

Research Article**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF RELATED IMPURITIES OF ASPIRIN AND PRASUGREL HYDROCHLORIDE IN ITS DOSAGE FORM BY RP-HPLC**Shweta A. Mishra <sup>a\*</sup>, Dr. Ashlesha J. Chauhan <sup>b</sup><sup>a\*</sup> Department of Pharmaceutical Chemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023, Gujarat, INDIA.<sup>b</sup> Assistant Professor, Department of Pharmaceutical Chemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023, Gujarat, INDIA.

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**ABSTRACT**

**Objective:** 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.

**Methods:** A gradient reverse phase high performance liquid chromatography (RP-HPLC) analysis was performed on Hypersil BDS C18 column (250mm X 4.6mm, 5 $\mu$ m) 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 detection was carried out at 224nm.

**Results:** The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 4-22.5 $\mu$ g/ml for related impurities of Aspirin and 0.5-3 $\mu$ g/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%. The LOD value was found minimum of 0.09 $\mu$ g/ml and maximum of 1.49 $\mu$ g/ml for all known impurities. The LOQ value was found minimum of 0.28 $\mu$ g/ml and maximum of 4.52 $\mu$ g/ml for all known impurities.

**Conclusion:** The proposed method was found to be specific, linear, sensitive, precise, accurate and robust in nature.

**KEYWORDS:** Aspirin, Prasugrel Hydrochloride, Impurities, RP-HPLC, Method Development, Validation.

**INTRODUCTION**

Aspirin chemically is 2-(acetyloxy)benzoic acid used as an analgesic, antipyretic, anti-inflammatory and an anti-platelet agent. Aspirin act as cyclooxygenase inhibitor. It suppresses the production of prostaglandins and thromboxanes due to inactivation of cyclooxygenase enzyme. Its molecular formula is C<sub>9</sub>H<sub>8</sub>O<sub>4</sub> and its molecular weight is 180.16 gm/mole [1]. 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.

Prasugrel Hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4H,5H,6H,7H-thieno[3,2 c]pyridine-2-yl acetate hydrochloride. Prasugrel Hydrochloride is a member of the thienopyridine class of ADP receptor inhibitors. Its molecular formula is C<sub>20</sub>H<sub>21</sub>ClFNO<sub>3</sub>S and molecular weight is 409.902 gm/mole [3]. Prasugrel Hydrochloride is 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 2.

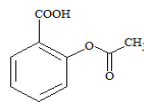
The combination of aspirin and prasugrel hydrochloride is used for the treatment of the reduction of thrombotic cardiovascular events in patients with acute coronary syndrome. Literature review reveals that many analytical methods have been developed for the estimation of aspirin and prasugrel hydrochloride in combined dosage form [6-9]. But no related impurities method has been reported for the 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 determination of related impurities of aspirin and prasugrel hydrochloride in combined

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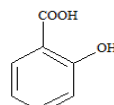
dosage form. The developed method was also validated in compliance with ICH Guidelines.

**Name of Compounds****Chemical Structure**

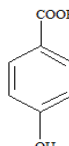
Aspirin



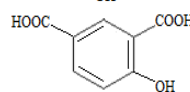
Salicylic Acid



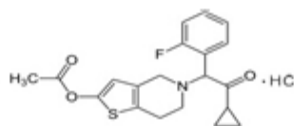
4-Hydroxybenzoic acid



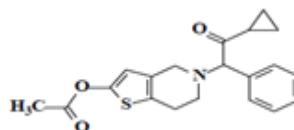
4-Hydroxyisophthalic acid

**Fig. 1: Chemical Structure of Aspirin and its Related Impurities****Name of Compounds****Chemical Structure**

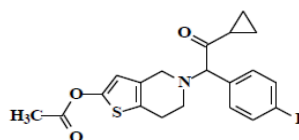
PrasugrelHydrochloride



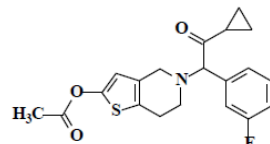
Desfluoro Prasugrel



4-Fluoro Prasugrel



3-Fluoro Prasugrel

**Fig. 2: Chemical Structure of Prasugrel Hydrochloride and its Related Impurities****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
Orthophosphoric 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 Fig. 5 and Table No. 1 and 2.

#### Preparation of Standard Solution of Aspirin and Prasugrel Hydrochloride Related Impurities:

**Standard stock solution of Salicylic acid (150 ppm):** Take 15mg of Salicylic acid working standard into a 100ml volumetric flask and 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 acid working standard into a 25ml volumetric flask and 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 Prasugrel working 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 of Aspirin and Prasugrel Hydrochloride Related Impurities:

**Working standard solution of Aspirin Related Impurities:** (Salicylic acid-15ppm, 4-hydroxybenzoic acid-16ppm and 4-hydroxyisophthalic acid-16ppm): Take 1ml each from standard stock solutions of Salicylic acid, 4-hydroxybenzoic acid and 4-hydroxyisophthalic 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 and 20mg of Prasugrel

Hydrochloride 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:<sup>[10]</sup>

The proposed method for estimation of related impurities of Aspirin and Prasugrel Hydrochloride was validated as per ICH guidelines by studying several parameters such as specificity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), linearity and robustness.

