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Development and Validation for Related Substances of Tartaric Acid Base Pellets of Dipyridamole Modified Release Capsules by Using High Performance Liquid Chromatography

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A simple, sensitive, robust stability indicating gradient high performance liquid chromatographic method was developed for the quantitative determination of related substance of dipyridamole in tartaric acid based pellets of dipyridamole modified release capsules. This method is able to separate all process known, degradation impurities which includes three newly identified potential degradant impurities. The method was developed by using YMC pack pro C8 (150 mm × 4.6 mm) 5 µm column with mobile phase containing mixture of mobile phase A (0.02 M buffer, pH 4.70, acetonitrile and methanol solution) and mobile phase B (0.02 M buffer, pH 4.70, acetonitrile and methanol solution). The flow rate was 1.5 mL/min with column temperature of 30 °C and detection wavelength at 295 nm. The dipyridamole substance and drug product was subjected to the forced degradation conditions and impurities were well resolved from dipyridamole and its impurities. This proved the stability-indicating nature of the method and validated as per ICH guidelines.

Keywords: Dipyridamole, Tartaric acid, Impurities, Forced degradation.

INTRODUCTION

Dipyridamole (Fig. 1) is used in combination with "blood thinners" such as warfarin to keep clots from forming after heart valve replacements. Clots are a serious complication that can cause strokes, heart attacks or blocked blood vessels in the lungs (pulmonary embolisms). Dipyridamole is an anti-platelet drug. It is an odourless yellow crystalline powder, having a bitter taste. It is soluble in dilute acids, methanol and chloroform and practically insoluble in water.

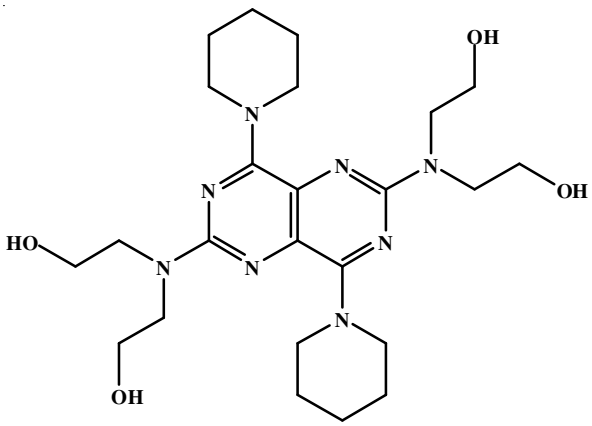

Fig. 1. Structure of dipyridamole

Dipyridamole in tartaric acid based pellets of dipyridamole modified release 150 and 200 mg capsules are available. Each capsule contains dipyridamole 200 and 150 mg respective dosage strength. The adults including the elders recommended dose is one capsule twice daily, usually one in the morning and one in the evening preferably with meals. The capsules should be swallowed whole without chewing as per emc [1].

In the literature survey, there were quite a few LC methods have been reported for determination of dipyridamole in pharmaceutical preparation [2] and few methods were reported for dipyridamole and its degradation product [2-7]. However, several methods were reported for determination of dipyridamole in combination with other drug [8-10]. Estimation of dipyridamole and its metabolites in human plasma by LC-MS and HPLC has been performed [10-13]. In this method, the reported impurities and degradation impurities which includes three newly identified potential degradant impurities in our drug product during force degradation studies. The newly three potential impurities in our drug product named as the impurity-1, impurity-2 and impurity-3. The impurity-1, impurity-2 and impurity-3 levels were found in the forced degradation study. These three-product degradations and their impurities may increase in the stability studies and shelf life of the product.

Hence, the monitoring of these degradants is required and very important during the release and stability studies of our

drug product. The analytical method was developed and validated and proven the power of the stability indicating HPLC method. All the known impurities process and degradation impurities were well separate from the dipyridamole and its impurities. The impurities are of dipyridamole namely as impurity-A, impurity-B, impurity-C, impurity-D, impurity-E, impurity-F and impurity-G impurities and drug product impurities namely as impurity-1, impurity-2 and impurity-3 (Table-1). The HPLC method was developed and validated with respect to specificity, linearity, precision, accuracy, LOD, LOQ, robustness and ruggedness. The force degradation studies were performed on the drug substance, placebo and drug products during the analytical method validation as ICH guidelines [14,15].

