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Estimation of Dacomitinib in Tablet Dosage Form by High-Performance Thin-Layer Chromatography

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Pushpani Parve[1*], Bhushan Sonawane[2], Vijay Kumar M.[2], Sayali Warde[2], R. Mohan Singh[2], Smita Nayak[1]

[1]Department of Quality Assurance, Gahlot Institute of Pharmacy, Plot no. 59, Sector-14, Koparkhairne, Navi Mumbai – 400709, Maharashtra, India.

[2]Analytical Research and Development, Central Drug Testing Laboratory, Zonal FDA Bhavan, GMSD Compound, Belasis Road, Mumbai Central, Mumbai – 400008, Maharashtra, India.

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ABSTRACT

A new, simple, accurate, fast, economic, and precise high-performance thin-layer chromatographic method was developed and validated for the estimation of Dacomitinib in a tablet dosage form. Separation of active ingredient from Dacomitinib tablet was done on pre-coated HPTLC plate (silica gel 60 F_{254}) and mobile phase consisting of Hexane: Ethyl acetate: Methanol in the ratio of 6:3:1*v/v/v*. Densiometric analysis of Dacomitinib was carried out at the wavelength of 282nm. This system was found well-resolved bands for Dacomitinib at a R_f value of 0.24. The method was developed and valid per ICH guidelines. Linearity was found to be in the concentration range of 25-135 ng/band with the correlation coefficient of 0.9990 for Dacomitinib. Good accuracy and precision were obtained as revealed from %RSD value less than 2. No interference was observed from excipients while doing study. It can be successively applied in pharmaceutical solid dosage formulation with potential application in the pharmaceutical analysis of Dacomitinib tablet dosage form.

1 INTRODUCTION

Dacomitinib, chemically (2E)-N-{4-[(3-Chloro-4-fluorophenyl)amino]-7-methoxy-6-quinazolinyl}-4-(1-piperidinyl)-2-butenamide (Fig. 1), is an anti-cancer agent with molecular weight of 469.94 [1]. It is an oral small molecule second-generation pan-HER TKI that irreversibly and selectively binds to the ATP binding pockets of EGFR, ERBB2 and ERBB4 at low nanomolar affinities (CAS 1110813-31-4) [4,5]. Recently, in a phase III clinical trialdacomitinib was found to produce a remarkable increase in progression free survival (PFS) in the first line treatment of patients with advanced EGFR-mutated non-small cell lung cancer as compared with gefitinib [6]. Compared to gefitinib, dacomitinib recorded the longer duration of response in patients who responded to treatment might be due to the irreversible binding of dacomitinib to its targets in contrast to the reversible binding of gefitinib [7]. Patients received a range of dacomitinib dose from 0.5 to 60 mg/day. Dacomitinib had a long half-life of 59–85 h and apparent volume of distribution of approximately 2,600 L over the 30-60 mg dosage range. Given the long half-life, accumulation was expected and top accumulation was discovered during cycle 1. No effects of food or locally acting antacids on the pharmacokinetics of dacomitinib were seen in this study [8].

Literature reports, to the best of our knowledge a single validated LC-MS assay was lately published reporting the assay for quantification of Dacomitinib and application t investigating its metabolic stability [3]. Literature survey reveals few HPLC methodologies for the determination of Dacomitinib. But no method found on high performance thin layer chromatography (HPTLC) for the estimation of Dacomitinib in tablet dosage form. The present study was to develop an alternative HPTLC method for the determination of Dacomitinib in tablet dosage form.

The Food and Drug Administration (FDA) approved dacomitinib (DMB) on September 27, 2018, a potent and irreversible second-generation EGFR-targeted TKI agent in the form of VIZIMPRO tablets. The irreversible pan-human epidermal growth factor receptor (HER) family inhibitor includes the first-line treatment of patients with metastatic NSCLC harboring EGFR Del19 or exon 21 L858R mutations [2,3].

