Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(2):285-293

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Prasugrel charactherization: Reference substance and pharmaceutical

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ABSTRACT

The prasugrel characterization of reference substance described in this work ensures its purity and identity. The melting point by DSC (122.03 °C) and capillary method (120.5- 121.9 °C) demonstrated consistent results in both methods. In addition, the IR spectrum, ^{1}H NMR and ^{13}C NMR spectroscopy results are presented and discussed in this study. For qualitative analysis of pharmaceutical, Rf (0.64) results obtained by TLC, chromatographic (6.02 minutes) and spectroscopic methods verified the identity of the samples. The methods employed in this paper were suitable for the identification and qualitative quantification of prasugrel and provides important information for quality and the conformity of products containing this drug. The information available emphasized the important role the qualification, especially, of the secondary reference standards.

Keywords: prasugrel; spectroscopic method; differential scanning calorimetry; thin layer chromatography; liquid chormatography; nuclear magnetic resonance spectrometry.

INTRODUCTION

Prasugrel (Fig. 1) is a thienopyridinic compound approved in 2009 by Food and Drug Administration (FDA) for the management of acute coronary syndromes and in patients undergoing percutaneous coronary intervention [1- 3]. The synthesis of racemic mixture is common but the coated tablets containing prasugrel were marketed in the hydrochloride form due its better hydrolytic stability and satisfactory solubility in physiological pH. Prasugrel hydrochloride is a white to light brown crystalline solid and slightly hygroscopic. Its solubility is influenced by pH, with the drug soluble or slightly soluble in the pH range 1.0 - 4.0, very slightly soluble at pH 5.0 and practically insoluble at pH 6.0 -7.0 range. The pKa value of prasugrel hydrochloride is 5.1. Besides, studies indicate that the compound exhibits polymorphism [4].

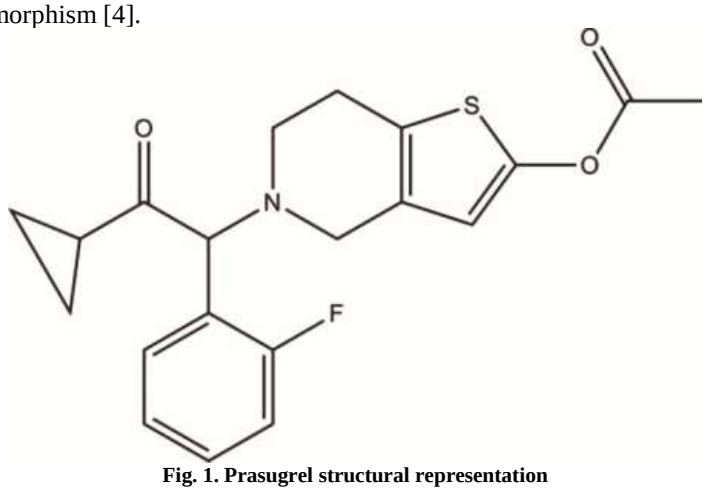

Fig. 1. Prasugrel structural representation

There are methods that provide important physicochemical information for identification and structural elucidation of reference standards, ensuring their characterization for further analysis of drugs present in pharmaceuticals.

Melting range is a physical test employed to identify chemical substances such as drugs. This parameter can be determined by capillary method and differential scanning calorimetry (DSC). The DSC is a useful thermoanalytical study for the thermal characterization of drugs, determination of purity, incompatibility of the components of the formulation and detection of polymorphic forms [5-6].

Infrared (IR) spectrophotometry is a important tool for the identification of drugs. The analysis carried out in the electromagnetic radiation range of 400 – 4.000 cm^{-1} allows the verification of characteristic bands related to chemical compounds [7].

Nuclear magnetic resonance (NMR) is a technique used to characterize and identify atoms, such as, ^{1}H and ^{13}C. The information about number of this atoms and its electronic environment is provided in this analysis, associate with the IR spectrum, accomplish structural elucidation of entirely unknown molecules [8].

