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Review Article

Analysis of Prasugrel by Chromatography - Review

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ABSTRACT

Prasugrel, being a potent platelet aggregation inhibitor, is used widely around the world to reduce cardiovascular risks in patients with stroke, myocardial infarction, and atherosclerosis. The aim of this review firstly to focus on a comprehensive update of chromatography determination of Prasugrel and its metabolites in human plasma, and in pharmaceutical preparations. It has been described using TLC, HPLC/MS, RP-HPLC, and UV methods. Secondly to localize the chromatographic conditions for separation and quantification. This review provides detailed information on separation conditions for Prasugrel alone, with Aspirin, and in the presence of its related compounds.

Keywords: Chromatography, HPLC, MS, Prasugrel, TLC, UV.

INTRODUCTION

Prasugrel chemically is 5-[2-cyclopropyl-1-(2-fluoro-phenyl)-2-oxoethyl]-4,5,6,7-tetra hydrothieno [3,2-c] pyridin-2-yl acetate (Figure 1). Its empirical formula is $C_{20}H_{20}FNO_3S$and its molecular weight is 373.442 g/mol. Prasugrel is a member of the thienopyridine class of ADP receptor inhibitors, like Ticlopidine and Clopidogrel.[1] These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to P2Y12 receptors. Prasugrel inhibits adenosine diphosphate–induced platelet aggregation more rapidly, more consistently, and to a greater extent than do standard and higher doses of Clopidogrel in healthy volunteers and in patients with coronary artery disease.[2-4]

A pharmacodynamic study suggests that acute coronary syndrome (ACS) patients can be safely switched from Clopidogrel to Prasugrel and that doing so results in a further reduction in platelet function after one week.[5] When patients receive a loading dose of Prasugrel prior to switching from Clopidogrel, the reduction in platelet function occurs within two hours.[6]

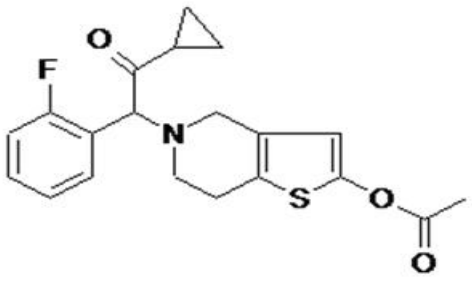

Figure 1: Chemical Structure of Prasugrel

Because Prasugrel is a pro-drug, it must be converted to its pharmacologically active form in order for it to exert effects in the body (Figure 2). The conversion of Prasugrel to its active component takes place via rapid hydrolysis by esterase, followed by a single cytochrome P450 (CYP) dependent step. The drug's active metabolite contains a thiol group that binds to a free cysteine on the P2Y12 receptor. It is through this mechanism that ADP binding and activation are irreversibly blocked.

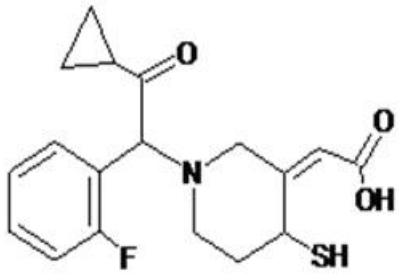

Figure 2: Chemical Structure of the Active Metabolite of Prasugrel (R-138727)

Prasugrel reduces the tendency of platelets to stick or clump together by blocking the P2Y12 ADP receptor on the platelet's surface. Clumping of platelets can cause clogged arteries and may lead to a heart attack.[7]

Pharmacokinetics of Prasugrel

Prasugrel's pharmacokinetic profile is summarized in Table 1.[8] Prasugrel is rapidly absorbed and extensively hydrolyzed to an inactive thiolactone metabolite (R-95913) (Figure 2). The thiolactone metabolite is metabolized by hepatic cytochrome P450 to the active metabolite R-138727 (Figure 3), which is further metabolized into another inactive metabolite.[9] No drug is recovered unchanged in the urine. The major route of elimination of Prasugrel metabolites is renal excretion (70%), with the remaining metabolites excreted in the feces; this suggests that Prasugrel is fully absorbed and metabolized before excretion.[8]

