation (µg/ml)	Std. spiked (µg/ml)	Total conc. taken (µg/ml)	Total conc. Found* (µg/ml)	% Recovery
10	2	12	11.84	99.05 ± 0.42
10	3	13	12.92	99.46 ± 0.33
10	4	14	14.01	100.12 ±0.42
30	2	32	32.10	100.32 ±0.52
30	3	33	32.92	99.76 ± 0.34
30	4	34	33.75	99.28 ± 0.36

*Average of three determination.

The precision of the proposed method was checked in terms of inter-day and intra-day, where method was repeated on three different days and also repeated for three different time periods in the same day. The results given in Table 4.17 showing % CV of less than 1% at each level clearly indicate that the proposed method is precise enough for the analysis of drug. The reproducibility of the method was checked by getting the proposed method performed by three different analysts and by taking solvent from three different manufacturers. The values of % RSD less than 1% (Table 4.17) indicate that the proposed method is reproducible for the analysis of DTB.

Table 4.17: Precision data and reproducibility data

Interday precision (%CV) *	Intraday precision (%CV) *	Reproducibility (%RSD) *
0.698	0.782	0.526

*Average of three determination.

The optical characteristics, such as Beer's law limit, molar absorptivity, Sandell's sensitivity, are recorded in Table 4.18. The regression analysis using the method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations. The results are summarized in Table 4.18. Recovery analysis of the results shows that the presence of excipients in tablet formulation did not interfere with the final determination of the active component, DTB. This reveals the potential utility of this developed method for the routine analysis of DTB in bulk and in pharmaceutical preparations.

Table 4.18: Summarized optical characteristics and other parameters

Parameters	Result
λ max (nm)	745
Color of chromogen	Blue
Beer's law limit (µg/ml)	10 to 80
Molar extinction coefficient (l/mol.cm)	$1.026 X10^4$
Sandell's sensitivity (µg/cm² per 0.001 absorbance unit)	0.01996
Regression equation (Y= mX + c)	0.0111x + 0.0098
Slope	0.0111
Intercept	0.0098
Limit of detection (µg/ml)	0.156
Limit of quantification (µg/ml)	0.489
Coefficient of determination	0.9991
% RSD	< 1%
Accuracy	> 99%

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