For qualitative analysis of pharmaceutical, spectrophotometric and chromatographic methods provide important information for quality dosage forms, such as the identification and characterization of drugs. In thin layer chromatography (TLC) the qualitative determination occurs through differential migration of the components of a mixture on a stationary surface in the presence of an eluent system. The determination of the retention factor (Rf) allows the identification of compounds [9].

The ultraviolet (UV) spectrophotometry is a technique highly applied in pharmaceutical analysis justify since their analysis are rapid, inexpensive and easy to perform. For the identification of drugs in pharmaceuticals, the UV spectrum of the sample and reference substance obtained in the same experimental conditions are compared [7].
The high performance liquid chromatography (HPLC) is applied for the drugs identification through the comparison of the retention time obtained in the sample and reference substance analysis in the same experimental conditions [7, 9].

A literature review reported bioanalytical methods described for active and inactive prasugrel metabolites determination [10-12] and for chiral separation method in human plasma by liquid chromatography tandem mass spectrometry (LC-MS) [13]. Other studies reported prasugrel determination in bulk and tablets using UV spectrometric [14-18] and LC methods [19-28]. Stability profile of prasugrel and degradation products were performed in few studies [28-31]. The dissolution profile of prasugrel were determined by Rigobello and colleagues [28] which developed and validated a dissolution testing for pharmaceutical containing this drug. However, it is important to note that any official monograph is described in literature for prasugrel identification and determination. In addiction, considering the lack of information avaiable in literature about the characterization of this compound this work aims to present widely discussion about the technical results for qualitative analysis of this compound and provide data to facilitate the study of dosage forms containing this antiplatelet.

EXPERIMENTAL SECTION

2.1 Chemicals

Prasugrel base reference substance with an assigned purity of 98.0% was purchased from ONTARIO® Inc (Canada). The coated tablets of prasugrel (as hydrochloride) were obtained commercially (Effient ®).

2.2 Characteristics and identification of prasugrel

2.2.1 Melting range by capillary method

For melting range determination by the capillary method was used Mettler Toledo® equipment, FP 90, previously calibrated. The sample was compacted into capillary (1 mm in diameter and 6 mm long) with heating of 5 ° C $minute^{-1}$, and the analysis performed in duplicate.

2.2.2 Differential scanning calorimetry (DSC)

The exploratory differential calorimeter consisted of Shimadzu® DSC-60 model with FC-60A flow controller, integrating TA 60WS, with head flow and software TA 60 version 2.0. The equipment was calibrated with indium (156.6 °C transition energy - 28.45 J g^{-1}) and zinc (419.58 ° C transition energy - 100.50 J g^{-1}). The heating ramp was 10 ° C $minute^{-1}$. It was weighed about 1 mg of reference substance in aluminum sample holder with 4 µL capacity, which was sealed and subjected to analysis.

2.2.3 IR Spectrophotometry

The characterization by IR spectrophotometry was performed in Varian® FTIR spectrophotometer 640-IR and Resolutions-Pro software. For analysis was transferred approximately 1.5 mg of reference substance to agate mortar containing 150 mg of potassium bromide. The mixture was transferred to pastillator and, posteriorly, it was subjected to analysis.

2.2.4 Nuclear magnetic resonance of hydrogen (^{1}H NMR) and carbon (^{13}C NMR)

The ^{1}H NMR and ^{13}C NMR spectrum were performed in Varian® equipment VNMRS- 300 MHz using as solvente dimethyl sulfoxide-d_6 (DMSO d_6).

2. 3 Prasugrel qualitative analysis in pharmaceutical

2.3.1 Thin layer chromatography (TLC)

Prasugrel sample and reference substance solutions were prepared in methanol at a concentration of 1.0 mg mL^{-1}. The reference substance solution of caffeine at 1.0 mg mL^{-1} in methanol was used as internal standard. Silica gel 60 (Merck®) coated with fluorescent indicador F254 with the plates size of 12.0 X 5.0 cm was used as stationary phase. A mixture of n-hexane:acetone (60: 40 v v^{-1}) was employed as eluting system. The application of the solutions was performed using capillary tubes at a distance of 1.0 cm from the bottom edge of the plates. After migration and drying at room temperature a 254-nm UV light was employed for visualization of the compounds and obtained the Rx and retention factor (Rf) values.

