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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF RELATED IMPURITIES IN COMBINED DOSAGE FORM OF ASPIRIN AND PRASUGREL HYDROCHLORIDE BY RP-HPLC

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ABSTRACT

A simple, economic, selective and precise RP-HPLC method has been developed and validated for the estimation of related impurities of Aspirin and Prasugrel Hydrochloride in combined dosage form. A gradient reverse phase high performance liquid chromatography (RP-HPLC) analysis was performed on Hypersil BDS C18 column (250mm X 4.6mm, 5µm) using mobile phase A: 0.05M Ammonium acetate buffer pH-3.0 and mobile phase B:Acetonitrile at a flow rate of 1.0 ml/min and the detection wavelength was 224nm. The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 4-22.5µg/ml for related impurities of Aspirin and 0.5-3µg/ml for related impurities of Prasugrel Hydrochloride with correlation coefficient more than 0.990 for related impurities of Aspirin and Prasugrel Hydrochloride. The % recovery value was found minimum of 95.62% and maximum of 104.50% for all known impurities of Aspirin and Prasugrel Hydrochloride. The relative standard deviation value for repeatability, interday precision and intraday precision was less than 5%. The LOD value was found minimum of 0.09µg/ml and maximum of 1.49µg/mlfor all known impurities. The LOQ value was found minimum of 0.28µg/ml and maximum of 4.52µg/mlfor all known impurities.The proposed method was found to be specific, linear, sensitive, precise, accurate and robust in nature.

INTRODUCTION

Aspirin chemically is 2-(acetyloxy) benzoic acid is cyclooxygenase inhibitor. The molecular formula of aspirin is $C_9H_8O_4$ and the molecular weight is 180.16 gm/mole[1]. Aspirin inhibits the activity of cyclooxygenase to decrease the formation of precursors of prostaglandins and thromboxanes from arachidonic acid. It is used as non-steroidal anti-inflammatory drug and inhibits platelet aggregation. The related impurities of aspirin are Salicylic acid, 4-Hydroxybenzoic acid and 4-Hydroxyisophthalic acid[2]. Structure of aspirin and its related impurities are given below in Figure 1, 2, 3 and 4.

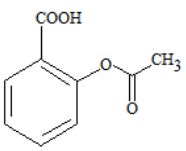

Fig 1 Chemical Structure of Aspirin

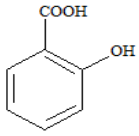

Fig 2 Chemical Structure of Salicylic Acid

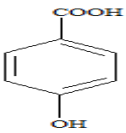

Fig 3 Chemical Structure of 4-Hydroxybenzoic Acid

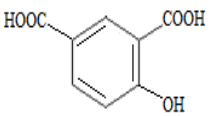

Fig 4 Chemical Structure of 4-Hydroxyisophthalic Acid

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Prasugrel Hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4H,5H,6H,7H-thieno[3,2c]pyridine-2-yl acetate hydrochloride. The molecular formula of Prasugrel Hydrochloride is $C_{20}H_{21}ClFNO_3S$and the molecular weight is 409.902 gm/mole [3].PrasugrelHydrochlorideinhibits adenosine diphosphate induced platelet aggregation and used in the treatment of coronary artery disease. The related impurities of Prasugrel Hydrochloride are Desfluoro Prasugrel, 4-Fluoro Prasugrel and 3-Fluoro Prasugrel[4-5].Structure of Prasugrel Hydrochloride and its related impurities are given below in Figure 5, 6, 7 and 8.