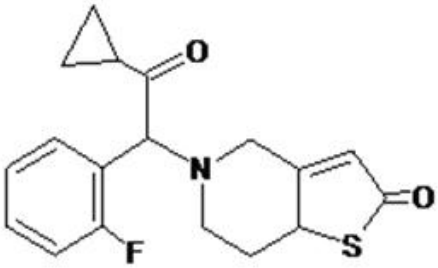

Figure 3: Chemical Structure of the Inactive Metabolite of Prasugrel (R-95913)

Table 1: Pharmacokinetic parameter of R-138727 in healthy volunteers

Parameter	R-138727[a]
AUC_{0-24h}(ng/mL/h)	122
C_{max}(ng/mL)	80
$t_{1/2}$ (h)	3.7
T_{max}(h)	0.5

AUC, area under the curve; C_{max}, maximum serum concentration; $t_{1/2}$: Half-life; T_{max}: Time to C_{max}; [a] Active metabolite of Prasugrel.

Pharmacodynamics of Prasugrel

Prasugrel produces inhibition of platelet aggregation to 5-20 µM ADP, as measured by light transmission aggregometry.[6] Following a 60 mg loading dose of the drug, about 90% of patients had at least 50% inhibition of platelet aggregation by one hour. Maximum platelet inhibition was about 80%. Mean steady-state inhibition of platelet aggregation was about 70% following three to five days of dosing at 10 mg daily after a 60 mg loading dose. Platelet aggregation gradually returns to baseline values over five to 9 days after discontinuation of Prasugrel, this time course being a reflection of new platelet production rather than pharmacokinetics of Prasugrel. Discontinuing Clopidogrel 75 mg and initiating Prasugrel 10 mg with the next dose resulted in increased inhibition of platelet aggregation, but not greater than that typically produced by a 10 mg maintenance dose of Prasugrel alone. Increasing platelet inhibition could increase bleeding risk. The relationship between inhibition of platelet aggregation and clinical activity has not been established.[10]

Indication of Prasugrel

Prasugrel is co-administered with acetylsalicylic acid (ASA), and is indicated for the prevention of athero-thrombotic events in patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI).[11-13]

Dosage and administration

Treatment with Prasugrel is initiated with a single 60 mg oral loading dose after cardiac catheterization, followed by a maintenance dose of 10 mg orally once daily, with or without food. For patients weighing less than 60 kg, 5 mg once daily can be considered. Patients should also take aspirin (75–325 mg) daily.[2,10]

Determination of the Active and Inactive Metabolites of Prasugrel in Human Plasma

Liquid Chromatography/Tandem Mass Spectrometry

Two fast and sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS)-based bioanalytical assays were developed and validated to quantify the active and three inactive metabolites of Prasugrel. After extraction and separation, the analytes were detected and quantified using a triple quadru-pole mass spectrometer using positive electro spray ionization. The validated concentration range for the inactive metabolites assay was from 1 to 500 ng/mL for each of the three analytes. Additionally, a 5X dilution factor was validated. The inter-day accuracy ranged from -10.5% to 12.5% and the precision ranged from 2.4% to 6.6% for all three analytes. All results showed accuracy and precision within ±20% at the lower limit of quantification and ±15% at other levels. The validated concentration range for the active metabolite assay was from 0.5 to 250 ng/ml. Additionally, a 10X dilution factor was validated. The inter batch accuracy ranged from -7.00% to 5.98%, while the precision ranged from 0.98% to 3.39%. Derivatization of the active metabolite in blood with 2-bromo-3'-methoxyacetophenone immediately after collection was essential to ensure the stability of the metabolite during sample processing and storage. These methods have been applied to determine the concentrations of the active and inactive metabolites of Prasugrel in human plasma.[14]

Electro spray Ionization LC-MS/MS

In this method, a rapid and sensitive liquid chromatography tandem mass spectrometry method has been developed and validated for the determination of the active metabolite (R-138727) of Prasugrel in human plasma. Because R-138727 contains a thiol group, it requires stabilization by derivatizing with N-ethyl maleimide. Commercially available trandolapril was used as the internal standard (IS). The derivatives of R-138727 and IS were extracted from human plasma using a liquid-liquid extraction technique. Chromatography was performed on a Hypurity C18, 5 µ (50 mm × 4.6 mm, i.d.) column, with the mobile phase consisting of acetonitrile and 10 mM ammonium formate (pH 3.0, 50:50 V/V), followed by detection using mass spectrometry. No significant endogenous peaks corresponding to R-138727 or IS were detected in the blank human plasma samples and no significant matrix effect was observed for R-138727 and IS in the human plasma samples. The mean recovery for R-138727 ranged from 90.1 to 104.1%, with the lower limit of quantification set at 1 ng/ml. Linearity was established for concentrations in the range of 1.0-500.12 ng/ml, with a coefficient of determination r^2 of 0.9958. The derivatised R 138727 was stable in human