2.3.2 UV Spectrophotometry

The prasugrel identification in reference substance and pharmaceutical by UV spectrometry was performed according the analysis conditions developed and validated by Rigobello and colleagues [18]. The absorption spectrum was obtained at a range of 200 - 400 nm using as blank the solvent employed in the preparation of the solutions.

2.3.3 High-performance liquid chromatography (HPLC)

The chromatographic separation was performed according the analysis developed and validated by Rigobello and colleagues [28]. Reference substance and sample stock solutions were prepared at concentration of 500 µg mL^{-1} in methanol. Aliquots of 2 ml were transferred to 25 mL volumetric flask containing mobile phase, obtaining a concentration of 40 µg mL^{-1}.

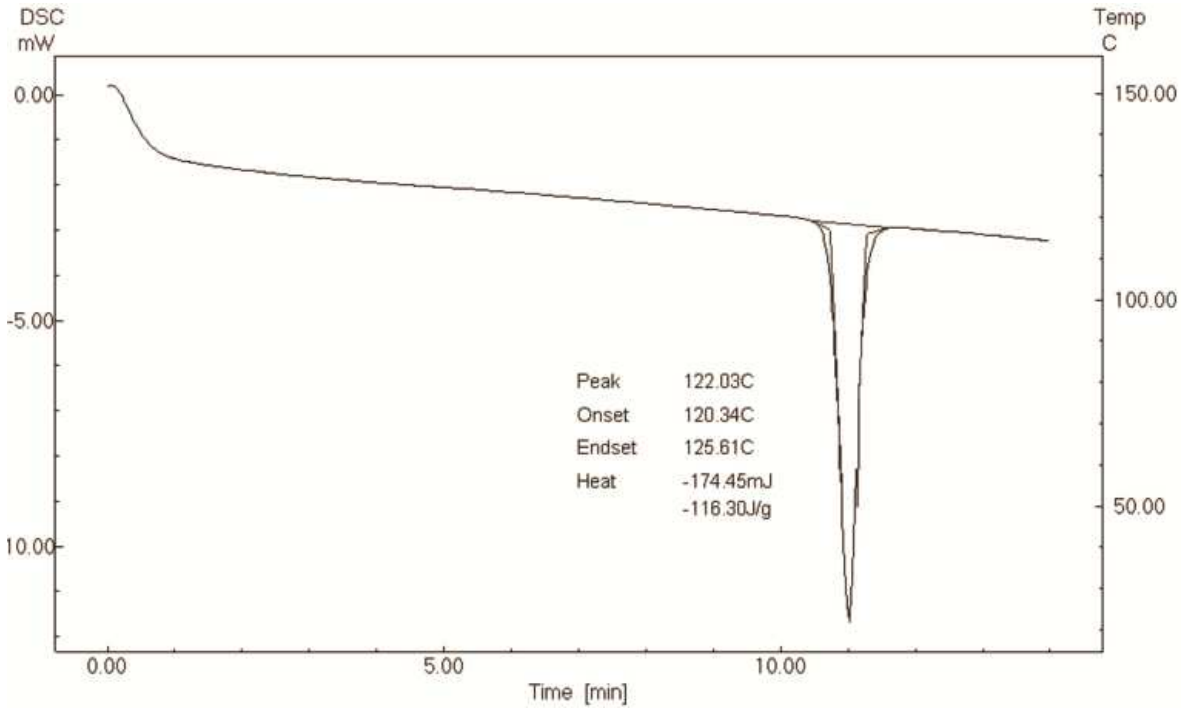

Fig. 2. DSC measurement curve of prasugrel reference substance

RESULTS AND DISCUSSION

The results obtained through these analytical methods are important for the characterization of prasugrel reference substance and coated tablets containing this drug. The melting point determination by DSC was an indicator of identity and purity of the drug. The analysis showed sharp peak, where is characteristic of purity, and the melting point of 122.03 °C as observed in Fig. 2. The DSC results were consistent with the melting range obtained by the capillary method (120.5- 121.9 °C). The enthalpy of fusion (ΔH) of 116.30 J g^{-1} indicates an endothermic process, since there is heat absorption for the fusion compound. The conformity of the results by capillary method and DSC demonstrated that both procedure are suitable for the characterization and purity determination of prasugrel.