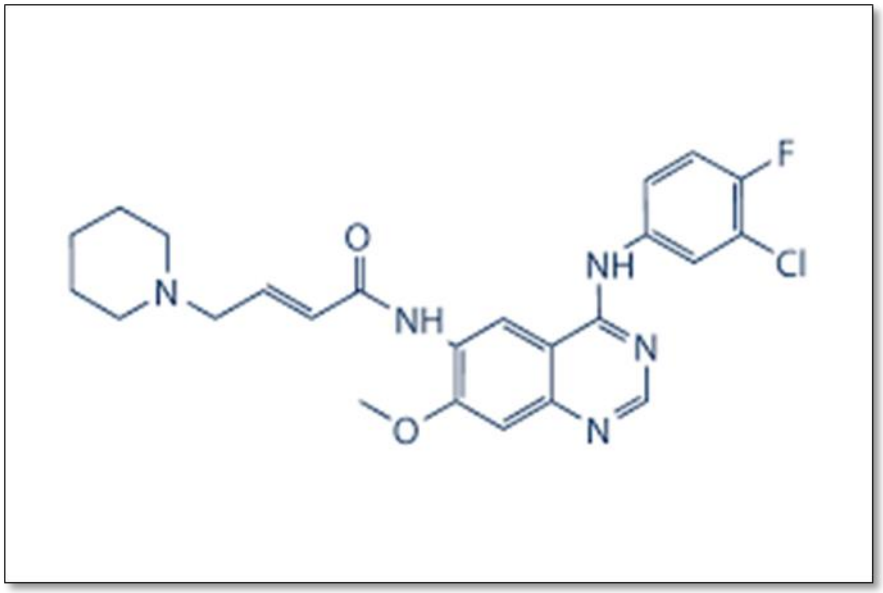

FIGURE NO. 1: CHEMICAL STRUCTURE OF DACOMITINIB

2 EXPERIMENTS

2.1 Instrumentations

The HPTLC system comprising of Camag Linomat 5, sample applicator (CAMAG, Switzerland), coupled with Camag Hamilton Bonaduz microlitre syringe (100 µl), UV chamber with dual-wavelength UV lamps, and CAMAG TLC scanner 4 controlled by vision CATS software (CAMAG) was used for the application and detection of spots respectively. The Chromatographic separations of drugs were performed using pre-coated HPTLC plates with silica gel 60 F254 (10 × 20 cm with 250 µm thickness) Sigma Aldrich and a CAMAG twin-trough developing chamber was used for chromatographic method development.

2.2 Materials and reagents

Dacomitinib, reference standards were procured from Central Drug Testing Laboratory, Mumbai with defined potency 96.1 %. Marketed formulation of tablet dosage form DACOPLICE® 45 mg (Pfizer) was procured from local market whereas analytical grades such as hexane, ethyl acetate, and methanolwere procured from Molychem. Silica gel 60 F254 plates from Sigma-Aldrich (Mumbai, India) were used.

2.3 Preparation of standard solution

Reference standard of Dacomitinib was accurately weighed to 18.0 mg and transferred into a 100 ml volumetric flask, diluted with methanol up to the mark to get a stock solution having the strength of 180 µg/ml. Further dilutions were made using methanol to get a concentration of Dacomitinib (18 µg/ml).

2.4 Analysis of marketed formulation

To determine the concentration of Dacomitinib in tablet dosage form (45mg per tablet), the content of 20 tablets were accurately weighed and average weight was determined. An accurately weighed powder, equivalent to 18mg of Dacomitinib was weighed and transferred into a 100ml volumetric flask containing 50ml methanol and sonicated for 15min and volume was makeup to the mark with methanol. The solution is filtered through Whatman filter paper No. 4. Further dilutions were made to get a concentration (18 µg/ml).

2.5 Chromatographic conditions

The Plate was activated at 110 °C for 20 min before chromatography. For saturation of chamber, 30ml of the mobile phase was transferred into the development tank closed with a lid. The assembly was aside for 45 min under room temperature. The samples were spotted in the form of narrow bands having a length of 8 mm. The application positions X and Y were kept at 8 mm and 20 mm, respectively, to avoid edge effects. The distance between the two bands was 20mm. Bands were applied at a constant rate of 5 nL/s using a nitrogen aspirator.

