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VALIDATED RP-HPLC METHOD FOR DETERMINATION OF DACOMITINIB IN PURE FORM AND TABLET DOSAGE FORM BY USING RP-HPLC

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Abstract:
A new simple, selective, rapid, precise RP- HPLC method is described for the determination of Dacomitinib in bulk and marketed pharmaceutical dosage form. Chromatography was carried on a Symmetry C18 (4.6×150mm) 5µm column using a mixture of Methanol: Phosphate Buffer (pH-4.6) (25:75) V/V as the mobile phase at a flow rate of 1.0mL/min with detection at 260 nm. The retention time of the drug was 2.379min. The detector response was linear in the concentration of 10-50mcg/mL the limit of detection and limit of quantification was 2.13 and 6.31mcg/mL respectively. The % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The percentage assay of Dacomitinibwas 99.86%. The proposed method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, fast, accurate and precise and hence can be applied for routine quality control of Dacomitinib in bulk and marketed pharmaceutical dosage form.
Keywords: *Dacomitinib, RP-HPLC, Method Development, Validation, ICH Guidelines.*

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INTRODUCTION:

DISCOVERY OF CHROMATOGRAPHY

Chromatography is a physicochemical method for separation of complex mixtures was discovered at the very beginning of the twentieth century by Russian–Italian botanist M. S. Tswett. [1]. In his paper "On the new form of adsorption phenomena and its application in biochemical analysis" presented on March 21, 1903 at the regular meeting of the biology section of the Warsaw Society of Natural Sciences, Tswett gave a very detailed description of the newly discovered phenomena of adsorption-based separation of complex mixtures, which he later called "chromatography" as a transliteration from Greek "color writing" [2]. Serendipitously, the meaning of the Russian word "tswett" actually means color. Although in all his publications Tswett mentioned that the origin of the name for his new method was based on the colorful picture of his first separation of plant pigments (Figure 1), he involuntarily incorporated his own name in the name of the method he invented. The chromatographic method was not appreciated among the scientists at the time of the discovery, as well as after almost 10 years when L. S. Palmer in the United States and C. Dhere in Europe independently published the description of a similar separation processes.

Twenty-five years later in 1931, Lederer read the book of L. S. Palmer and later found an original publications of M. S. Tswett, and in 1931 he (together with Kuhn and Winterstein) published a paper [4] on purification of xantophylls on $CaCO_3$ adsorption column following the procedure described by M. S. Tswett. In 1941 A. J. P. Martin and R. L. M. Synge at Cambridge University, in UK discovered partition chromatography for which they were awarded the Noble Prize in 1952. In the same year, Martin and Synge published a seminal paper which, together with the paper of A.T. James and A. J. P. Martin, laid a solid foundation for the fast growth of chromatographic techniques that soon followed.

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification timesof compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge.

By the 2000 very fast development was undertaken in the area of column material with small particle size technology and other specialized columns. The dimensions of the General Introduction typical HPLC column are 100-300 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 µm to 200 µm. In this decade, sub 2 micron particle size technology (column material packed with silica particles of < 2µm size) with modified or improved HPLC instrumentation becomes popular with different instrument brand name like UPLC (Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid Resolution Liquid Chromatography) of Agilent.

Today, chromatography is an extremely versatile technique; it can separate gases, and volatile substances by GC, in-volatile chemicals and materials of extremely high molecular weight (including biopolymers) by LC and if necessary very inexpensively by TLC. All three techniques, (GC), (LC) and TLC have common features that classify themas chromatography systems. Chromatography has been defined as follows,

"Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. As a consequence, solutes are eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase; ipso facto a separation is achieved".

VARIOUS TYPES OF CHROMATOGRAPHY

Chromatography can be classified by various ways (I) On the basis of interaction of solute to the stationary phase, (II) On the basis of chromatographic bed shape, (III) Techniques by physical state of mobile phase.

ON THE BASIS OF INTERACTION OF SOLUTE TO STATIONARY PHASE

ADSORPTION CHROMATOGRAPHY

Adsorption chromatography is probably one of the oldest types of chromatography around. It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase. The equilibration between the mobile and stationary phase accounts for the separation of different solutes.