EXPERIMENTAL

The HPLC grade acetonitrile, methanol and potassium dihydrogen phosphate, orthophosphoric acid analytical grade and HPLC grade water. The dipyridamole drug substance, placebo samples of dipyridamole modified release capsules and samples of dipyridamole modified release capsules and impurities impurity-A, impurity-B, impurity-C, impurity-D, impurity-E, impurity-F, impurity-G, impurity-1, impurity-2 and impurity-3 were supplied by Bluefish Pharmaceuticals Pvt. Ltd., Bangalore, India.

The analytical method was developed by using the HPLC from Waters Alliance 2695 separation module equipped with 2489 UV/visible detector or 2998 PDA detector. The output signal was monitored and processed using Empower2 software.

Chromatographic conditions: The method was developed by using YMC pack pro C8 (150 mm × 4.6 mm) 5 µm column with mobile phase containing mixture of mobile phase A (0.02 M potassium dihydrogen phosphate, pH adjusted to 4.70 with diluted orthophosphoric acid, acetonitrile and methanol solution) and mobile phase B (0.02 M potassium dihydrogen phosphate, pH adjusted to 4.70 with diluted orthophosphoric acid, acetonitrile and methanol solution). The flow rate was 1.5 mL/min with column temperature of 30 °C and detection wavelength at 295 nm. The injection volume was 10 µL with the gradient program (Table-2).

Preparation of solutions

Diluent solution: Diluent used as mixture of 50:50 % v/v ratio of 0.01N hydrochloric acid and methanol.

TABLE-2
GRADIENT PROGRAM

Time	% Mobile phase-A (v/v)	% Mobile phase-B (v/v)
0	60	40
10	60	40
15	40	60
25	40	60
30	0	100
55	0	100
60	60	40
65	60	40

Dipyridamole system suitability solution: The dipyridamole system suitability solution in the concentration of 1 mg/mL prepared by using the ethanol sonicate to dissolve the material and followed makeup to the volume with diluent.

Preparation of standard solution of dipyridamole (stock): The standard stock of dipyridamole were prepared with ethanol followed by in diluent with a concentration of 0. 2 mg/mL.

Preparation of standard solution of dipyridamole: The standard solution of dipyridamole were prepared by diluting dipyridamole standard solution (stock) to get the final working concentration of 0.003 mg/mL

Preparation of sample solution: Weighed and transferred the pellets equivalent to 100 mg of dipyridamole and sonicate the sample with the known volume of the ethanol for 15 min with intermittent shaking. Further add 50 mL of diluent and sonicate for 15 min with intermittent shaking. Filter the solution in 0.45 µ membrane filter. The concentration of the solution is 1 mg/mL.

System suitability criteria: The theoretical plate count for system suitability solution of dipyridamole peak should not be less than 5000. The present relative standard deviation of dipyridamole peak area for six replicate injections should not more than 5.0. The theoretical plate for dipyridamole peak in standard solution should not be less than 2000. The tailing factor for dipyridamole peak in standard solution should be in between 0.8 to 1.5.

RESULTS AND DISCUSSION

Method development and optimization: The analytical method was developed to resolve all the known impurities from each known impurity and dipyridamole. The spiked sample was prepared by spiking all the known impurities, impurity-A, impurity-B, impurity-C, impurity-D, impurity-E, impurity-