Linear ascending development of chromatogram was carried out in a Camag twin trough glass chamber saturated with the mobile phase consisting of Hexane: Ethyl acetate: Methanol [6:3:1 *v/v/v*] for 30 min and chromatogram run was kept up to 90 mm. Following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Spectrodensitometric analysis of the separated components was carried out using Camag TLC Scanner 4 in the reflectance–absorbance mode at 282 nm and the source of radiation utilized was a deuterium lamp. The slit dimension used was 6.0mm×0.3mm and sensitivity was kept at auto mode. The scanning speed was 100 nm/s. The evaluation was achieved by linear regression of the peak area response against the amount of drug by using vision CATS (CAMAG) software for peak area measurement and data processing.

2.6 Method optimization

Considering molecular structure and solubility data of Dacomitinib silica gel F254 plate were used as a stationary phase. Different mobile phase containing solvents such as toluene and methanol in a different composition. Initial trial was done with a mobile phase comprising of Toluene: Methanol (8:2 v/v) but low R_f value was found. Further trial was made by modifying mobile phase containing Toluene: Methanol (9:2 v/v) but symmetrical peak shape was not found. Finally symmetrical peak shape and acceptable SST parameters were found with the mobile phase comprising of Hexane: Ethyl acetate: Methanol- 0.2% ammonia (6:3:1 v/v/v).

2.7 Method validation

The proposed method was validated in accordance with ICH guidelines Q2 (R1) and various parameters such as specificity, linearity, precision, accuracy, LOD, LOQ and robustness were carried out.

2.7.1 Specificity

The specificity of the developed method was determined by analyzing standard drug and test sample solutions containing Dacomitinib from marketed tablets. The band for Dacomitinib in the sample was confirmed by comparing the *R*F value and spectrum of the spot with that of the standard. The peak purity of Dacomitinib was determined by comparing the spectrum at three different regions of the spot, i.e., peak start(S), peak apex (M), and peak-end (E).

The results are summarized in Table 8 and Fig 6.

2.7.2 Calibration curve

From the above standard solution, different volumes were applied and linear relationship between peak area and concentration of the drugs was determined over the concentration range of 25- 135 ng/band by making three replicates for each concentration. The results are shown in Table 2 and Fig 4, 5.

2.7.3 Precision

Precision of the developed method was verified by performing repeatability and intermediate precision, peak area measured and calculated in terms of percent relative standard deviation (% RSD). The data are given in Table 3 and Table 4.

2.7.4 Sensitivity

The sensitivity of the developed method is expressed as the limit of detection (LOD), the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value under the experimental conditions, as well as the limit of quantification (LOQ), which is the lowest amount of analyte that can be detected and quantified with suitable precision, accuracy, and reproducibility. The LOD and LOQ are calculated based on the standard deviation of the regression lines and slope of the calibration curves using the below equations: LOD = 3.3 ×σ/S.

$$LOQ = 10 \times \sigma/S$$

Where σ is the standard deviation of the regression line and S is the slope of the calibration curve. The results are summarized in Table 2.

2.7.5 Accuracy

This experiment can be performed by the recovery test. The method's recovery was assessed using known amounts of standard preparation at three different concentration levels i.e. 110, 120 and 130. From three sets, each concentration in duplicates were made and injected. The drug reference standards were added to the formulation (pre-analyzed sample) at levels of 110, 120, 130. The % recovery and % mean recovery for the drug were calculated three times, as shown in table 5.

2.7.6 Robustness

The effect of deliberate variations in method parameters like the composition of mobile phase, saturation time, development distance were evaluated. The RF and standard deviation of peak areas were calculated for each parameter and the % RSD was viewed as < 2% and the results are shown in Table 6.