PARTITION CHROMATOGRAPHY

This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile phase and the stationary liquid

ION EXCHANGE CHROMATOGRAPHY

In this type of chromatography, the use of a resin (the stationary solid phase) is used to covalently attach anions or cations onto it. Solute ions of the opposite charge in the mobile liquid phase are attracted to the resin by electrostatic forces.

MOLECULAR EXCLUSION CHROMATOGRAPHY

Also known as gel permeation or gel filtration, this type of chromatography lacks an attractive interaction between the stationary phase and solute. The liquid or gaseous phase passes through a porous gel which separates the molecules according to its size. The pores are normally small and exclude the larger solute molecules, but allow smaller molecules to enter the gel, causing them to flow through a larger volume. This causes the larger molecules to pass through the column at a faster rate than the smaller ones.

ON THE BASIS OF CHROMATOGRAPHIC BED SHAPE

COLUMN CHROMATOGRAPHY

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

In 1978, W.C.Still introduced a modified version of column chromatography called flash column chromatography. The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

PLANAR CHROMATOGRAPHY

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific Retention factor (Rf) of each chemical can be used to aid in the identification of an unknown substance.

PAPER CHROMATOGRAPHY

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to

allow for quantification, high-performance TLC can be used.

DISPLACEMENT CHROMATOGRAPHY

The basic principle of displacement chromatography is, “A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities”.

There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings.

Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”.

Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

INTRODUCTION TO HIGH PERFORMANCE LC

The acronym HPLC, coined by the late Prof. Csaba Horvath for his 1970 Pittcon paper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi. This was called high pressure liquid chromatography, or HPLC.

The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi of pressure, and incorporated improved injectors, detectors, and columns. HPLC really began to take hold in the mid-to late-1970s. With continued advances in performance during this time (smaller particles, even higher pressure), the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High performance liquid chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. The components of a basic high-performance liquid chromatography (HPLC) system are shown in the simple diagram in Figure 5 (Schematic Diagram).

A reservoir (Solvent Delivery) holds the solvent (called the mobile phase, because it moves). A high-pressure pump solvent manager is used to generate and meter a specified flow rate of mobile phase, typically milliliters per minute.

An injector (sample manager or auto sampler) is able to introduce (inject) the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column (most compounds have no color, so we cannot see them with our eyes).

The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the eluate containing that purified compound for further study. This is called preparative chromatography. The high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the conduit for the mobile phase, sample, and separated compound bands.

The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitate the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector (ELSD). The most powerful approach is the use multiple detectors in series. For example, a UV

and/or ELSD detector may be used in combination with a mass spectrometer (MS) to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

ISOCRATIC AND GRADIENT LC SYSTEM OPERATION

Two basic elution modes are used in HPLC. The first is called isocratic elution. In this mode, the mobile phase, either a pure solvent or a mixture, *remains the same throughoutthe run.* A typical system is outlined. The second type is called gradient elution, wherein, as its name implies, *the mobile phasecomposition changes during the separation.* This mode is useful for samples that containcompounds that span a wide range of chromatographic polarity. As the separation proceeds, the elution strength of the mobile phase is increased to elute the more strongly retained sample components.

In the simplest case, shown in Figure 3, there are two bottles of solvents and two pumps. The speed of each pump is managed by the gradient controller to deliver more or less of each solvent over the course of the separation. The two streams are combined in the mixer to create the actual mobile phase composition that is delivered to the column over time. At the beginning, the mobile phase contains a higher proportion of the weaker solvent [Solvent A]. Over time, the proportion of the stronger solvent [Solvent B] is increased, according to a predetermined timetable. Note that in Figure 3, the mixer is downstream of the pumps; thus the gradient is created under *high pressure*. Other HPLC systems are designed to mix multiple streams of solvents under *low pressure*, ahead of a single pump. A gradient proportioning valve selects from the four solvent bottles, changing the strength of the mobile phase over time.

