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Development of a new rapid and sensitive HPTLC method for estimation of Dasatinib in bulk and its pharmaceutical formulations

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ABSTRACT

A sensitive, fast, and reproducible high performance thin-layer chromatographic method has been developed for the estimation of Dastinibanti cancer drug, using TLC aluminium plates precoated with silica gel G60F254. Among the different combinations of mobile phases used, best separation was achieved in Toluene-methanol-0.1% acetic acid (3.4.1.5, v/v/v). Densitometric scanning of the plates directly at 288nm was used for analysis of Dasatinib. The R f value was found to be 0.45±0.02. The methods were validated in terms of linearity, accuracy and precision. The linearity curves were found to be linear over 10-150 µg/ml for high performance thin layer chromatography and 200-1000 ng/band for high performance thin layer chromatography. The limit of detection and limit of quantification for, 20 and 60 ng/band, respectively. The proposed methods were successfully used for estimation of dasatinib in tablet dosage form.

Keywords: HPTLC, Dasatinib, ICH guidelines.

INTRODUCTION

HPTLC is the most simple separation technique and usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography. HPTLC is an important alternative method to HPLC or gas chromatography. The present work explain the method development and validation of HPTLC method for Dasatinib (anti- cancer drug)[1]in bulk and its pharmaceutical formulations.

Objective of the present work

Dasatinib[1] known as BMS-354825. It is an anticancer drug produced by Bristol-Myers Squibb and marketed under the trade name Sprycel. Dasatinib is an oral protein and inhibits tyrosine kinase also approved for first line use in patients with chronic myelogenous leukemia (CML) and Philadelphia. Extensive literature survey revealed that there is no official high performance thin layer chromatography (HPTLC) method reported in major pharmacopeias like USP, EP, BP and IP.

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There are very few analytical methods available [2-9] for estimation of DAS by different instrumental techniques. Nowadays, HPTLC has become a routine analytical technique due to its advantages of reliability in quantitation of analytes at micro and even in nanogram levels and cost effectiveness. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase unlike HPLC [2]. This reduces the time and cost of analysis. The objective of the present study was to develop and validate a new, simple, accurate, specific and reproducible HPTLC method for determination of DAS in bulk, pre formulation studies and pharmaceutical formulation to help the industries as well as researchers for their sensitive determination.

EXPERIMENTAL METHODS

Instrumentation and chromatographic conditions

Chromatography was performed on aluminum-backed silica gel 60F $_{254}$ HPTLC plates (20 × 10 cm) prewashed with methanol; plates were developed with Toluene-methanol-0.1% acetic acid (3:4:1.5, v/v) in a Camag twin-trough chamber (10 × 20). Chromatography was performed on 20X10cm aluminum packed HPTLC silica gel 60 F_{254} plates (MERCK). Before use, the plates were dried in an oven at 50^0c for 5min. Sample were applied as 8mm bands by spraying at a rate of 2µl/s by means of Camag Linomat 5 sample applicator equipped with a 100µl syringe. The distance between bands was 18.8mm. The developing solvent was allowed to ascend to 8cm, with Methanolas a mobile phase in a Twin Though Chamber was saturated for 20min lined with whatmann filter paper. The room temperature was 25 ± 2^0c. The average development time was 15minutes. After chromatography, the plates were dried in a current of air by using air-blowing drier. Densitometric scanning at λ=288nm was then performed for Dasatinib Hydrobromide with a Camag TLC scanner-3 equipped with winCATS software, using a Deuterium light source, the slit dimensions were 6.00X0.45mm having scanning speed was 20 mm s-1. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatograph were determined from the intensity of the diffused light. Evaluation was done by peak areas with linear regression.

Reagents and chemicals requirements

All chemicals and reagents used were of analytical grade. The authentic sample of dasatinib was procured from Intas Pharmaceuticals Ltd., Ahmedabad. The pure drug obtained had 99.9% w/w assay value, and was used without further purification. Dasatinib is available as commercial tablets under the brand name dasnat –tab 50 mg from oddway exports, was procured from the local pharmacy.

Preparation of standard stock solutions

An accurately weighed quantity (100 mg) of DAS was transferred to 100 ml volumetric flask containing 4 ml methanol and volume was adjusted to mark with methanol to obtain a concentration of 1000ng / µl of DAS.

Preparation of Linearity solutions

A stock solution containing 100 ng/µl (100µg/ml) of DAS was prepared from above stock solution. Different volumes of stock solution (2, 4, 6, 8, 10 µl) were spotted on an HPTLC plate in triplicate to obtain concentrations of 200, 400, 600, 800, and 1000ng/band of DAS , respectively.

RESULTS AND DISCUSSION

Optimization chromatographic conditions for HPTLC

The mobile phase in TLC is generally selected by controlled trial and error method. In normal phase, TLC separation is carried out on a non-aqueous mobile phase (silica gel) using a non-aqueous mobile phase. Developing solvent usually, is a mixture of non-polar organic solvent with a polar modifier such as methanol, ethyl acetate, acetone, acetonitrile to control the solvent strength and selectivity.