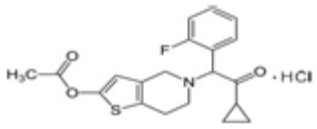

Fig 5 Chemical Structure of Prasugrel Hydrochloride

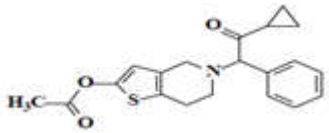

Fig 6 Chemical Structure of Desfluoro Prasugrel

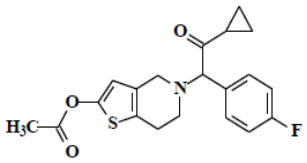

Fig 7 Chemical Structure of 4-Fluoro Prasugrel

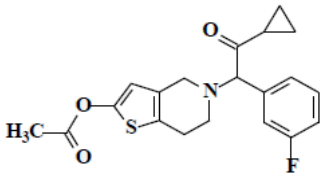

Fig 8 Chemical Structure of 3-Fluoro Prasugrel

Literature review reveals that a number of analytical methods have been developed for the estimation of aspirin and prasugrel hydrochloride in combined dosage forms[6-9]. But no related impurities method has been reported forthe estimation of aspirin and prasugrel hydrochloride in combined dosage form by RP-HPLC.The present aim of the work is to develop RP-HPLC method for the estimationof related impurities of aspirin and prasugrel hydrochloride in combined dosage form. The developed method was also validated to ensure the compliance in accordance with International Conference on Harmonization Guidelines.

MATERIALS AND METHODS

Instruments

- ✓ HPLC - Shimadzu LC-20 AT consisting of BDS Hypersil C18 column.
- ✓ UV Visible spectrophotometer - Systronic 119
- ✓ Electronic Balance – Shimadzu ATX-224
- ✓ pH meter –Analab Scientific Pvt Ltd
- ✓ Sonicator–Frontline Ultrasonic Cleaner

Chemicals

Chemicals	Make	Grade
Methanol	Merck	HPLC
Acetonitrile	Merck	HPLC
Ammonium acetate	Merck	HPLC
Orthophosphic acid	Rankem	AR

Raw Materials

Aspirin and its Related Impurities (Salicylic acid, 4-Hydroxybenzoic acid and 4-Hydroxyisophthalic acid) were obtained as a gift samples from Rivan Pharmaceutical Pvt Ltd, Ahmedabad, India.

Prasugrel Hydrochloride and its Related Impurities (Desfluoro Prasugrel, 4-Fluoro Prasugrel and 3-Fluoro Prasugrel) were obtained as a gift samples from Remus Pharmaceutical Pvt Ltd, Ahmedabad, India.

Method Development

Optimized Chromatographic Conditions

Stationary Phase: Hypersil BDS C18 (250 x 4.6mm, 5 µm)
MobilePhase:
Mobile Phase A: 0.05M Ammonium acetate buffer pH-3.0
Mobile Phase B: Acetonitrile

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-7	90	10
7-22	50	50
22-30	90	10

Diluent: 0.05M Ammonium acetate buffer pH 3.0: Acetonitrile (50:50)
Flow rate: 1ml/min
Wavelength: 224nm
Injection volume: 20 µl

System Suitability Solution: Prepare a combine standard preparation of all known impurities with aspirin and prasugrel hydrochloride of known concentration of 0.1mg/ml. Results for system suitability were shown in Figure 11 and Table 1 and 2.

Preparation of Standard Solution

- ✓ Standard stock solution of Salicylic acid(150 ppm):
- ✓ Take 15mg of Salicylic acid working standard into a 100ml volumetric flaskand dissolve with diluent upto the mark.
- ✓ Standard stock solution of 4-Hydroxybenzoic acid(160 ppm):
- ✓ Take accurately 4.0mg of 4-Hydroxybenzoic acid working standard into a 25ml volumetric flask and dissolve with diluent upto the mark.
- ✓ Standard stock solution of 4-Hydroxyisophthalic acid(160 ppm):
- ✓ Take accurately 4.0mg of 4-Hydroxyisophthalic acidworking standard into a 25ml volumetric flaskand dissolve with diluent upto the mark.
- ✓ Standard stock solution of Desfluoro Prasugrel(20 ppm):
- ✓ Take accurately 4.0mg of Desfluoro Prasugrel working standard into a 200ml volumetric flask and dissolve with diluent upto the mark.
- ✓ Standard stock solution of 4-Fluoro Prasugrel(20 ppm):

- ✓ Take accurately 4.0mg of 4-Fluoro Prasugrelworking standard into a 200ml volumetric flask and dissolve with diluent upto the mark.
- ✓ Standard stock solution of 3-Fluoro Prasugrel(20 ppm):
- ✓ Take accurately 4.0mg of 3-Fluoro Prasugrel working standard into a 200ml volumetric flask and dissolve with diluent upto the mark.