plasma for 3 months at -20 °C. This method increased the sensitivity and selectivity, resulting in high-throughput analysis of R-138727 using trandolapril as the internal standard in pharmacokinetic and bioequivalence studies, with a chromatographic run time of 3.7 min.[15]

Stereo selective metabolism of Prasugrel in humans using chiral LC-Tandem Mass Spectrometry

A liquid chromatography-tandem mass spectrometry method was developed to chromatographically separate the four stereoisomers of the active metabolite of Prasugrel, R-138727, in human plasma after derivatization with bromo-methoxy-acetophenone to stabilize the molecule. This technique was designed to determine the relative contribution of each stereoisomer, based on statistical analyses of each stereoisomer's chromatographic peak areas. The methodology was validated and used for the analysis of clinical samples in which R-138727 had been derivatised at the time of blood collection. This technique can be useful to determine the ratios of stereoisomers in biological samples (e.g., plasma) especially in situations in which authentic standards of each individual stereoisomer are scarce or unavailable. In humans, the metabolic formation of R-138727 from Prasugrel was found to be stereo selective, where 84% of R-138727 was present as RS and RR, the two most pharmacologically potent isomers, whereas the SR and SS enantiomers accounted for about 16%. The ratios of the R-138727 stereoisomer's were consistent among subjects, regardless of the dose or time of sample collection or whether the blood was sampled after the first dose or after 4 weeks of therapy.[14,16]

Determination of Prasugrel as Bulk Drug from Pharmaceutical Dosage Forms

High-Performance Thin Layer Chromatography (TLC)

The developed HPTLC method was sensitive, selective, precise and stability indicating (in accordance with ICH guidelines) for analysis of Prasugrel, to resolve drug response from that of their degradation products. The method employed TLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of Dichloromethane: Methanol (9.9:0.1v/v). This system was found to give compact spot for Prasugrel (R_f value 0.58±0.03). Prasugrel was subjected to stress test conditions like acid, alkali, neutral hydrolysis, oxidation, dry heat and photo degradation. The spot for product of degradation was well resolved from the drug. Densitometric analysis of drug was carried out in the absorbance mode at 254 nm. The linear regression data for the calibration plots showed good linear relationship andR^2 was 0.995 in the concentration range of 300-1500ng/band. The result indicates that the drug was susceptible to degradation, to different extent in differentconditions.[17]

High Performance Liquid Chromatography (HPLC)

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Prasugrel Hydrochloride in pharmaceutical dosage forms. The mobile phase consist of buffer (0.02M sodium dihydrogen phosphate, pH-3 adjusted with ortho phosphoric acid):methanol:acetonitrile in the ratio of 45:20:35 v/v delivered at a flow rate of 1.0 ml/min and wavelength of detection at 220 nm. The retention times of Prasugrel Hydrochloride was 8.20 min. The developed method was validated according to ICH guidelines. The result indicates that the method was found to be simple, rapid, and accurate and can be adopted in routine analysis of Prasugrel in formulation.[18]

Another stability indicating method was simple, rapid and precise, the method was developed for the quantitative estimation of Prasugrel hydrochloride in pharmaceutical dosage form. A chromatographic separation of Prasugrel and its degradants was achieved with Zorbax XDB C_8, 150 x 4.6 mm, 3.5µm analytical column using aqueous solution of 0.05 M ammonium acetate pH 4.5 with acetic acid-acetonitrile (40:60 v/v). The instrumental settings include flow rate of 1.0 ml/min, column temperature at 30°C and detector wavelength of 254 nm using a photodiode array detector. Theoretical plates for Prasugrel were 7023. Tailing factor for Prasugrel was 1.11. Prasugrel was exposed to thermal, photolytic, hydrolytic and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. Peak homogeneity data of Prasugrel was obtained using photodiode array detector in the stressed sample chromatograms, which demonstrated the specificity of the method for the estimation in presence of degradants. The described method showed excellent linearity over a range of 10–300 µg/ml for Prasugrel. The correlation coefficient was 0.999. The relative standard deviation of peak area for six measurements was always less than 2%. Overall, the proposed method was found to be suitable and accurate for quantitative determination and stability study of Prasugrel in pharmaceutical dosage form.[19]