Today's HPLC requires very special apparatus which includes the following.

1. Extremely precise gradient mixers.
2. HPLC high pressure pumps with very constant flow.
3. Unique high accuracy, low dispersion, HPLC sample valves.
4. Very high efficiency HPLC columns with inert packing materials.
5. High sensitivity low dispersion HPLC detectors.
6. High speed data acquisition systems.
7. Low dispersion connecting tubes for valve to column and column to detector.

MATERIALS AND METHODS:

Dacomitinib (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck.

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Dacomitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.72ml of the above Dacomitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate Buffer (pH-4.6 adjusted with orthophosphoric acid) in proportion 25:75 v/v respectively.

Optimization of Column:

The method was performed with various C18columns like ODS column, Xterra, and X Bridge C18 column. Symmetry C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.6) (25:75) V/V
Column : Symmetry C18 (4.6×150mm) 5µm
Column temperature : 40ºC
Wavelength : 260nm
Flow rate : 1.0ml/min
Injection volume : 10µl
Run time : 6minutes

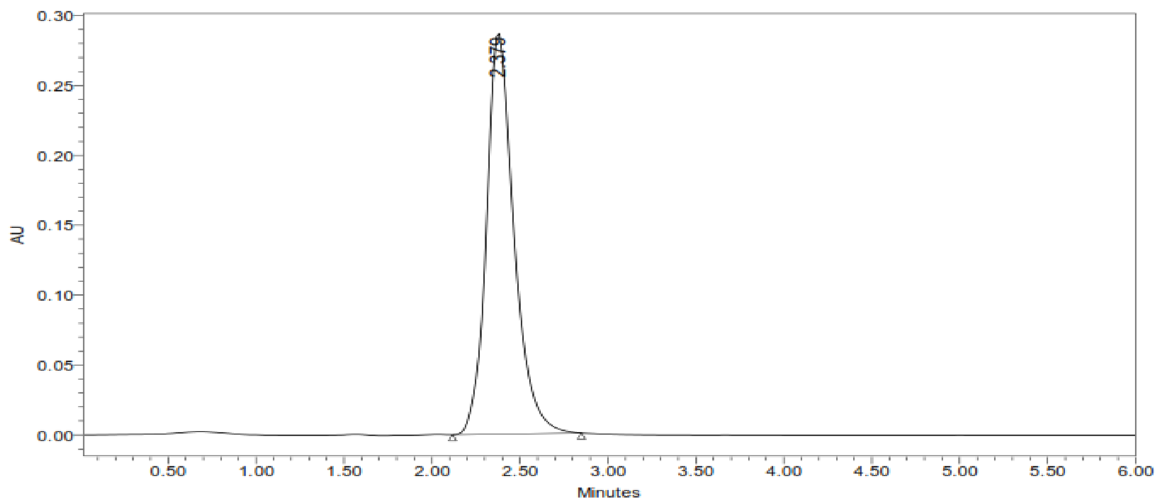

Figure: Optimized Chromatogram (Standard)

Table: Results of Optimized Chromatogram (Standard)

S. No.	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Dacomitinib	2.379	3265415	285645	1.27	6854

Observation: In this trail it shows well peak shape and proper plate count and tailing under limit in the chromatogram. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