Preparation of Working Standard Solution

Working standard solution of Aspirin Related Impurities

(Salicylic acid-15ppm, 4-hydroxybenzoic acid-16ppm and 4-hydroxyisopthalic acid-16ppm):
Take 1ml each from standard stock solutions of salicylic acid, 4-hydroxybenzoic acid and 4-hydroxyisopthalic acid into a 10ml volumetric flask and make up with diluents upto the mark.

Working standard solution of Prasugrel Hydrochloride Related Impurities

(Desfluoro Prasugrel-2ppm, 4-Fluoro Prasugrel-2ppm and 3-Fluoro Prasugrel-2ppm):
Take 1ml each from standard stock solutions of Desfluoro Prasugrel, 4-Fluoro Prasugrel and 3-Fluoro Prasugrel into a 10ml volumetric flask and make up with diluents.

Preparation of Test Solution

Weigh and take content of 20 capsules. Take capsule powder equivalent to 150mg of Aspirin into a 100ml volumetric flask. Add 60ml diluent and shake for 15minutes and sonicate for 10minutes. Make up volume with diluent. Filter this solution with 0.45micron membrane filter.

Method Validation [10]

The proposed method for estimation of related impurities of Aspirin and Prasugrel Hydrochloride is validated as per ICH guidelines.

Specificity

Specificity is a measure of relative separation of the individual components. The test is useful for showing separation / estimation of impurity peaks from the principal peak.Specificity is carried out to demonstrate that individual expected known peaks of the impurities are completely separated from Aspirin and Prasugrel Hydrochloride peak. The working standard solution ofrelated impurities of Aspirin and Prasugrel Hydrochloride were spiked with the test solutionand injected into the chromatographic system to develop a chromatograph.The chromatogram of blank is not interfering with all known impurities of Aspirin and Prasugrel Hydrochloride peaks.

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Precision was carried out at three levels i.e. repeatability, intra-day precision and inter-day precision

Repeatability

The working standard solution of related impurities of Aspirin and Prasugrel Hydrochloride was injected six times and areas of peaks were measured and % R.S.D. was calculated. Results for repeatability were shown in Table 3.

Intraday Precision

The working standard solution of related impurities of Aspirin and Prasugrel Hydrochloridewere analyzed three times on the same day and % R.S.D was calculated. It was carried out at three different levels i.e. LOQ, 100% and 150% under specified chromatographic conditions. Results for intraday precision were shown in Table 4 and 5.

Interday Precision

The working standard solution of related impurities of Aspirin and Prasugrel Hydrochloridewere analyzed three times on the different day and % R.S.D was calculated. It was carried out at three different levels i.e. LOQ, 100% and 150% under specified chromatographic conditions. Results for interday precision were shown in Table 6 and 7.

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy for all related impurities was determined by analyzing Aspirin and Prasugrel Hydrochloride test solution spiked with all the related impurities at four different concentration levels of LOQ, 80%, 100%, and 120% of each in triplicate at the specified limit. The % recovery of related impurities ofAspirin and Prasugrel Hydrochloride was calculated by injecting standard solution for each level. Results for accuracy were shown in Table No. 8 and 9.

LOD and LOQ

LOD (Limit of Detection) is the lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. LOQ (Limit of Quantitation) is the lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions.LOD and LOQ were determined by measuring the standard deviation of the response and the slope which is obtain from the linearity data. Results for LOD and LOQ were shown in Table 10.