In other method, the HPLC separation was carried out by reverse phase chromatography on inertsil ODS-3V column (5µm; 250x4.6mm) with a mobile phase composed of 0.02M potassium dihydrogen orthophosphate, 0.02M dipotassium hydrogen orthophosphate in water: Acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1ml/min. The detection was monitored at 210nm. The calibration curve for Prasugrel was linear from 100 to 600ng/ml. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Prasugrel in bulk and its tablet dosage forms. LOD and LOQ for Prasugrel were found to be 0.25 µg/ml and 0.75 µg /ml respectively. Accuracy (recoveries: 99.8-101.2%) and reproducibility were found to satisfactory. The proposed method is simple, fast,

accurate and precise for the simultaneous quantification of Prasugrel in dosage form, bulk drugs as well as for routine analysis in quality control.[20]

A new reverse-phase liquid chromatographic (RP-LC) method was developed for the assay of Prasugrel in bulk. The Chromatography was performed on Kromasil C_{18} column. The eluted compounds were monitored by UV detection at 257nm using mobile phase methanol-potassium dihydrogen orthophosphate (pH 2.2; 10mM) (70:30, v/v). The method was statistically validated for linearity, accuracy, precision and repeatability. The linearity of Prasugrel was demonstrated in concentration range 15-75µg/ml. The limit of detection and quantitation were 10 and 50ng/ml, respectively. The method developed was precise, accurate and specific for estimation of Prasugrel in bulk.[21]

Other methods were developed to determine Prasugrel in the presence of its impurities; the first method describes the development of reverse phase HPLC method for Prasugrel hydrochloride in the presence of impurities and degradation products generated from the forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of Prasugrel hydrochloride was observed under neutral, acid, base and oxidation environment. The drug was found more sensitive to basic condition. Successful separation of the drug from the process related impurities and degradation products were achieved on Gemini C18 (250 x 4.6 mm) 5 µm particle size column using reverse phase HPLC method. The isocratic method employed with a mixture of buffer and (10%v/v water in acetonitrile) mixture of ratio 30:70, respectively. Potassium dihydrogen orthophosphate (0.05M) is used as buffer. The HPLC method was developed and validated with respect to linearity, accuracy, precision, specificity and robustness, which is useful for the routine determination of Prasugrel hydrochloride.[22]

The second method used a gradient reverse-phase high performance liquid chromatographic (RP-HPLC), the method was developed and validated for the determination of Prasugrel hydrochloride and its related substances. The chromatographic separation of Prasugrel from its seven related substances and degradation products achieved on Sunfire C18, 5µm (250x4.6mm) column temperature maintained at 45°C with a mobile phase A: 0.1% v/v orthophosphoric acid in water and mobile phase B: 0.1% v/v orthophosphoric acid in acetonitrile. The flow rate was 1.0 ml/min, and the detection wavelength was 220nm. The developed method was validated for specificity, forced degradation studies, sensitivity (LOD and LOQ), linearity, precision (system precision, method precision and intermediate precision), accuracy, stability of standard and sample solutions and robustness. The method was linear with a concentration range of 0.085-3.218µg/ml with correlation coefficients more than 0.9997 for Prasugrel and its related substances. The method recoveries obtained ranged between 96.4% -101.1% for LOQ levels and 94.7%-103.3% for remaining levels. The method was found to be specific, linear, sensitive, precise, rugged, accurate, robust and stability indicating in nature.[23] For details see Table 2.