3 RESULTS

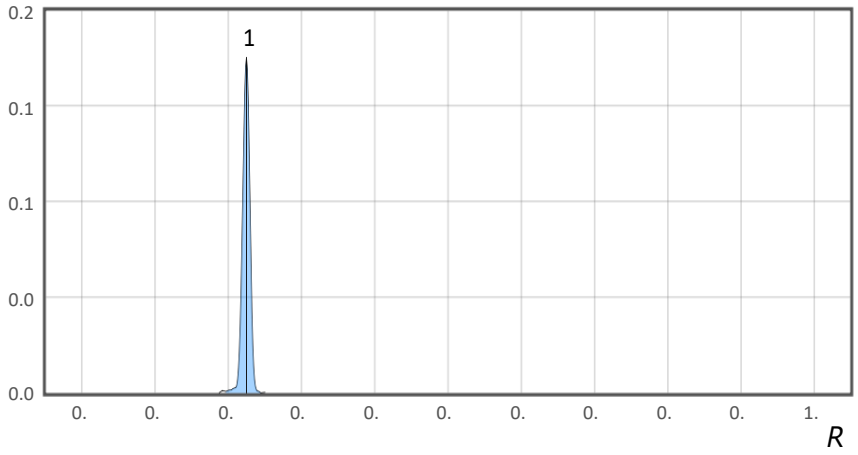

FIGURE NO. 2: TYPICAL DENSITOGRAM OF DACOMITINIB

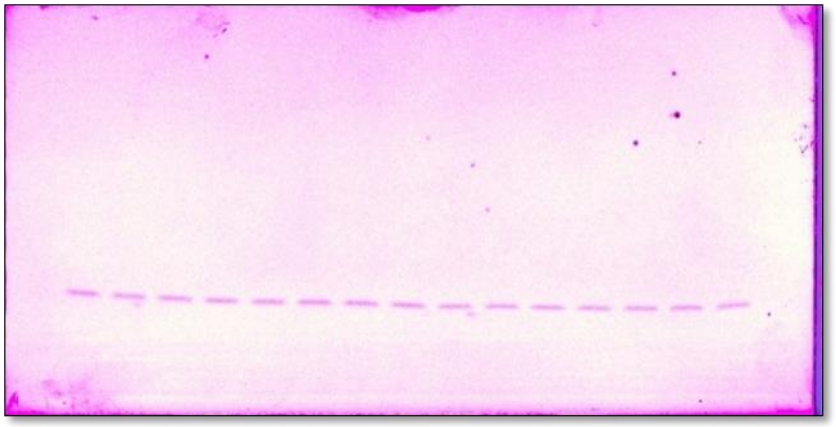

FIGURE NO. 3: TLC VISUALIZATION

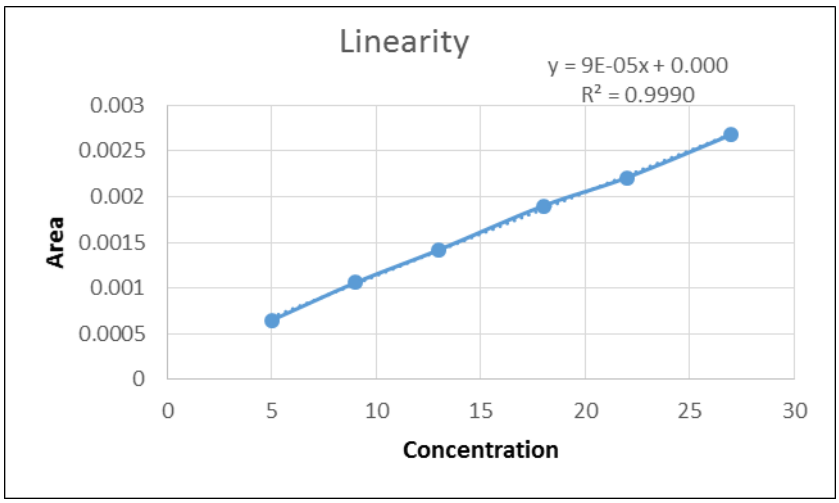

FIGURE NO. 4: CALIBRATION CURVE OF DACOMITINIB AT 282 NM

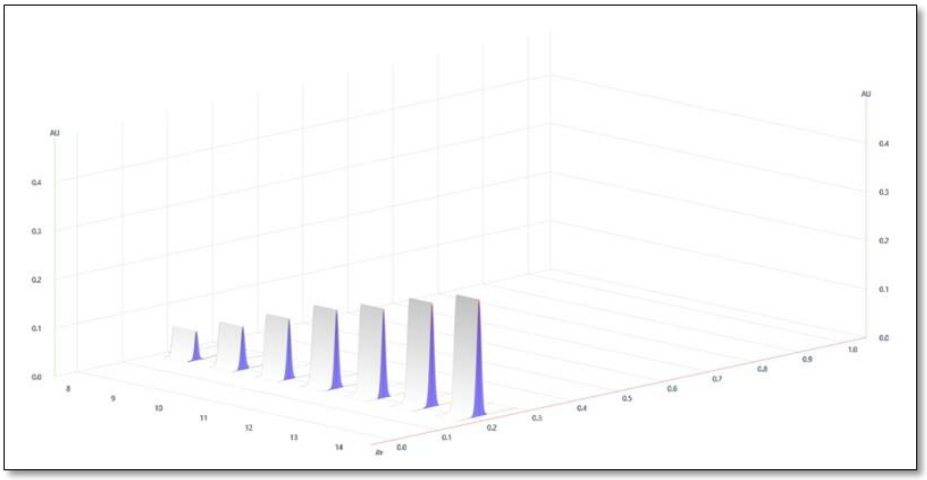

FIGURE NO. 5: THREE-DIMENTIONAL DENSITOGRAM FOR LINEARITY SPECTRA OF DACOMITINIB

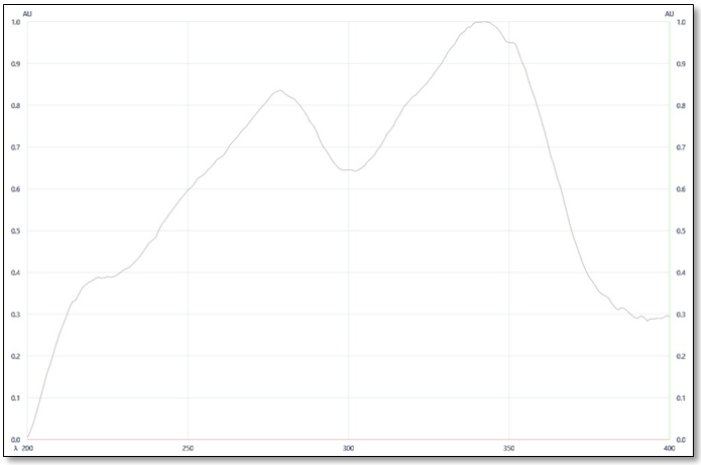

FIGURE NO. 6: PEAK PURITY SPECTRA OF DACOMITINIB

TABLE NO. 1: SYSTEM SUITABILITY DATA		
Parameters	(*R*F =0.24) (n = 6)	Limit Criteria (Reference Value 16)
% RSD of peak area	0.26	<2
% RSD of Retention Factor	1.87	<2
Tailing Factor	0.75	<2
Capacity Factor	1.50	0-10
Plate Height	576	-

TABLE NO. 2: REGRESSION ANALYSIS DATA FOR PROPOSED METHOD	
Parameter	HPTLC method
Detection wavelength (nm)	282
Concentration range (ng/band)	25-135 ng/band
Regression equation	y = 9E-05x + 0.000