Linearity

The Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. The linearity of the method for all the related impurities was determined by analyzing solution of related impurities of Aspirin and Prasugrel Hydrochloride at six different concentration levels of LOQ, 50, 75, 100, 125 and 150% of each at specified limit. The correlation coefficient was calculated for each known impurities.Results for linearity were shown in Table 11.

Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is expected that such change

should not alter the performance of the analytical method. A study was conducted to determine the effect of variation in flow rate, mobile phase composition and mobile phase pH. Working standard solutions was prepared of related impurities asper the test method and was injected into the HPLC system using flow rates 0.8ml/min and 1.2ml/min, mobile phase composition consists of +2% solvent and -2% solvent in gradient run and pH of buffer was sets at 2.8 and 3.2. Then % RSD of related impurities of Aspirin and Prasugrel Hydrochloride were calculated. Results for robustness were shown in Table 12, 13, 14, 15, 16 and 17.

Calculation of Known and Unknown Impurities of Aspirin and Prasugrel Hydrochloride

Analyzed test solution for three times and calculate % of each known and unknown impurities in comparison with standard preparations of Aspirin and Prasugrel Hydrochloride. The amount of known and unknown related impurities present in the formulation of aspirin and prasugrel hydrochloride is calculated by using the formula given below.

For each known impurities of Aspirin and Prasugrel Hydrochloride

% of each known impurities = (Cu/Cs) X (Ru/Rs) X 100
Where,
Cu= Concentration of each impurity in standard preparation
Cs= Concentration of each impurity in testpreparation
Ru: Area of each impurity in test preparation
Rs: Area of each impurity in standard preparation

For each unknownimpurities of Aspirin and Prasugrel Hydrochloride

% of each unknown impurities = (Ru/Rs) X 100
Where,
Ru: Area of unknown impurity in testpreparation.
Rs: Total area in test preparation.

For all unknown impurities ofAspirin and Prasugrel Hydrochloride

% of all unknown impurities = (Ru/Rs) x 100
Where,
Ru: Sum of area of unknown impurities in testpreparation.
Rs: Total area in test preparation.

Results for % of each known and unknown impurities ofAspirin and Prasugrel Hydrochloride were shown in Figure 12 and Table 18.

RESULTS AND DISCUSSIONS

The simultaneous estimation for related impurities of aspirin and prasugrel hydrochloride was done by RP-HPLC and in the optimized method the mobile phase A consist of 0.05M Ammonium acetate buffer pH-3.0 and mobile phase B consist of Acetonitrile at a flow rate of 1ml/min and detection wavelength was 224nm. The retention time of aspirin, prasugrel hydrochloride, salicylic acid, 4-hydroxybenzoic acid, 4-hydroxyisophthalic acid, desfluoro prasugrel, 4-fluoro prasugrel and 3-fluoro prasugrel are 5.453 min, 8.467 min, 6.620 min, 3.903 min, 4.320 min, 7.410 min, 13.530 min and 18.940 min respectively as shown in Figure 11. The linearity was observed in the range of 4-22.5 µg/ml for related impurities of Aspirin and 0.5-3 µg/ml for related impurities of Prasugrel Hydrochloride with correlation coefficient more than 0.990 for related impurities of Aspirin and Prasugrel Hydrochloride. The % recovery value was found minimum of 95.62% and maximum of 104.50% for all known impurities. The relative standard deviation value for repeatability, interday precision and intraday precision was less than 5%.