Ultra-Violet and Colorimetric Spectroscopy (UV/VIS)

A simple and cost effective colorimetric method is described for the determination of Prasugrel in pure form and in pharmaceutical formulations. The determination of the drug in pharmaceutical formulations is based on the drugs redox reaction with Folin-Ciocalteu reagent (Phenol reagent). The linearity range for Prasugrel of blue chromogen produced at wavelength of detection 725 nm was obtained as 10–50 µg/ml. The linear regression equation obtained by least square regression method, was Y=0.0173.X – 0.1671, where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution. The absorbance was found to increase linearly with increasing concentration of Prasugrel, which is corroborated by the calculated correlation coefficient value of0.999. The limit of detection and limit of quantification was found to be 1.6145µg/ml and 4.8925 µg/ml, respectively. The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of Prasugrel in pharmaceutical formulations without any interference from common recipients.[24]

Another simple, economical, precise, reliable and reproducible Visible Spectrophotometric method was developed for the estimation of Prasugrel. The developed method is based on formation of chloroform extractable complex of Prasugrel with Bromocresol green which shows absorbance maximum at 418nm.The absorbance-concentration plot is linear over the range of 100-1000µg/ml. The different experimental parameters affecting the development and stability were studied carefully and optimized. Results of analysis for the method were validated statistically and by recovery studies.[25]

A simple, accurate, precise, specific and highly sensitive method for the determination of Prasugrel present in pharmaceutical dosage forms. The method is validated for the determination of Prasugrel in bulk and tablet dosage form. The solvent used is 0.1N HCl and the λ_{max} or the absorption maxima of the drug was found to be 249 nm. A linear response was observed in the range of 1-50µg/ml with a regression coefficient of 0.9993. The method was then validated for different parameters as per the ICH guidelines. This method can be used for the determination of Prasugrel in quality control of formulation without interference of the excipients.[26]

Three simple, precise and accurate Spectrophotometric methods were developed for the estimation of Prasugrel in pharmaceutical formulations. Prasugrel exhibits

maximum absorbance (λ_{max}) at 254.0 nm (Method A). In Method B (D1) is a first derivative method showing minima at 272 nm and Method C is an Area under Curve (AUC) method (244.0-264.0 nm). The drug obeys the Beer-Lambert's law in the concentration range of 1-50 µg/ml in all the three methods. The methods were validated and can be successfully applied to estimate Prasugrel in pharmaceutical dosage forms.[27]

Table 2: Summary of chromatographic Conditions used in HPLC methods

Column	Mobile Phase	Elution	Flow Rate (ml/min)	Temp. (°C)	Retention Time (min)	LOQ	Detection	Ref.
GraceSmart C_{18} (150X4.6mm,5µm)	(0.02M sodium dihydrogen phosphate, pH=3), methanol, acetonitrile	(45:20:35) v/v Isocratic	1.0	Amb.	8.20	ND	UV 220nm	[18]
Zorbax $XDBC_8$, (150X4.6mm,3.5µm)	(0.05 M ammonium acetate pH=4.5):acetonitrile	(40:60) v/v Isocratic	1.0	30	~ 6.8	0.364 µg/ml	UV 254 nm	[19]
Inertsil ODS-3V (250X 4.6mm, 5µm)	0.02M potassium dihydrogenphosphate, 0.02M dipotassium hydrogen phosphate: Acetronitrile	(30:70) v/v Isocratic	1.0	Amb.	10.59	0.75 µg/ml	UV 210nm	[20]
Kromasil C_{18}, (250X4.6 mm, 5µm)	Methanol:10mM potassium dihydrogen orthophosphate pH =2.2	(70:30) v/v Isocratic	1.0	Amb.	5.35	50 ng/mL	UV 257nm	[21]
Kromasil C_{18}, (100X 4.6mm; 5µm)	Methanol:potassium dihydrogen phosphate, pH=2.1	(70:30) v/v Isocratic	0.8	Amb.	1.9	ND	UV 220nm	[31]
Xterra C_{18}, (250X4.6 mm, 5 µm)	0.03M K_2HPO_4, pH=3.2: Acetonitrile	(25:75) v/v Isocratic	1.0	Amb.	4.762	7.5 µg/ml	UV 210 nm	[32]
C_{18} (150X4.6 mm, 5 µm)	Acetonitrile: Trifluroacetic acid	45:55% v/v Isocratic	1.0	Amb.	3.36	10.03 µg/ml	UV 252 nm	[33]
C_{18} (250X4.6 mm, 5µm)	Methanol: Acetonitrile	(90: 10) v/v Isocratic	1.0	Amb.	3.78	1.69 µg/ml	UV 240 nm	[34]
Luna C_{18} (150X4.60mm, 5µm)	Acetonitrile:0.05M ammonium acetate buffer(pH=4.5)	(75:25) v/v Isocratic	0.6	25	8.72	ND	245 nm	[30]
Sunfire C_{18} (250X4.6mm, 5µm)	**A**: 0.1% v/v H_3PO_4acid in water **B**: 0.1% v/v H_3PO_4acid in acetonitrile.	Gradient	1.0	45	22	0.204 µg/ml	UV 220 nm	[23]
Xterra C_{18}, (150X4.6 mm, 5 µm)	KH_2PO_4, pH=3 : Acetonitrile	(40:60) v/v Isocratic	1.0	Amb.	2.216	0.2 µg/ml	UV 210 nm	[35]