Correlation coefficient	R2 = 0.9990
slope	0.000090
LOD (ng/band)	0.202
LOQ (ng/band)	0.611

TABLE NO. 3 : REPEATABILITY OF DACOMITINIB (n=6)	
Sr. No.	**Peak area of standard**
1	0.00280
2	0.00281
3	0.00276
4	0.00284
5	0.00281
6	0.00284
AVERAGE	0.00281
SD	0.000030
% RSD	1.056

n = no. of replicates

SD = standard deviation

RSD = relative standard deviation

TABLE NO. 4 : RESULT OF PRECISION STUDIES (n = 3)				
Concentration (ng/band)	**Intraday Precision**		**Interday Precision**	
	Mean area ± SD	**% RSD**	**Mean area ± SD**	**% RSD**
80	0.0017 ± 0.0000096	0.55	0.0020 ± 0.000017	0.83
90	0.0023 ± 0.0000058	0.25	0.0022 ± 0.0000086	0.39
100	0.0025 ± 0.0000096	0.40	0.0022 ± 0.000014	0.65

TABLE NO. 5: RESULT OF ACCURACY (n=3)					
% Level	**Std spiked (ng/band)**	**Amount recovered (mg/tab)**	**% amount recovered**	**% recovery**	**Mean % recovery**
110	9	49.33	109.62	99.65	100.43
120	18	54.03	120.08	100.06	
130	27	59.68	132.04	101.56	