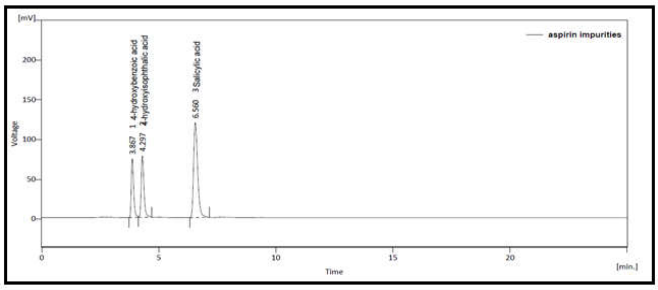

Fig 9 Chromatogram for Aspirin Related Impurities

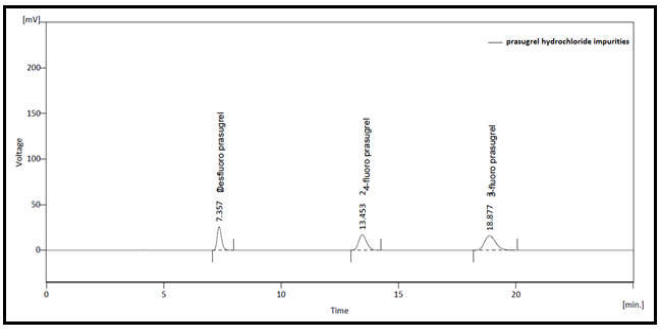

Fig 10 Chromatogram for Prasugrel Hydrochloride Related Impurities

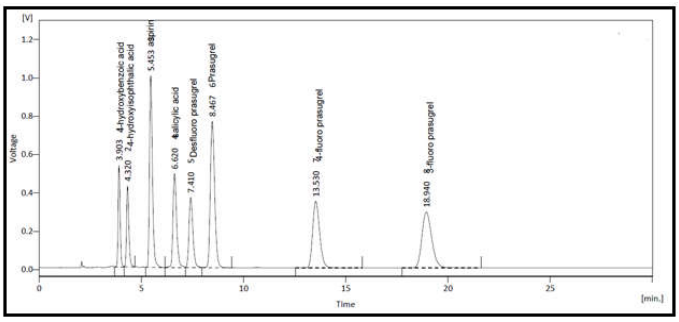

Fig 11 Chromatogram for System Suitability

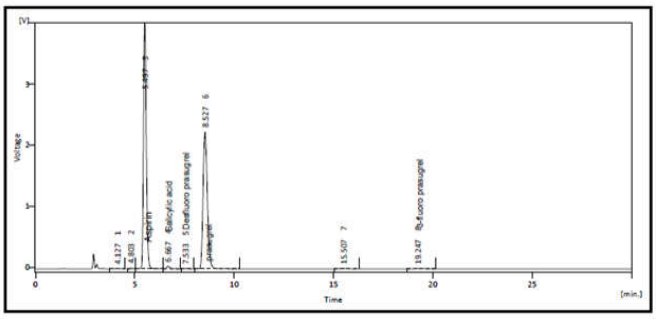

Fig 12 Chromatogram of Test Solution for Known and Unknown Related

Table 1 Results for System Suitability Parameters of Aspirin and its Related Impurities

S. No.	System Suitability Parameters	Aspirin	Salicylic acid	4-Hydroxybenzoic acid	4-Hydroxy isophthalic acid	Range
1	Retention time	5.453	6.620	3.903	4.320	-
2	Column Efficiency (N)	7007	6968	7419	6797	N > 2000
3	Tailing Factor (T)	1.588	1.512	1.308	1.500	T < 2

Table 2 Results for System Suitability Parameters of Prasugrel Hydrochloride and its Related Impurities

S. No.	System Suitability Parameters	Prasugrel Hydrochloride	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel	Range
1	Retention time	8.467	7.410	13.530	18.940	-
2	Column Efficiency (N)	7294	7357	7408	7350	N > 2000
3	Tailing Factor (T)	1.345	1.396	1.368	1.361	T < 2

Table 3 Results for Repeatability of Aspirin and Prasugrel Hydrochloride Related Impurities

Impurity	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
Avg Area	1480.64	540.55	644.32	340.07	411.53	538.93
%R.S.D	1.95	1.81	2.07	1.78	1.85	1.67

Table 4 Results for Intraday Precision of Aspirin Related Impurities

S.No.	IntradayLevel	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	LOQ	486.43	1.40	140.92	3.65	206.03	2.77
2	100%	1460.96	1.28	537.12	1.76	642.10	1.86
3	150%	2180.28	1.50	801.87	1.29	960.18	1.33