Sometimes small amounts of third component such as acetic acid, ammonia, triethylamine and formic acid are added to mobile phase because they partially modify the surface of silica gel. Keeping the acidic and the basic centers in a molecule nonionised; leads to

decrease in the tailing of polar sample components. The selection of the mobile phase is of prime importance in the development of a chromatographic technique for proper elution, resolution, spot definition, symmetrical peak shapes and Rf reproducibility of the analytes.

In present research work, initial trials were done using mobile phase with used these solvent like chloroform, methanol, ethyl acetate and hexane.it was observed that resolution between the two actives was good but the Rf value was not meeting the acceptance criteria.

Finally, the mobile phase consisting of Toluene-methanol-0.1% acetic acid (3:4:1.5, v/v) was found to be optimum. In order to reduce the neckless effect, TLC chamber was saturated for 25 min using saturation pads. The mobile phase was run up to a distance of 8 cm, which takes approximately 20 min for complete development of the TLC plate. During optimization of the HPTLC method, we had achieved good separation of the drug as well as the degradation products on normal phase TLC plates, which are more economical compared with reverse phase TLC plates. The spot appeared more compact and peak shape more symmetrical when the TLC plates were pretreated with methanol and activated at 110^0Cfor5min.Well- defined spots of standard was obtained when the chamber saturation time was optimized at 20min at room temperature. The optimized chromatographic condition were shown in table.1.The obtained peaks of standard was showed in Figure.1 respectively.

Table1.Optimized chromatographic conditions

Parameters	**Chroma to graphic Conditions**
Developmentchamber	CAMAGE Twin TroughChamber
Stationaryphase	Silicagel GF254 pre coated on aluminum sheet
Mobilephase	Toluene-methanol-0.1% acetic acid (3.4.1.5, v/v)
Chambersaturation	20 min
Sample applicator	CAMAGELINOMATV
Band	8mm
Space	18mm
Scanningspeed	20mm/sec
Development distance	8cm
Dryingofplate	current of air by using air-blowing drier
Densitometricscanner	CAMAGE TLC SCANNER
Lamp	Deuterium
Wavelength	288nm
Volume	2µl/spot
Developing technique	Linear ascending technique
Developing chamber	Twin trough glass chamber
Rf value	4.51

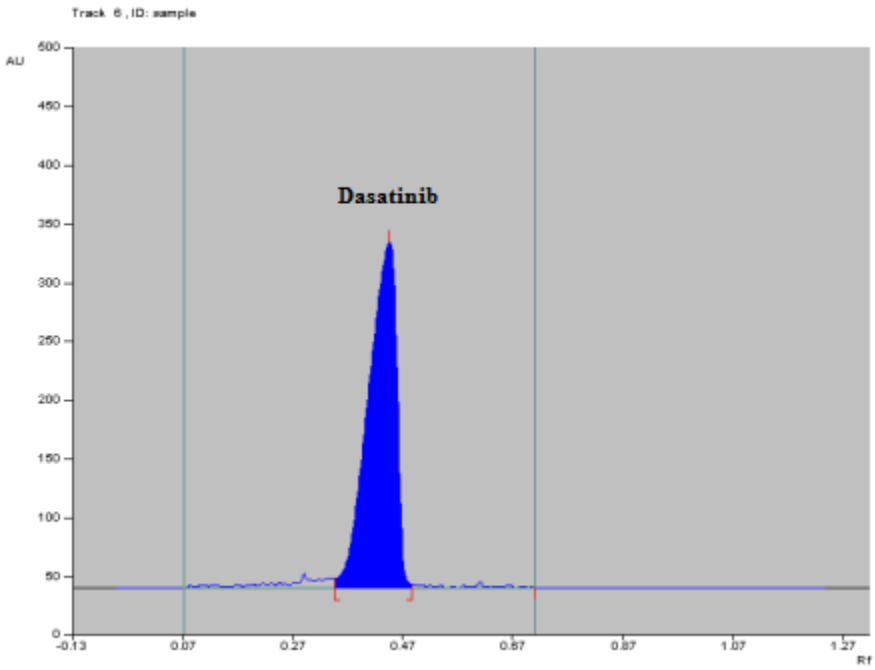

Figure.1.Typical HPTLC chromatogram of Dasatinib Hydrobromide standard

Analysis of tablet formulation

Twenty tablets were weighed accurately and average weight was determined. Then all the 10 tablets were ground to a fine powder in mortar. A quantity equivalent to 100mg of Dasatinib is transferred to a 100ml volumetric flask. The contents were ultrasonicated for 10min with methanol, made to volume and filtered through whatmann filter paper and make up with methanol. Thesamplesolutionwasfurtherdiluted to getsolutionsof 150 ngµL-1 of DAS. 1, 2, 3, 4,5microlitresofsample and standard solutionswereapplied as band8mm at8.8mmintervalunderstreamofnitrogen. The standard solution prepared from standard stock solution to give the same concentration of sample solution. The assay is calculated by area comparison method. Thedevelopedchromatogramswereevaluatedbyscanningindensitometricmodeat287nm. The obtained analytical report is denoted Table.2 The amount of DAS presentpertablet was calculated and results were documented in table No.2. The 3D fingerprinting report for DAS standard and sample in all the ten tracks were shown in Figure 2.That is, track 1&2 is 150 ng/band of standard and sample , track 3&4 is 300 ng/band of standard and sample, track 5&6 is 450 ng/band of standard and sample, track 7&8 is 600ng/band of standard and sample, track 9&10 is 750ng/band of standard and sample. The visualization of spots at 287nm shown in Figure 3.