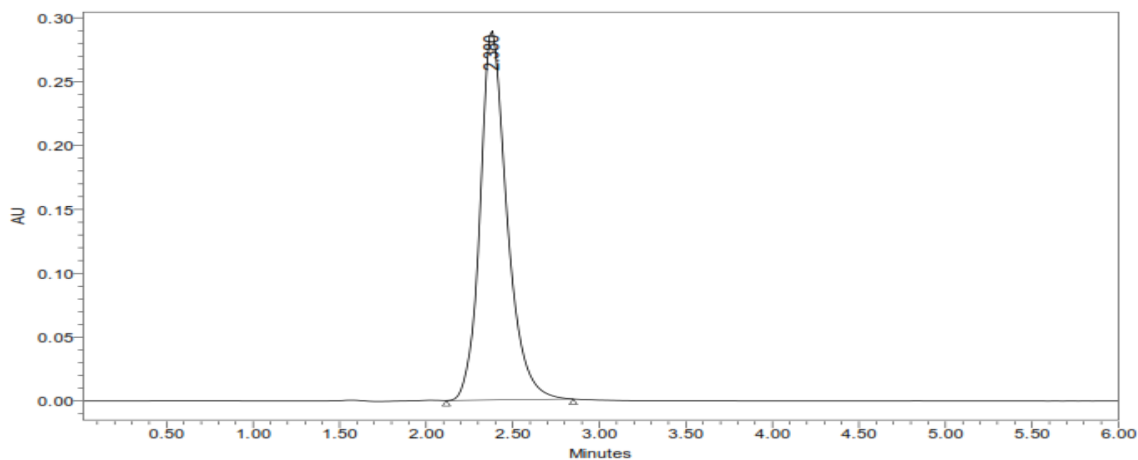

Figure: Optimized Chromatogram (Sample)

Table: Results of Optimized Chromatogram (Sample)

S. No.	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Dacomitinib	2.380	3254587	278795	1.28	6857

Acceptance criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

System suitability:

Table: Results of system suitability for Dacomitinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dacomitinib	2.317	3266542	286598	6935	1.29
2	Dacomitinib	2.302	3259878	287547	6974	1.28
3	Dacomitinib	2.323	3265844	286587	6897	1.29
4	Dacomitinib	2.343	3259854	287844	6987	1.29
5	Dacomitinib	2.321	3268475	286594	6935	1.28
Mean			3264119			
Std.Dev.			3999.925			
%RSD			0.122542			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Standard):

Table: Peak results for assay standard

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Dacomitinib	2.354	3262547	285698	1.28	6985	1
2	Dacomitinib	2.350	3265415	286947	1.27	6895	2
3	Dacomitinib	2.354	3268547	285412	1.29	6874	3

Assay (Sample):

Table-14: Peak results for Assay sample

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Dacomitinib	2.354	3254154	284562	1.26	6785	1
2	Dacomitinib	2.350	3254856	284751	1.25	6751	2
3	Dacomitinib	2.354	3258747	284913	1.26	6746	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Dacomitinib in pharmaceutical dosage form was found to be 99.86%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Table-15: Chromatographic Data for Linearity Study

Concentration µg/ml	Average Peak Area
10	1056841
20	2057454
30	3102468
40	4048547
50	4988746