Amb. : Ambient temperature; ND: Not Detected; Temp.: Temperature; Ref.: Reference.

Determination of Prasugrel and Aspirin combination in dosage forms

Two new, simple, accurate, precise and reproducible UV Spectrophotometric methods have been developed and validated for the simultaneous determination of Prasugrel and Aspirin in their combined dosage forms. Method- I is based on simultaneous equation method using two wavelengths, 254 nm (λ_{max} of Prasugrel) and 276 nm (λ_{max} of Aspirin). Method - II Q-absorption ratio method using two wavelengths, 274.7 nm (Isoabsorptive point) and 254 nm (λ_{max} of Prasugrel). Methanol was the solvent used in all methods. This method obeyed Beer's law in the concentration range of 5-60 µg /ml for Prasugrel and 20-140 µg/ml for Aspirin. All methods were validated statistically and recovery studies were carried out. Hence, the described methods can be successfully applied in quality control of combined pharmaceutical dosage forms.[28]

Another article described two simple, accurate, and precise UV derivative Spectrophotometric methods for the simultaneous determination of Prasugrel and Aspirin in synthetic mixture form have been developed. The first

method involves measurement of second order derivative spectra of Prasugrel and Aspirin. The zero crossing wavelengths 267.62nm and 252.40 nm were selected for estimation of Prasugrel and Aspirin, respectively. In the second method, the first order derivatives of ratio spectra were calculated and used for the determination of Prasugrel and Aspirin by measuring the peak intensity at 268 nm and 290 nm, respectively. The methods were validated as per the ICH guideline Q2 (R1). Beer's lawis followed in the range of 5–45 μg/ml for Prasugrel and 25–150 μg/ml for Aspirin by second order derivative method and 6–22 μg/ml for Prasugrel and 45–165 μg/ml for Aspirin by ratio first order derivative method. The recovery studies confirmed the accuracy of the methods. Relative standard deviations for repeatability and inter- and intraday assays were less than 2%. Hence, the described derivative Spectrophotometric methods are simple, accurate, precise, and excellent alternatives to sophisticated chromatographic techniques and can be potentially used for the simultaneous determination of Prasugrel and Aspirin in combined dosage forms.[29]

A new RP-HPLC method has been developed and validated to determine Aspirin and Prasugrel in synthetic mixture form. The method was simple, reliable, rapid, precise, sensitive. Chromatographic separation achieved isocratically on Luna C18 column (5µm, 15×4.60mm) and acetonitrile: 0.05M ammonium acetate buffer (pH 4.5) in the ratio of 75:25 (v/v) as the mobile phase, at a flow rate of 0.6 ml/min. Detection was carried out at 245 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for Aspirin and Prasugrel were found to be 2.25±0.5 and 8.72±0.5 min, respectively. Linearity for Aspirin and Prasugrel was in the range of 75-375 g/ml and 10-50µg/ml, respectively. The mean recoveries obtained for Aspirin and Prasugrel were 99.58 and 99.48 % respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Aspirin and Prasugrel.[30]

CONCLUSION

A few methods for determination of Prasugrel or its metabolites have been reported. Some HPLC assay methods were also used to monitor Prasugrel. Methods For the analysis of active and inactive metabolites of Prasugrel in plasma has also been reported. Some articles related to the determination of Prasugrel alone or in combination with Aspirin in pharmaceutical dosage forms has been mentioned. Other articles reported HPLC methods for determination of Prasugrel in the presence of its related compounds.

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