TABLE-1
IMPURITIES OF DIPYRIDAMOLE AND ITS CHEMICAL NAMES

Name of the impurity	Chemical Name
Impurity-A	2,2′-[[4,6,8-tri(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-2-yl]nitrilo]diethanol
Impurity-B	2,2′,2″,2‴,2⁗,2⁗′[[8-(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,4,6-triyl]trinitrilo]hexaethanol
Impurity-C	2,2′-[[6-chloro-4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-2-yl]nitrilo]diethanol
Impurity-D	2,2′-[[6-[(2-hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-2-yl]nitrilo]diethanol
Impurity-E	2,2′,2″,2‴-[[6,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,4-diyl]dinitrilo]tetraethanol
Impurity-F	2,2′,2″,2‴-[[4-[(2-hydroxyethyl)amino]-8-(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,6-diyl]dinitrilo]tetraethanol
Impurity-G	2,6-dichloro-4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidine
Impurity-1	2,6-*bis*(*bis*(2-hydroxyethyl)amino)-8-(piperidin-1-yl)pyrimido[5,4d]pyrimidin-4-ol
Impurity-2	2,6-*bis*(*bis*(2-hydroxyethyl)amino)-8-(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-4-amine-*n*-pentanal
Impurity-3	2,3-Di hydroxy-succinic acid mono [2-[[6-[*bis*-(2-hydroxy-ethyl)-amino]-4-piperidin-1-yl-pyrimido [5,4-d] pyrimidin-2-yl] –(2-hydroxy-ethyl)-amino]-ethyl] ester

F, impurity-G, impurity-1, impurity-2 and impurity-2 with test concentration.

The gradient method employed using mobile phase-A, 1 g of potassium dihydrogen orthophosphate in 1000 mL of water adjusted the pH 7.0 with diluted sodium hydroxide, Mobile phase-B as methanol, HPLC column Intertsil ODS-2 (100 mm × 4.0 mm), 5 µm, flow 1.4 mL/min, column temperature 35 °C with the HPLC instrument, the know impurity-2 is co-eluting with the dipyridamole peak.

The gradient method employed using mobile phase-A, 2.72 g of sodium acetate dihydrate in 1000 mL of water adjusted the pH 4.4 with diluted orthophosphoric acid, mobile phase-B as methanol, HPLC column Intertsil ODS-2 (250 mm × 4.6 mm), 5 µm, flow 1.2 mL/min, column temperature 30 °C with the HPLC instrument, all the known impurities were eluted with less resolution. The gradient method employed using mobile phase-A mixture of, 6.8 g of potassium dihydrogen orthophosphate in 1000 mL of water adjusted the pH 4.4 with diluted orthophosphoric acid, methanol, acetonitrile in the ratio of 90:5:5, mobile phase-B mixture of above buffer, methanol, acetonitrile in the ratio of 30:40:30, HPLC column Prontosil C8 (150 mm × 4.6 mm), 5 µm, flow 1.5 mL/min, column temperature 30 °C with the HPLC instrument, the resolution between impurity-2 and impurity-C is less and impurity-G is not eluting. Base on above trail, the gradient program was modified and found that all the known impurities were well separated and less resolution was observed between impurity-E and impurity-2.

The gradient program was optimized using mobile phase-A mixture of, 6.8 g of potassium dihydrogen orthophosphate in 1000 mL of water, added 1 mL of triethylamine, mix, adjusted the pH 4.7 with diluted orthophosphoric acid, methanol, acetonitrile in the ratio of 90:5:5, mobile phase-B mixture of above buffer, methanol, acetonitrile in the ration of 30:30:40, HPLC column YMC pack pro C8 (150 mm × 4.6 mm), 5 µm, flow 1.5 mL/min, column temperature 30 °C with the HPLC instrument. It is observed that the dipyridamole peak shape and all the impurities peak shapes were good. The resolution between dipyridamole and all the impurities are well separated and there is no blank and placebo interference was observed at the retention time of main peak and impurities.

Based on the optimization of the trials, the below mentioned chromatographic conditions were finalized for the quantification of the known impurities in the dipyridamole in tartaric acid based pellets of dipyridamole modified release capsules as follows, The gradient using mobile phase-A mixture of, 6.8 g of potassium dihydrogen orthophosphate in 1000 mL of water, added 1 mL of trirthylamine, mix, adjusted the pH 4.7 with dil. orthophosphoric acid, methanol, acetonitrile in the ration of 90:5:5, mbile phase-B mixture of above buffer, methanol, acetonitrile in the ratio of 30:30:40, HPLC column YMC pack pro C_8 (150 mm × 4.6 mm), 5 µm, flow 1.5 mL/min, column temperature 30 °C with the HPLC instrument. The injection volume is 10 µL. The relative retention times (RRT) of the impurities (Table-3).