Based on the literature [8; 32], it was performed the interpretation of IR spectrum illustrated in Fig. 3. The results confirmed the identity of prasugrel reference substance for the presence of bands that markers of functional groups present on the drug structure. Besides, the IR spectrum obtained in this study and it described in the literature for prasugrel hydrochloride [33] are similar to the presence of intensive bands in the region between 1820 – 1660 cm^{-1} characteristic of the carbonyl group.

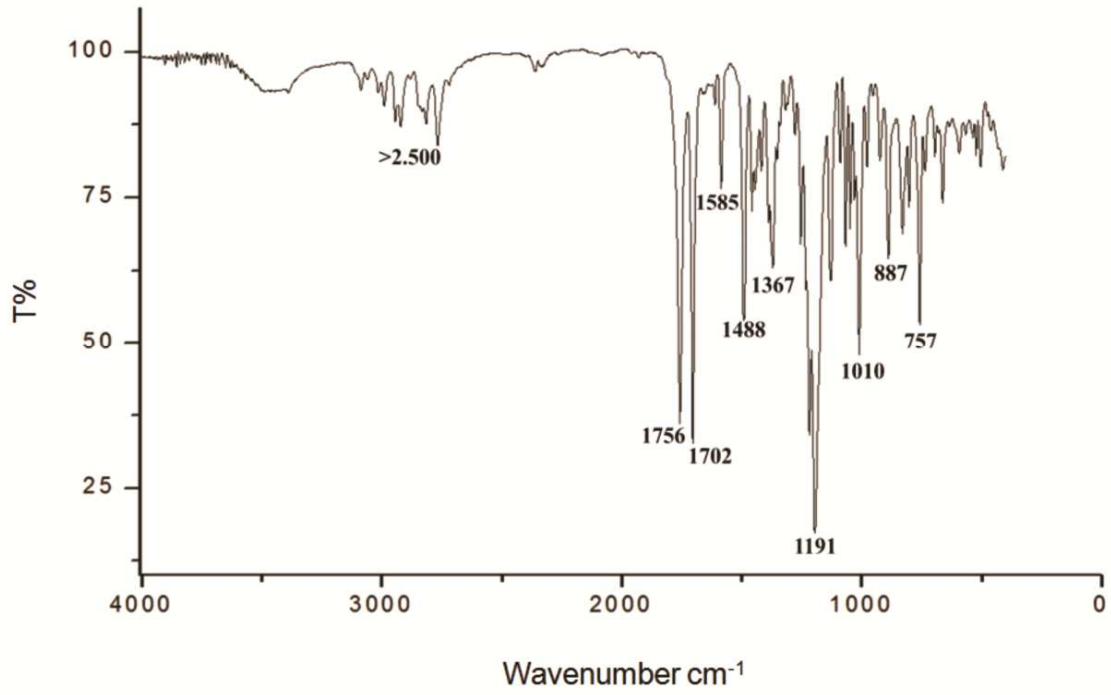

Fig. 3. IR spectrum in the area to prasugrel the chemical reference

The IR bands and their main assignments are described in Table 1.

Table 1. Designation of the main bands obtained in the IR spectrometric analysis of the prasugrel reference substance

Frequence (cm^{-1})	Atributtion
757	Ortho substitution of the aromatic ring
1191	C-O of the ester function
1702	C = O of the ketone function
1756	C=O of the ester function
>2.500	C-H

Hydrogens and carbons attribution of prasugrel reference substance were determined based on literature [8; 34] and the results are consistent with the chemical compound studied and confirmed the identity of this drug.