#### Specificity:

Specificity is used to measure relative separation of the individual components. 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 of related impurities of Aspirin and Prasugrel Hydrochloride was prepared as per the test method and 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. 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 No. 3.

#### Intraday precision:

Intraday precision was carried out at three different levels i.e. LOQ, 100% and 150% under specified chromatographic conditions. The working standard solution of related impurities of Aspirin and Prasugrel Hydrochloride were analyzed three times on the same day and % R.S.D was calculated. Results for intraday precision were shown in Table No. 4 and 5.

#### Interday precision:

Interday precision was carried out at three different levels i.e. LOQ, 100% and 150% under specified chromatographic conditions. The working standard solution of related impurities of Aspirin and Prasugrel Hydrochloride were analyzed three times on the different day and % R.S.D was calculated. Results for interday precision were shown in Table No. 6 and 7.

#### Accuracy:

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. 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 of Aspirin 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 No. 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 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 No. 11.

**Robustness:**

Robustness of analytical method was studied by small changes in the method like altering the flow rate, mobile phase pH and mobile phase composition. It is expected that such change should not alter the performance of the analytical method. Working standard solutions was prepared of related impurities as per 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 No. 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 preparation 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:

$$\% \text{ of each known impurities} = (\text{Cu/Cs}) \times (\text{Ru/Rs}) \times 100$$

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 unknown impurities of Aspirin and Prasugrel Hydrochloride:

$$\% \text{ of each unknown impurities} = (\text{Ru/Rs}) \times 100$$

Where,

Ru: Area of unknown impurity in testpreparation.

Rs: Total area in test preparation.

For all unknown impurities of Aspirin and Prasugrel Hydrochloride:

$$\% \text{ of all unknown impurities} = (\text{Ru/Rs}) \times 100$$

Where,

Ru: Sum of area of unknown impurities in testpreparation.

Rs: Total area in test preparation.

Results for % of each known and unknown impurities of Aspirin and Prasugrel Hydrochloride were shown in Fig. 6 and Table No. 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. 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%.