TABLE-3
RELATIVE RETENTION TIMES OF IMPURITIES

Impurity name	RRT	Impurity name	RRT
Impurity-A	2.55	Impurity-G	2.64
Impurity-B	0.20	Impurity-1	0.36
Impurity-C	1.75	Impurity-2	1.47
Impurity-D	0.80	Impurity-3	0.70
Impurity-E	1.43	–	–
Impurity-F	0.33	–	–

Method validation: The developed analytical method for quantification of the known impurities in the dipyridamole in tartaric acid based pellets of dipyridamole modified release capsules was validated as per ICH guidelines [15,16]. The following validation characteristics were addressed: specificity, estimation of LOD and LOQ, accuracy, precision, linearity, range, ruggedness and robustness.

System suitability: System suitability is defined as the checking of a system, before or during analysis of unknowns, to ensure system performance. The all the parameters were found well within the acceptance criteria (Table-4). The chromatograms of the blank (Fig. 2), placebo (Fig. 3), system suitability solution (Fig. 4), standard solution (Fig. 5), as such sample (Fig. 6) and all the known impurities spiked blend solution chromatograms in Fig. 7.

Limit of detection and limit quantification: The limit of detection (LOD) and limit of quantification (LOQ), concentrations of 1 to 50 % of dipyridamole and all individual known impurity solutions were prepared within the range of specification level and injected in to HPLC as per test method. Calculated correlation coefficient, LOD and LOQ values from the linearity slope method (Table-5).

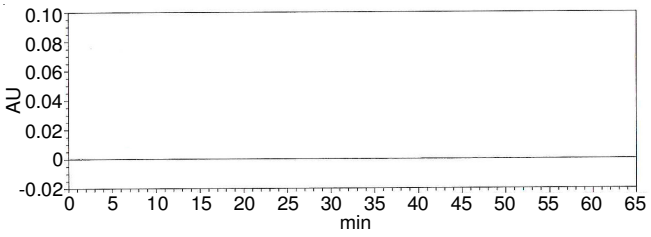

Fig. 2. Typical chromatogram of blank

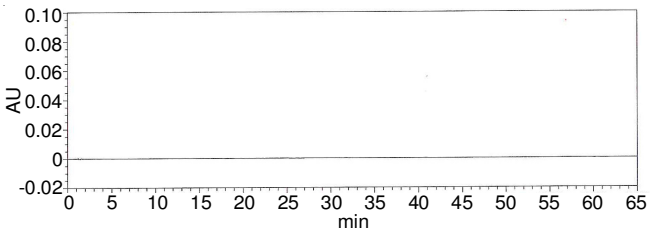

Fig. 3 Typical chromatogram of placebo

TABLE-4
SYSTEM SUITABILITY CRITERIA AND RESULTS

Parameter	Acceptance criteria	Result
Theoretical plate count for system suitability solution of dipyridamole peak.	≥ 5000	26250
Present relative standard deviation of dipyridamole peak area for six replicate injections.	≤ 5.0	0.8
Theoretical plate for dipyridamole peak in standard solution.	≥ 2000	50072
Tailing factor for dipyridamole peak in standard solution.	≥ 0.8 ≤ 1.5	1.0