The ^{1}H NMR spectrum of prasugrel reference substance is represented in Fig. 4 and spectrum assignments are shown in Table 2.

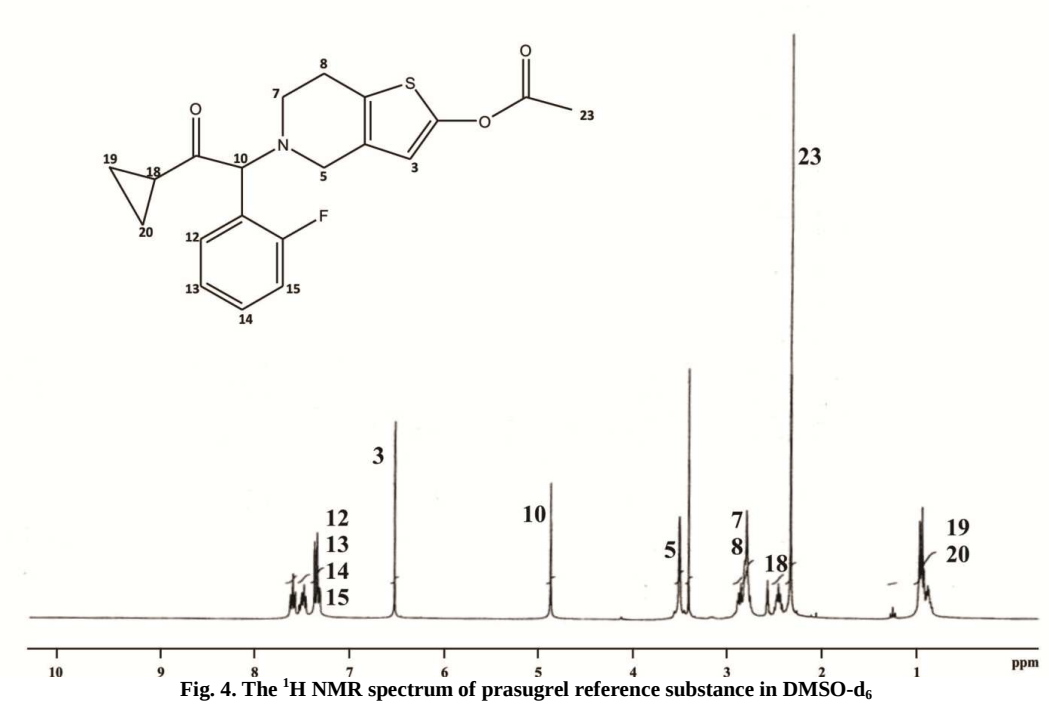

Fig. 4. The ^{1}H NMR spectrum of prasugrel reference substance in DMSO-d_6

Table 2. Peak attribution of prasugrel reference substance analysis by ^{1}H NMR spectrometry employed DMSO-d6

Position	Chemical Shift (ppm)	Multiplicity	Hydrogens numbers	Attribution
19, 20	0.8 – 1.0	Multiplet	4H	2CH2
23	2.1	Singlet	3H	CH3
18	2.4	Multiplet	1H	CH
-	2.5	Multiplet	-	Solvent (DMSO $_{d6}$)
7, 8	2.6 – 2.8	Multiplet	4H	2CH2
-	3.3	Singlet	-	Solvent (water)
5	3.5	Singlet	2H	CH2
10	4.8	Singlet	1H	CH
3	6.4	Singlet	1H	CH
12, 13, 14, 15	7.2- 7.6	Multiplet	4H	CH aromatic ring

Fig. 5 shows the ^{13}C NMR spectrum obtained on analysis prasugrel reference substance and their assignments are shown in Table 3.