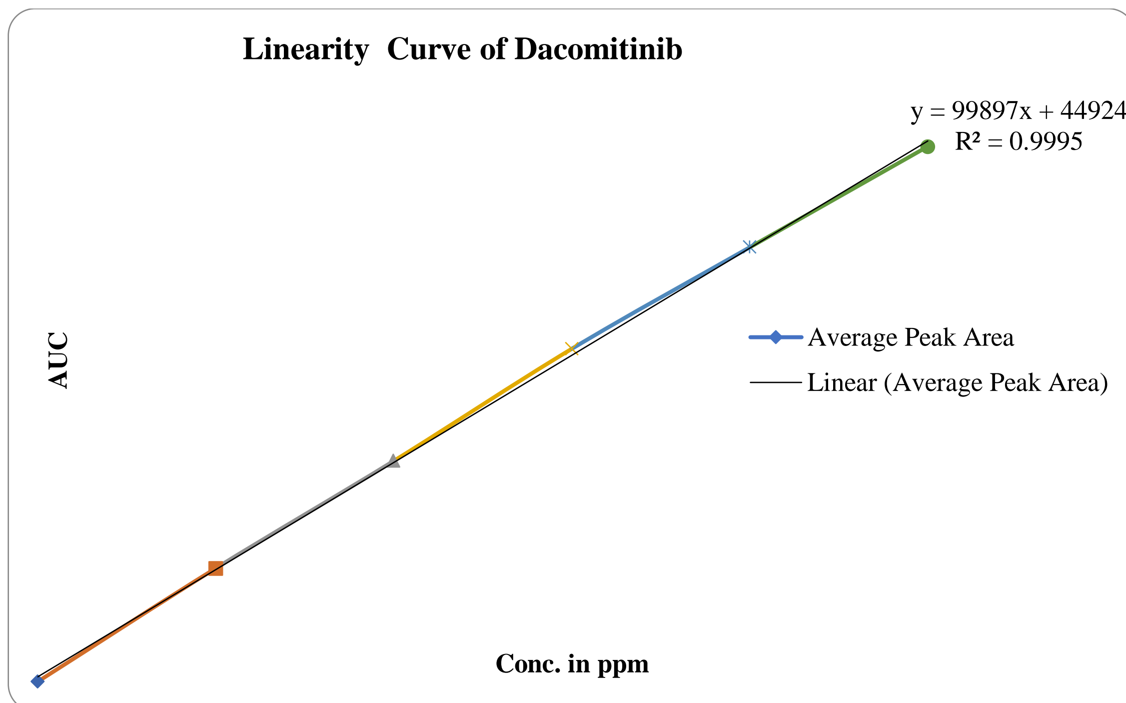

Fig: Calibration Curve of Dacomitinib

REPEATABILITY

Table: Results of repeatability for Dacomitinib:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dacomitinib	2.356	3265254	285874	1.29	6895
2	Dacomitinib	2.356	3265875	285471	1.28	6857
3	Dacomitinib	2.357	3275421	286352	1.29	6859
4	Dacomitinib	2.358	3274853	297451	1.28	6845
5	Dacomitinib	2.359	3268952	289542	1.29	6982
Mean			3270071			
Std.dev			4836.116			
%RSD			0.14789			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table: Results of Intermediate precision for Dacomitinib

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USPlate count	USP Tailing
1	Dacomitinib	2.380	3278547	284512	6985	1.29
2	Dacomitinib	2.383	3274585	286543	6952	1.30
3	Dacomitinib	2.385	3285471	286545	6941	1.29
4	Dacomitinib	2.385	3286598	287548	6895	1.30
5	Dacomitinib	2.389	3287547	289854	6983	1.29
6	Dacomitinib	2.389	3278958	287414	6935	1.30
Mean			3281951			
Std.Dev.			5293.457			
%RSD			0.16129			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table: Results of Intermediate precision Analyst 2 for Dacomitinib

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Dacomitinib	2.380	3285645	284514	6785	1.23
2	Dacomitinib	2.383	3278584	284754	6741	1.24
3	Dacomitinib	2.385	3289854	286542	6854	1.23
4	Dacomitinib	2.385	3278547	278471	6754	1.24
5	Dacomitinib	2.389	3285421	278541	6821	1.25
6	Dacomitinib	2.346	3278988	278965	6895	1.24
Mean			3282840			
Std.Dev.			4798.064			
%RSD			0.146156			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

ACCURACY:

Table: The accuracy results for Dacomitinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543693	15	15.003	100.02	100.194%
100%	3046549	30	30.047	100.156	
150%	4558585	45	45.183	100.406	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8mL/min	3265415	2.379	6854	1.27
Less Flow rate of 0.7mL/min	3125468	2.763	6785	1.26
More Flow rate of 0.9mL/min	3028985	2.234	6584	1.25
Less organic phase	3154754	2.765	6454	1.25
More organic phase	3068598	2.236	6784	1.26

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

The proposed study describes a new RP-HPLC method using simple mobilephase for the estimation of Dacomitinib in bulk and marketed pharmaceutical dosage formulations. The method was validated and foundto be simple, sensitive, accurate, robust and precise. It was also proved to beconvenient and effective for the determination of Dacomitinib in the pharmaceutical dosage form. The percentage of recoveryshows that the method is free from interference of the excipients used in marketed formulation. Moreover, the lower solvent consumption along with the shortanalytical run time leads to cost effective chromatographic method.

This method can be used for the routinedetermination of Dacomitinib in bulk drug and inPharmaceutical dosage forms.

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