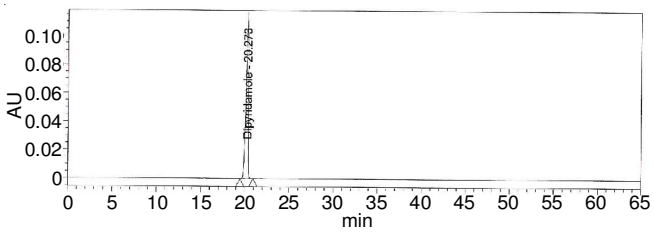

Fig. 4. Typical chromatogram of system suitability solution

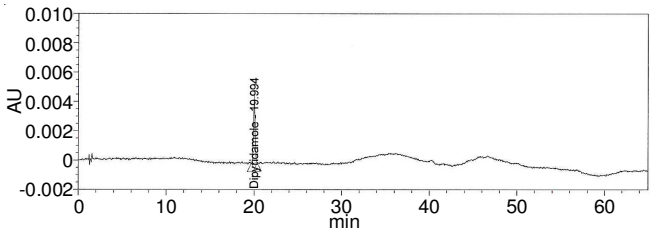

Fig. 5. Typical chromatogram of standard solution

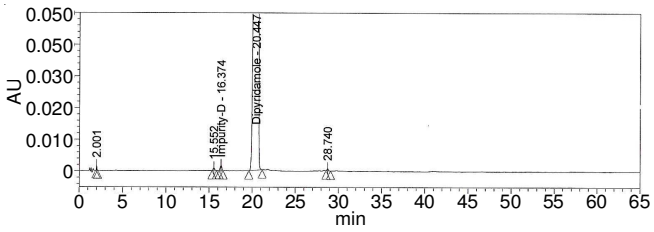

Fig. 6. Typical chromatogram of as such sample

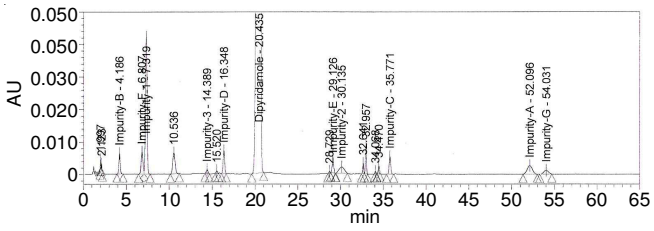

Fig. 7. Typical chromatogram of spiked sample

TABLE-5
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Name	Concentration (µg/mL)		Correlation co efficient
	Limit of detection	Limit of quantification	
Dipyridamole	0.03488	0.10571	0.999
Impurity-A	0.22021	0.66731	0.998
Impurity-B	0.05250	0.15909	0.999
Impurity-C	0.07269	0.22026	0.999
Impurity-D	0.03296	0.09988	0.999
Impurity-E	0.08525	0.25833	0.999
Impurity-F	0.02860	0.08666	0.999
Impurity-G	0.05206	0.15774	0.999
Impurity-1	0.06874	0.20831	0.999
Impurity-2	0.25746	0.78018	0.999
Impurity-3	0.11457	0.34719	0.998

Specificity: Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The below mentioned studies were conducted as part of the specificity study.

Blank interference: Blank was prepared and injected as per test method. It was observed that no peak interference at the retention time of analyte peak and known impurity peaks.

Placebo interference: Placebo solutions equivalent to the test concentration were prepared in duplicate and injected as per test method. It was observed that no peak interference at the retention time of analyte peak and known impurity peaks.

Impurity interference: Individual impurity solutions were prepared at shelf life specification limit and analyzed as per test method. It was observed that known impurities were not co-eluting with each other and with analyte peak.

As such and spiked sample: As such and spiked sample solutions were prepared by spiking all individual known impurities at self-life specification level and analyzed as per test method. It was observed that peak purity of dipyridamole and all known impurities in both as such and spiked sample were passing and there is no presence of purity flag in the purity results table.

Forced degradation: Forced degradation studies were performed on blank, drug substance; drug product and placebo for acid degradation, alkali degradation, peroxide degradation, water degradation, thermal degradation, humidity degradation, uv light degradation and visible light degradations and samples were injected as per test method. Degradation was calculated, analyte peak and known impurity peak purity was evaluated in all conditions. The peak purity of analyte peak and all known impurities in all the stressed samples were found to be within the acceptance criteria and there was no presence of the purity flag for the peaks in the purity results table. Mass balance assessment was also performed in all degradation conditions and found to be within the acceptance criteria. The summery of the forced degradation study were tableted in Table-6. The UV light and visible light degradations were conducted as per the ICH guidelines.