Table 3. Peak attribution of prasugrel reference substance analysis by ^{1}H NMR spectrometry employed DMSO-d6

Position	Chemical Shift (ppm)	Atribution
19, 20	11.1- 11.5	2(CH2)
18	17.7	CH
23	20.4	CH3
8	24.6	CH2
-	40.0	Solvent (DMSO $_{d6}$)
5, 7	47.9- 49.8	2 (NCH2)
10	71.4	CH
3	112.3	CH
15	115.6	C aromatic
11	121.9	C aromatic
9, 13	124.6- 125. 3	C + C aromatic
4, 12, 14,	129.4 – 130.8	C + 2C aromatic
2	148.6	C-0
16	162.2- 164.5	C-F
22	167.8	C=0
17	207.4	C=0

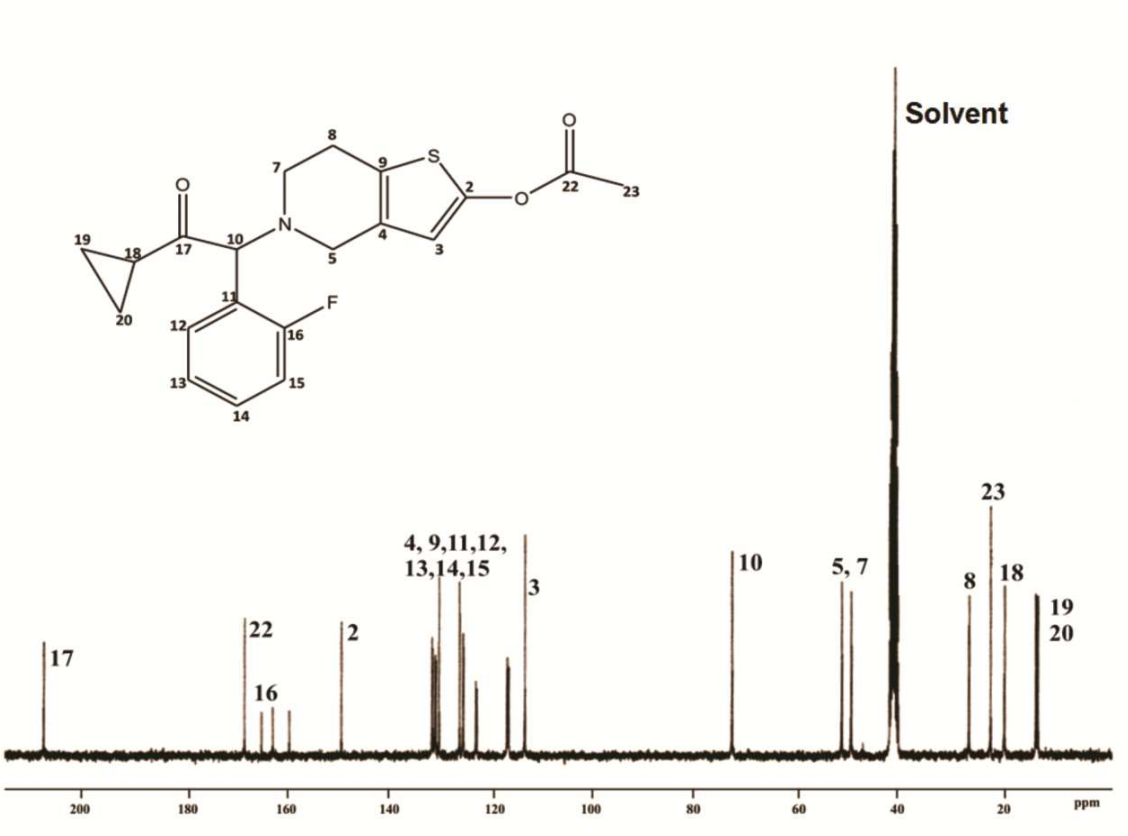

Fig. 5. The ^{13}C NMR spectrum of prasugrel reference substance

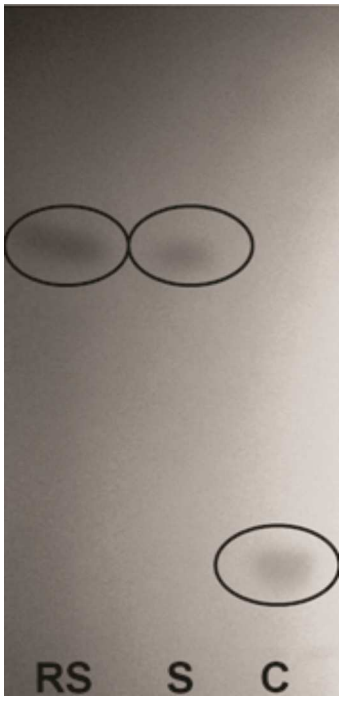

Fig. 6. TLC plate of prasugrel reference substance (RS), sample (S) and caffeine (C) analysis employed 254 nm UV lamp to detection of spots

In the qualitative analysis of tablets, Rf values obtained by the TLC technique proved the prasugrel identify and demonstrated that the developed method was suitable for the identification of the drug in the dosage form. The Rf values for the solutions analysed are shown in Table 4. The 254 nm UV lamp was employed to visualize the chromatographic migration of the analytes and the detected spots are demonstrated in Fig. 6. The Rx value of 3.10 was achieve between Rf value of prasugrel reference substance and Rf value of caffeine. The sample and prasugrel

reference substance solutions demonstrated similar migration patterns and Rx values. The TLC method developed was suitable for prasugrel identification in dosage form since showed good separation of compounds, with defined and well separated spots, characteristics that allowed its easy viewing.

Table 4. The Rf values of prasugrel reference substance (RS), sample (S) and caffeine (C).by chromatographic analysis

Substance	Rf
Reference substance (RS)	0.65
Sample (S)	0.64
Caffeine (C)	0.21

The overlay of UV spectra of prasugrel reference substance and sample are illustrated in Fig. 7. The similar UV absorption profile proved the identity of the analyzed solution, and the results demonostrated that the respective analytical method was appropriate of qualitative analysis of prasugrel in coated tablets.