Table 5 Results for Intraday Precision of Prasugrel Hydrochloride Related Impurities

S. No.	Intraday Level	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	LOQ	85.29	1.00	102.77	1.40	136.41	1.51
2	100%	338.17	2.23	410.59	1.76	537.75	1.52
3	150%	501.65	1.51	611.82	1.63	798.88	1.38

Table 6 Results for Interday Precision of Aspirin Related Impurities

S. No.	Interday Level	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	LOQ	487.80	1.33	143.29	1.27	210.17	1.46
2	100%	1467.68	1.32	539.04	1.29	640.15	1.18
3	150%	2181.23	1.14	799.28	1.47	951.96	1.23

Table 7 Results for Interday Precision of Prasugrel Hydrochloride Related Impurities

S. No.	Interday Level	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	LOQ	84.73	1.24	102.10	1.47	135.95	1.53
2	100%	338.69	1.38	411.59	1.28	539.56	1.17
3	150%	503.69	1.41	612.48	1.55	800.23	1.48

Table 8 Results for Accuracy of Aspirin Related Impurities

S. No.	Recovery Level	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		%Recovery	%R.S.D	%Recovery	%R.S.D	%Recovery	%R.S.D
1	LOQ	102.53		97.64		99.50	
2	LOQ	104.06	2.10	98.83	2.32	97.19	1.38
3	LOQ	99.83		102.10		97.15	
4	80%	97.97		104.50		98.23	
5	80%	98.73	1.04	102.94	2.56	98.03	3.06
6	80%	96.72		99.39		103.42	
7	100%	98.66		100.97		99.20	
8	100%	96.68	1.51	101.99	1.38	103.45	2.22
9	100%	95.81		103.76		102.69	
10	120%	98.21		103.64		100.04	
11	120%	97.21	0.62	103.43	3.53	103.94	2.16
12	120%	98.30		97.34		100.28	

Table 9 Results for Accuracy of Prasugrel Hydrochloride Related Impurities

S. No.	Recovery Level	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		%Recovery	%R.S.D	%Recovery	%R.S.D	%Recovery	%R.S.D
1	LOQ	102.69		103.46		102.41	
2	LOQ	101.08	3.21	104.23	3.24	103.86	3.81
3	LOQ	96.50		98.16		96.60	
4	80	95.62		100.88		101.40	
5	80	96.10	0.29	101.58	1.12	101.99	0.99
6	80	95.63		99.37		100.05	
7	100	96.65		101.25		101.71	
8	100	96.25	0.80	99.36	1.38	99.55	1.41
9	100	97.74		98.58		99.06	
10	120	95.66		100.82		101.11	
11	120	97.70	1.20	99.81	0.62	100.10	0.62
12	120	95.73		100.94		101.25	

Table 10 Results for LOD and LOQ of Aspirin and Prasugrel Hydrochloride Related Impurities

Impurity	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
LOD (µg/ml)	1.25	1.07	1.49	0.16	0.09	0.14
LOQ (µg/ml)	3.77	3.23	4.52	0.48	0.28	0.43

Table 11 Results for Linearity of Aspirin and Prasugrel Hydrochloride Related Impurities

Parameter	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
Conc- Range (µg/ml)	4-22.5	4-22.5	5-22.5	0.5-3	0.5-3	0.5-3
Correlation Coefficient (R^2)	0.9922	0.998	0.9973	0.9964	0.9915	0.9985
Slope	94.96	36.14	44.305	172.2	198.14	279.29
Intercept	33.841	10.301	31.774	11.793	12.69	30.15

Table 12 Results for Robustness (Change in Flow Rate) of Aspirin Related Impurities

S. No.	Flow Rate (ml/min)	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	0.8	1580.28	1.23	584.79	1.33	674.71	1.62
2	1.2	1312.42	1.26	507.90	1.39	583.69	1.41