Method precision (repeatability): Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. To evaluate the method precision for related substances method, six replicate test preparations (n = 6) of dipyridamole, 200 mg modified release capsules were prepared as such (Table-7) and by spiking all individual known impurities at shelf life specification level (Table-8) and analyzed as per test method. The % individual known impurity, % of total impurities and % RSD for % individual known impurity and % of total impurities were calculated and found to be within the acceptance criteria.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. A series of sample solutions were prepared in triplicate (six replicate test preparations for LOQ and about 300 % levels) by spiking the individual known impurities in placebo for LOQ level and on sample in the range of about 50, 100,150 and 300 % of shelf life specification level and injected into HPLC system and analyzed as per the test method. The concentrations of dipyridamole: 0.1, 1.0, 2.0, 3.0, 6.0 µg/mL; impurity-A: 0.6, 2.8, 5.1, 7.5, 16.2 µg/mL; impurity-B: 0.1, 2.5, 5.0, 7.4, 15.0 µg/mL; impurity-C: 0.2, 2.9, 5.7, 8.8, 17.7 µg/mL; impurity-D: 0.1, 3.1, 6.2, 9.3, 18.7 µg/mL; impurity-E: 0.3, 2.6, 5.1, 7.4, 15.1 µg/mL; impurity-F: 0.1, 2.7, 5.4, 8.1, 16.2 µg/mL; impurity-G: 0.1, 3.2, 6.0, 8.4, 19.2 µg/mL; impurity-1: 0.2, 19.7, 39.2, 59.0, 133.3 µg/mL;

TABLE-6
SUMMERY FOR FORCED DEGRADATION

Degradation condition	Drug product			
	Degradation (%)	Peak purity	Assay (%)	Mass balance
As such sample	0.39	Pass	100.4	N/A
2 HCl Heated at 60 °C for 4 h	0.77	Pass	95.8	96.18
2 N NaOH Heated at 60 °C for 4 h	3.21	Pass	95.8	98.62
% Peroxide heated at 60 °C for 4 h	3.36	Pass	95.1	98.07
Water degradation	0.43	Pass	97.8	97.84
Heated at 105 °C for 24 h	2.21	Pass	98.0	99.82
Humidity degradation 90 % RH for 6 h	9.97	Pass	92.4	101.98
UV light degradation 200 Watt. h/sq mt	0.39	Pass	98.3	98.30
Visible light degradation 1.2 million lux hours	2.85	Pass	96.9	99.36

TABLE-7
METHOD PRECISION DATA FOR AS SUCH SAMPLE

Sample No.	Impurity name		Total impurities
	Impurity-D	Single max unknown impurity	
1	0.14	0.05	0.24
2	0.14	0.05	0.24
3	0.14	0.05	0.24
4	0.15	0.05	0.25
5	0.14	0.05	0.24
6	0.14	0.05	0.24
Mean	0.14	0.05	0.24
RSD (%)	2.90	0.00	1.70

impurity-2: 1.0, 5.7, 11.5, 16.2, 30.4 µg/mL and impurity-3: 0.3, 1.9, 3.5, 5.0, 10.8 µg/mL. Individual % recovery, mean % recovery, % RSD and squared correlation coefficient for linearity of the test method were calculated and the results were found to be within the acceptance criteria (Table-9).

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

A series of solutions of dipyridamole and known impurity solutions were prepared in the range of LOQ to about 300 % of shelf life specification level and injected into the HPLC system. Linearity of detector response was established by plotting a graph between concentration *vs.* response of dipyridamole and all individual known impurity peaks. The detector response was found to be linear from about LOQ to 300 % of shelf life specification level and injected into HPLC system and analyzed as per the test method. The concentrations of dipyridamole: 0.1, 1.1, 2.3, 3.3, 6.6 µg/mL; impurity-A: 0.6, 2.4, 5.2, 7.6, 15.2 µg/mL; impurity-B: 0.1, 2.2, 4.6, 6.8, 13.6 µg/mL; impurity-C: 0.2, 2.4, 4.8, 7.5, 15.0 µg/mL; impurity-D: 0.1, 2.5, 5.2, 7.7, 15.3 µg/mL; impurity-E: 0.3, 2.3, 4.9, 7.3, 14.5 µg/mL;