TABLE NO. 6: ROBUSTNESS STUDIES			
Change in Mobile Phase ratio (6:3:1 % v/v/v ± 0.2)			
Ratio	**Rf**	**Mean % ± SD**	**% RSD**
5.8:3.2:1	0.23	97.72 ± 0.000022	1.13
6:3:1	0.24	99.91 ± 0.00001	0.48
6.2:2.8:1	0.22	99.46 ± 0.00001	0.74
Change in saturation time (45 min ± 10)			
Ratio	**Rf**	**Mean % ± SD**	**% RSD**
35	0.25	97.90 ± 0.00002	1.07
45	0.24	99.91 ± 0.00001	0.48
55	0.22	99.82 ± 0.00002	1.1
Change in development distance (8 cm± 1)			
Ratio	**Rf**	**Mean % ± SD**	**% RSD**
7	0.22	98.25 ± 0.000015	0.8
8	0.24	99.91 ± 0.000010	0.48
9	0.23	98.42 ± 0.000017	0.9

TABLE NO. 7: ANALYSIS OF DACOMITINIB IN TABLET DOSAGE FORM					
Drug	**Brand name**	**Label claim (mg/tab)**	**Amount found**	**Label claim Estimated (%)**	**% RSD**
Dacomitinib	Dacoplice	45 mg	44.88	99.74	0.48

TABLE NO. 8: ANALYTICAL VALIDATION PARAMETERS	
Parameters	**Dacomitinib**
Linearity	
Linearity range (ng/band)	25-135
Correlation coefficient (r2)	0.9990
Precision (%RSD)	
Repeatability	1.05
Intra-day precision	0.25-0.55
Inter-day precision	0.39-0.83
Sensitivity	
LOD (ng/band)	0.202
LOQ (ng/band)	0.611
Specificity	
r (S, M)	0.9998
r (M, E)	0.9995

4 DISCUSSION

A new, simple, accurate, fast, economic, and precise high-performance thin-layer chromatographic method was developed and validated for the estimation of Dacomitinib in a tablet dosage form. The chromatogram obtained by the proposed method is shown in **Fig. 2.** TLC visualization for Dacomitinib reference standard result obtained from the plate is given in **Fig. 3.**

The system suitability of the HPTLC system was demonstrated by comparing the obtained parameter values, with acceptance criteria of the ICH guidelines, such as percent relative standard deviation of peak area and retention factor, tailing factor and the obtained results found to be within the limits.

The calibration curve was found to be linear in between concentration range 25- 135 ng/band, regression coefficient was found to be 0.9990 with regression equation y = 9E-05x + 0.000.

Precision method was performed by repeatability and intermediate precision. % Relative deviation for repeatability of sample and references standard Dacomitinib and measurement of peak area was found to be 1.05 % as a result of system precision. % RSD values for intra and interday Precision were found to be in acceptable limits.

Proposed method was evaluated for accuracy in terms of percent recovery at level of 110, 120 and 130. Results for all different levels for percent recovery were found to be in range of 99-101 %.

By using the trendline equations derived from the experiments, the sensitivity of the method in terms of LOD and LOQ was calculated based on the standard deviation of the regression lines and slope of calibration curves. The LOD and LOQ were found to be 0.202 and 0.611 ng/b and indicating the sensitivity of developed method.

The peak purity of Dacomitinib was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the band. It demonstrates that the purity exceeded 0.999 for all peaks, indicating the specificity of the method in the presence of various excipients.

From obtained robustness result there was no difference or varied slightly significant difference between result obtained by applying various conditions. Thus method was shown to be robust for change in mobile phase ratio, change in saturation time and change in development distance.

The developed method was successfully applied for the analysis of marketed formulation i.e. Dacoplice and thus assay was found to be 99.74%.

5 CONCLUSION

The proposed HPTLC method was found to be simple, sensitive, specific, precise and accurate. This study reports a simple, fully validated HPTLC protocol for the quantification of Dacomitinib in pharmaceutical tablet formulation. It demonstrates that the method can accurately quantify the drug content of the tablet formulation without excipient interference or the necessity of a drug extraction step before analysis. Obtained results found to be in good agreement; thus, the developed method can be used in the quality control laboratories for the routine analysis of DACOPLICE in pharmaceutical tablet dosage form.

6 ACKNOWLEDGEMENT

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