Table 13 Results for Robustness (Change in Flow Rate) of Prasugrel Hydrochloride Related Impurities

S. No.	Flow Rate (ml/min)	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	0.8	368.10	2.63	439.19	2.63	558.25	2.62
2	1.2	315.31	1.97	383.26	1.96	528.72	1.73

Table 14 Results for Robustness (Change in pH of Mobile Phase) of Aspirin Related Impurities

S. No.	pH	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	3.2	1445.98	1.08	526.48	1.11	631.12	1.31
2	2.8	1440.35	1.36	523.76	1.56	638.11	1.72

Table 15 Results for Robustness (Change in pH of Mobile Phase) of Prasugrel Hydrochloride Related Impurities

S. No.	pH	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	3.2	341.59	2.25	423.79	2.47	544.13	2.04
2	2.8	342.17	3.17	425.02	2.66	541.27	3.23

Table 16 Results for Robustness (Change in Composition of Mobile Phase) of Aspirin Related Impurities

S. No.	Solvent	Salicylic acid		4-Hydroxybenzoic acid		4-Hydroxyisophthalic acid	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	+2%	1387.19	1.12	538.05	1.33	616.13	1.03
2	-2%	1606.82	2.11	563.01	2.51	697.00	2.51

Table 17 Results for Robustness (Change in Composition of Mobile Phase) of Prasugrel Hydrochloride Related Impurities

S. No.	Solvent	Desfluoro Prasugrel		4-Fluoro Prasugrel		3-Fluoro Prasugrel	
		Avg Area	%R.S.D	Avg Area	%R.S.D	Avg Area	%R.S.D
1	+2%	297.24	1.63	397.79	1.65	525.73	1.08
2	-2%	376.28	1.29	440.47	1.25	569.12	1.57

Table 18 Results for % Impurities of Aspirin and Prasugrel Hydrochloride

% Impurities	Aspirin Impurity	Prasugrel Hydrochloride Impurity		Other Impurity	
	Salicylic Acid (%)	Desfluoro Prasugrel (%)	3-Fluoro Prasugrel (%)	Unknown Impurity (%)	Total Unknown Impurity
Avg	0.323	0.100	0.099	0.142	0.287
R.S.D	3.52	4.32	0.86	3.65	1.71

Table 19 Results for Method Validation of Aspirin and Prasugrel Hydrochloride Related Impurities

Parameters	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxy isophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
Specificity	**Specific**					
Repeatability (%R.S.D)	1.95	1.81	2.07	1.78	1.85	1.67
Interday Precision (%R.S.D)	1.14-1.33	1.27-1.47	1.18-1.46	1.24-1.41	1.28-1.55	1.17-1.53
Intraday Precision (%R.S.D)	1.28-1.50	1.29-3.65	1.33-2.77	1.00-2.23	1.40-1.76	1.38-1.52
% Recovery	95.81-104.06	97.34-104.50	97.15-103.94	95.62-102.69	98.16-104.23	96.60-103.86
LOD (µg/ml)	1.25	1.07	1.49	0.16	0.09	0.14
LOQ (µg/ml)	3.77	3.23	4.52	0.48	0.28	0.43
Linearity-Range (µg/ml)	4-22.5	4-22.5	5-22.5	0.5-3	0.5-3	0.5-3
Correlation Coefficient (R^2)	0.9922	0.998	0.9973	0.9964	0.9915	0.9985
Robust-Ness	The system suitability parameters were found well within the acceptance criteria as per system suitability					

SUMMARY AND CONCLUSION

The RP-HPLC method developed for the determination of related impurities of aspirin and prasugrel hydrochloride is found to be specific, linear, sensitive, precise, accurate and robust in nature. The method was successfully validated in terms of specificity, precision, linearity, accuracy and robustness as per ICH guidelines. It can be concluded that the proposed method can be used for routine analysis for estimation of related impurities of aspirin and prasugrel hydrochloride in combined dosage form by RP-HPLC.

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