TABLE-8
METHOD PRECISION DATA FOR SPIKED SAMPLE

Sample No.	Impurity name											Total impurities
	A	B	C	D	E	F	G	1	2	3	Single max Unknown impurity	
1	0.52	0.59	0.55	0.62	0.60	0.47	0.61	3.74	1.08	0.27	0.09	9.14
2	0.52	0.58	0.55	0.62	0.61	0.47	0.62	3.77	1.07	0.27	0.10	9.18
3	0.52	0.58	0.54	0.61	0.60	0.47	0.59	3.77	1.07	0.27	0.11	9.13
4	0.50	0.57	0.54	0.62	0.60	0.47	0.60	3.74	1.08	0.27	0.10	9.09
5	0.52	0.58	0.55	0.62	0.60	0.47	0.61	3.78	1.08	0.27	0.10	9.18
6	0.52	0.58	0.55	0.62	0.60	0.46	0.59	3.75	1.09	0.26	0.10	9.12
Mean	0.52	0.58	0.55	0.62	0.60	0.47	0.60	3.76	1.08	0.27	0.10	9.14
RSD (%)	1.6	1.1	0.9	0.7	0.7	0.9	2.0	0.5	0.7	1.5	6.3	0.4

TABLE-9
ACCURACY DATA OF DIPYRIDAMOLE (API) AND KNOWN IMPURITIES

Spike level		% Recovery and relative standard deviation of dipyridamole and known impurities										
		A	B	C	D	E	F	G	1	2	3	API
Level-1 LOQ	Mean	100.1	99.2	108.4	91.3	103.9	105.2	96.3	106.6	100.0	100.3	103.6
	RSD (%)	2.5	4.1	2.2	8.0	7.0	6.3	3.7	3.3	7.0	9.0	2.5
Level-2	Mean	107.6	107.8	110.3	95.4	107.1	105.1	107.3	93.6	107.0	101.8	105.1
	RSD (%)	2.3	3.2	2.5	3.5	3.2	0.2	3.4	0.5	4.2	8.0	0.7
Level-3	Mean	108.0	108.2	106.3	95.7	101.5	103.3	107.7	101.4	108.0	111.4	101.1
	RSD (%)	1.1	3.2	0.4	0.9	0.8	0.9	2.7	1.0	1.3	1.3	1.1
Level-4	Mean	109.0	102.4	102.3	94.2	96.6	107.2	106.1	100.0	103.0	110.3	103.5
	RSD (%)	2.1	3.0	1.7	1.7	2.4	0.7	0.9	0.6	3.0	1.5	1.1
Level-5	Mean	107.8	102.1	106.0	95.7	99.2	103.3	105.9	92.5	104.4	107.1	102.9
	RSD (%)	2.5	3.0	3.1	3.3	3.5	3.1	3.2	11.3	3.4	3.3	1.1

TABLE-10
LINEARITY DATA OF DIPYRIDAMOLE AND KNOWN IMPURITIES

Description	Dipyridamole and known impurities					
	A	B	C	D	E	F
Square of correlation coefficient (r^2)	0.9987	0.9988	0.999	0.9992	0.9992	0.9993
Slope	23975.3	13144.6	15730.3	15145.6	10355.0	21850.9
Y-Intercept	619.1	1557.5	1128.7	1285.9	782.9	1586.2
% of Y-intercept	0.5	2.5	1.4	1.6	1.50	1.4
Residual sum of squares	94197074.7	22788431.7	33096597.0	25549046.4	10388004.8	43431053.1
	G	1	2	3	API	
Square of correlation coefficient (r^2)	0.9987	0.9991	0.9992	0.9983	0.9993	
Slope	10879.4	15273.1	8287.0	5821.8	21816.3	
Y-Intercept	1972.7	4076.0	4057.8	279.5	1423.8	
% of Y-intercept	3.6	0.6	4.6	1.3	2.9	
Residual sum of squares	18240160.7	1755718512	26532165.6	3392335.2	8871305.9	