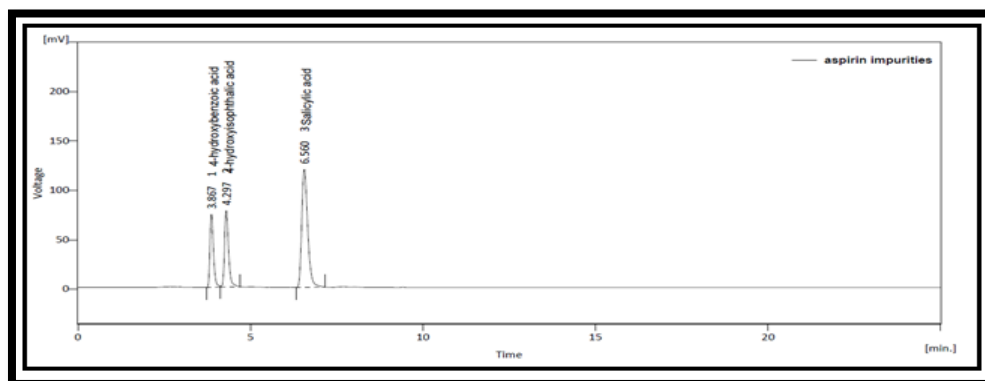


Fig. 3: Chromatogram for Aspirin Related Impurities

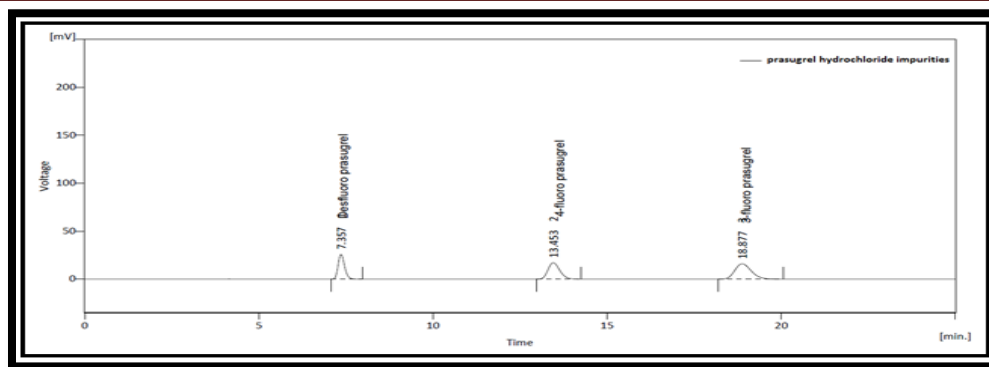


Fig.4: Chromatogram for Prasugrel Hydrochloride Related Impurities

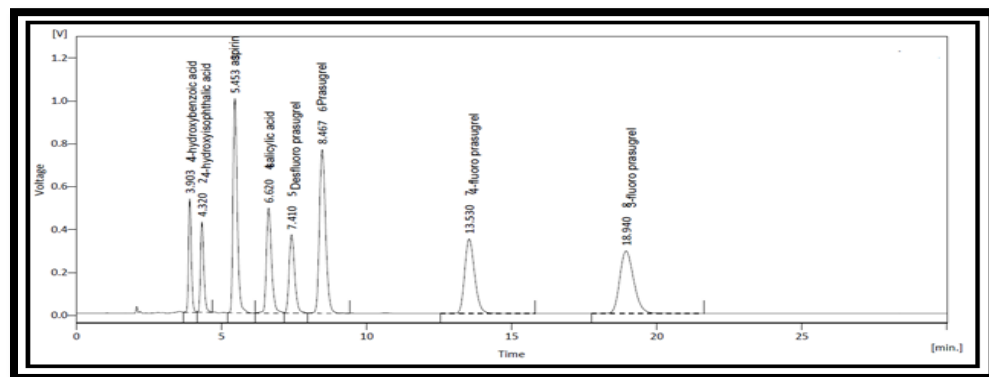


Fig.5: Chromatogram for System Suitability

Table No. 1: Results for System Suitability Parameters of Aspirin and its Related Impurities

S. No.	System Suitability Parameters	Aspirin	Salicylic acid	4-Hydroxy benzoic 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 No. 2: Results for System Suitability Parameters of Prasugrel Hydrochloride and its Related Impurities

S. No.	System Suitability Parameters	Prasugrel Hydro-chloride	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 No. 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 No. 4: Results for Intraday Precision of Aspirin Related Impurities

S. No.	Intraday 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	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 No. 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 No. 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 No. 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 No. 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 No. 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 No. 10: Results for LOD and LOQ of Aspirin and Prasugrel Hydrochloride Related Impurities

Impurity	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxyisophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
LOD ( $\mu\text{g/ml}$ )	1.25	1.07	1.49	0.16	0.09	0.14
LOQ ( $\mu\text{g/ml}$ )	3.77	3.23	4.52	0.48	0.28	0.43

Table No. 11: Results for Linearity of Aspirin and Prasugrel Hydrochloride Related Impurities

Parameter	Salicylic acid	4-Hydroxy benzoic acid	4-Hydroxyisophthalic acid	Desfluoro Prasugrel	4-Fluoro Prasugrel	3-Fluoro Prasugrel
Conc. Range ( $\mu\text{g/ml}$ )	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 No. 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 No. 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 No. 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 No. 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 No. 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 No. 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	3.2	297.24	1.63	397.79	1.65	525.73	1.08
2	2.8	376.28	1.29	440.47	1.25	569.12	1.57

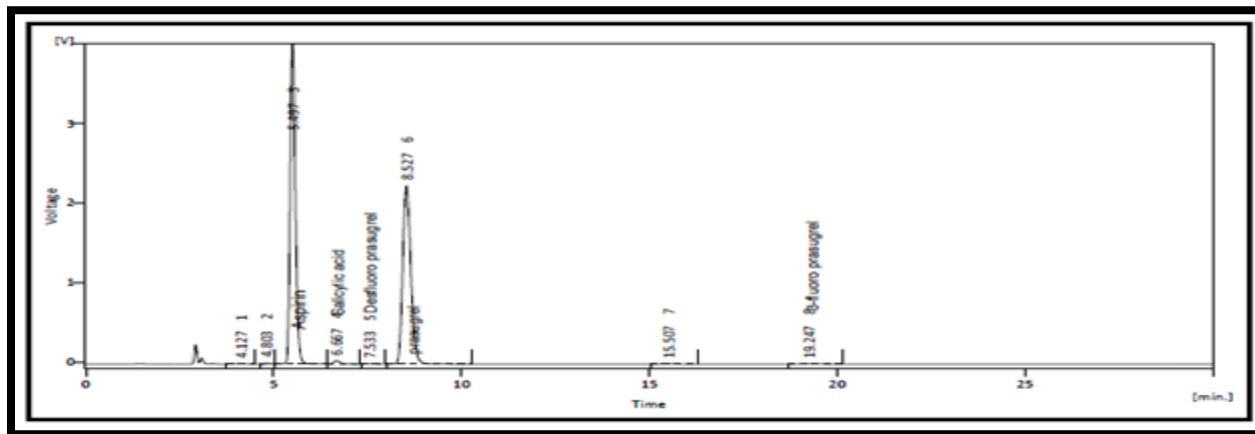


Fig. 6: Chromatogram of Test Solution for Known and Unknown Related Impurities of Aspirin and Prasugrel Hydrochloride

Table No. 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 No. 19: Results for Method Validation of Aspirin and Prasugrel Hydrochloride Related Impurities

Para-meters	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 <sup>2</sup> )	0.9922	0.998	0.9973	0.9964	0.9915	0.9985
Robustness	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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