Table 2. Report for assay @287nm

S.NO track	sample	QTY Applied(µl)	Dastinib	
			R_fValue	AREA (AU)
1	Std	1	0.45	2241
2	Tablet	1	0.45	2269
3	Std	2	0.45	4574
4	Tablet	2	0.45	4569
5	Std	3	0.45	6821
6	Tablet	3	0.45	6805
7	Std	4	0.45	9012

8	Tablet	4	0.45	9038
9	Std	5	0.45	11435
10	Tablet	5	0.45	11452

Figure 3.Visualisation of spots at 288nm

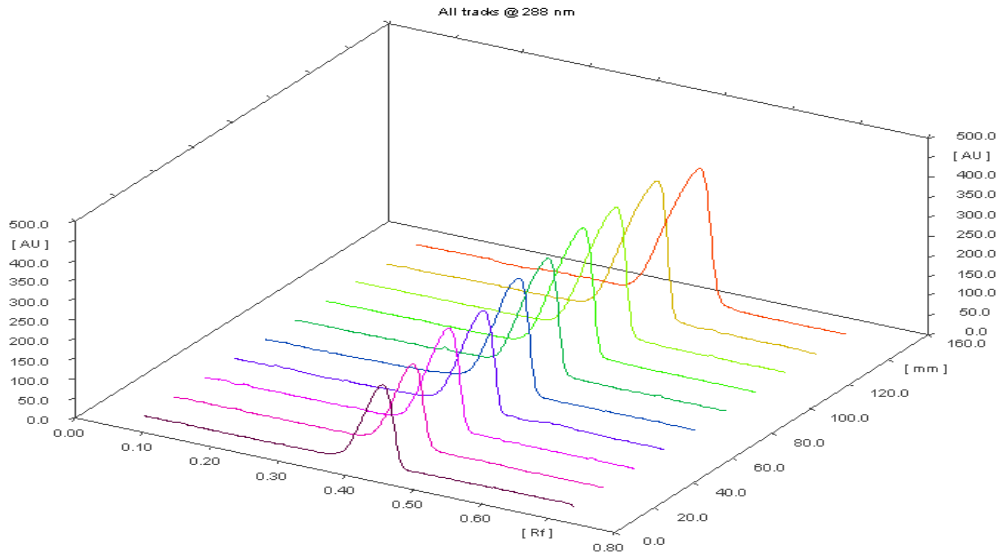

Figure. 2 Fingerprinting Report For Dasatinib Standard and Sample on different tracks: 3D DISPLAY @288nm

Table 3. Analysis of Formulation

Drug	Amount present(mg/tab)	Amount found (mg/tab)	% label claim	%RSD*
DAS	50mg	49.9	99.40	0.2458
DAS	100mg	100.42	100.28	0.2321

*** Each value is a mean of six observations**

VALIDATION OF THE METHOD

Method validation

The developed RP-HPLC method was validated according to ICH guidelines [10] to establish the suitability of the method for regular testing in quality control laboratory. The overall summarized validation report documented in Table 4.

Table 4:Overall Summarized Method Validation Results

S.no	Parameters		Dasatinib
1	**System Suitability**	Rf value	0.45
2	**Accuracy**	% Recovery	99.7-100.60%
3	**Linearity**	Correlation coefficient	0.9997
4	**System Precision**	%RSD	0.136
5	**Method Precision**	%RSD	0.057
5	**LOD**	ng/spot	20ng/spot
6	**LOQ**	ng/spot	60ng/spot
		Mobilephase composition (% RSD)	0.82
		Toluene-methanol-0.1% acetic acid (3.5.3.5.1.5, v/v)	
		Mobilephase composition (% RSD)	0.72
7	**Robustness**	Toluene-methanol-0.1% acetic acid (2.5.4.5.1.5, v/v)	
		Developmentdistance 7cm	1.12
		Developmentdistance 8cm	0.98
8	Ruggedness	% RSD	0.174

CONCLUSION

A new, simple, and sensitive HPTLC method has been successfully developed and validated for determination of DAS in bulk and pharmaceutical dosage form. The method was found to be accurate, precise, and reproducible with good stability under various processing and storage conditions. Chromatogramwasdevelopedandthepeak areaswere noted.Ateachlevelsoftheamount,three determinationswereperformed.Asshownfromthedatai nTable. 4goodrecoveriesoftheDASin the range from 99.7 to 100.6% were obtained at various addedconcentrations.All the validation results fall in the acceptable limits according to ICH guidelines.

Thus this developed and validated method will help the industries as well as researchers for their sensitive determination of DAS rapidly at low cost.

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