impurity-F: 0.1, 2.5, 5.2, 7.7, 15.4 µg/mL; impurity-G: 0.1, 2.3, 4.9, 7.2, 14.4 µg/mL; impurity-1: 0.2, 19.3, 41.0, 60.3, 120.7 µg/mL; impurity-2: 0.7, 4.7, 9.9, 14.6, 29.2 µg/mL and impurity-3: 0.4, 1.7, 3.5, 5.2, 10.3 µg/mL.

The square of correlation coefficient, slope and % y-intercept at 100 % level, intercept and residual sum of squares were calculated and the results were found to be within the acceptance criteria (Table-10).

Ruggedness: The ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by change in instrument, environment or the analyst and provides an indication of its reliability during normal usage.

To evaluate the intermediate precision for related substances method, six replicate test preparations (n = 6) of dipyridamole 200 mg modified release capsules were prepared by spiking all the known impurities at shelf life specification level and analyzed as per test method by using different HPLC system, different column of same make by different analyst on different day. The % individual known impurity, % of total impurities and % RSD for % of individual known impurity and % total impurities were calculated and found to be within the acceptance criteria. The overall % RSD for % of individual known impurity, % of total impurities for replicate preparations (n = 12) of method precision and intermediate precision were calculated and found to be within the acceptance criteria (Table-11).

TABLE-11
RUGGEDNESS DATA

Impurity	% RSD for six individual preparation	Overall RSD (%) (n = 12)
A	1.1	4.8
B	0.9	3.2
C	1.1	7.2
D	1.3	2.5
E	0.7	4.6
F	3.7	3.3
G	1.8	12.6
1	0.7	1.6
2	1.6	3.1
3	3.2	3.8
Single max unknown impurity	5.2	6.1
Total impurities	0.3	1.9
Impurity	% RSD for six individual preparation	Overall RSD (%) (n = 12)

Solution stability and mobile phase stability: The solution stability of dipyridamole and its impurities was determined by keeping sample solution and standard solutions in tightly capped volumetric flasks at room temperature for 1 day, 2 day and 3 days and measured against freshly prepared standard solution. The standard solution and sample solutions was found stable for 3 days at room temperature.

The stability of mobile phase was also determined by freshly prepared solutions of dipyridamole and its impurities at 1 day, 2 day and 3 day. The mobile phase was found stable for 3 days at room temperature.

Robustness: The 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 (Table-12).

TABLE-12
ROBUSTNESS DATA

Parameter variation	Theoretical plate count for system suitability solution of dipyridamole peak should not be less than 5000	Present relative standard deviation of dipyridamole peak area for six replicate injections should not more than 5.0	Theoretical plate for dipyridamole peak in standard solution should not be less than 2000	Tailing factor for dipyridamole peak in standard solution should be in between 0.8 to 1.5
Flow 1.30 mL/min	29405	1.0	55366	1.0
Flow 1.70 mL/min	22194	1.1	48702	1.0
Wavelength 293 nm	26237	1.9	50195	1.0
Wavelength 297 nm	26262	2.3	49273	1.0
Column temp. (25 °C)	24445	1.3	49253	1.0
Column temp. (35 °C)	30028	2.4	56597	1.0
pH 4.50	23579	2.7	63992	1.0
pH 4.90	40062	1.3	42147	1.0

Conclusion

A simple, sensitive, robust stability indicating gradient high performance liquid chromatographic method was developed for the quantitative determination of related substance of dipyridamole in tartaric acid based pellets of dipyridamole modified release capsules. This method is able to separate all process known impurities and degradation impurities which includes three newly identified potential degradant impurities. Be precise, accurate, linear, robust and rugged during validation. Satisfactory results were obtained from the validation of the method. The analytical method is stability indicating and can be used for routine analysis of production samples and to check the stability of the dipyridamole in tartaric acid based pellets of dipyridamole modified release capsules.

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