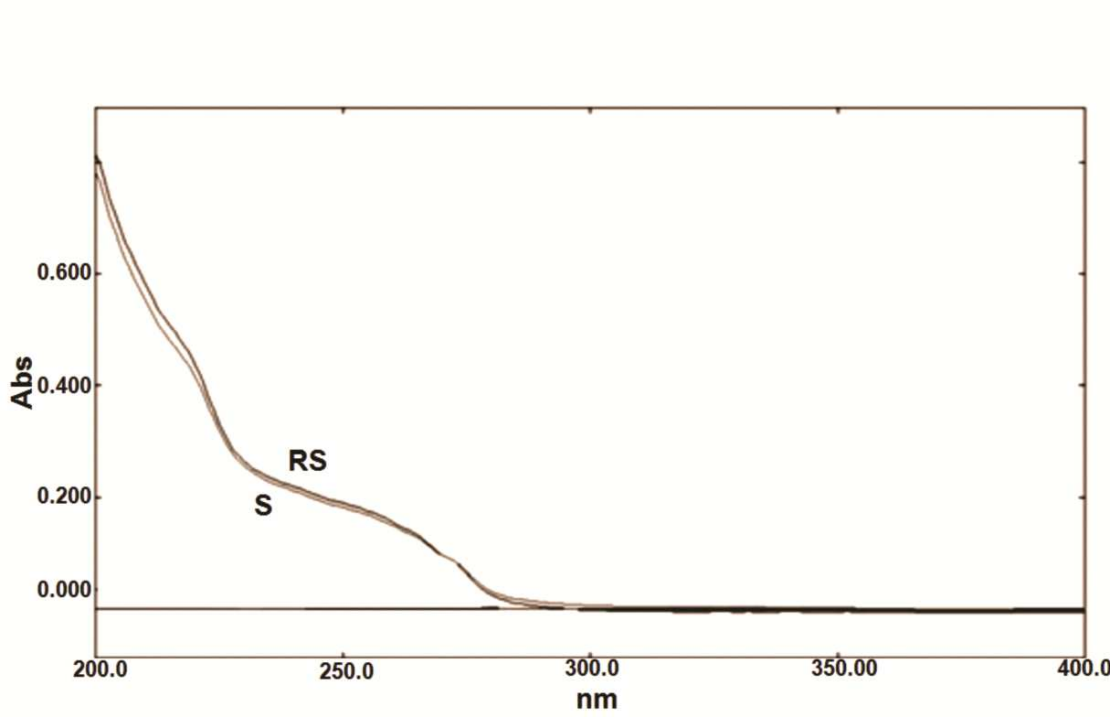

Fig. 7. Overlay UV spectra of prasugrel reference substance (RS) and sample (S) at concentration of 12 µg mL^{-1}

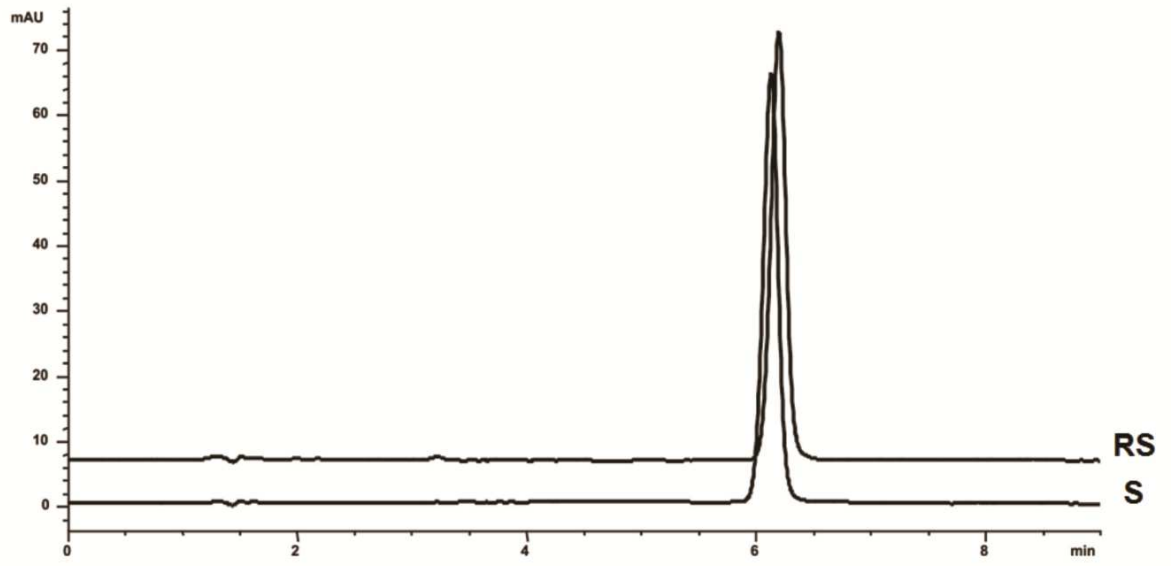

Fig. 8. The overlay of the chromatograms of prasugrel reference substance (RS) and sample (S) at a concentration of 40 µg mL^{-1}

The overlay chromatograms of prasugrel reference substance and samples at a concentration of 40 mg mL^{-1} are illustrated in Fig. 8. The similar retention times of the analyzed solutions (~6.2 minutes) in the same experimental conditions ensure the prasugrel qualitative determination.

Spectrophotometric and chromatographic methods were propely employed for the qualitative determination of prasugrel in pharmaceutical. The reference substance and sample solutions demonstrated smilar absorption profile by UV spectrometry analysis, and retention time of approximately 6.02 minutes in HPLC technique.

CONCLUSION

This study provides important information for the quality control of tablets containg prasugrel and to characterize the reference substance of this compound. This is a common procedure especially to ensure identify and purity of secondary reference substance in validation process, pharmaceutical analysis and equipment calibration. Moreover, the methods performed in this work afford reliable results can be applied in laboratorial routine of the analysis of products containing this antiplatelet drug.

Acknowledgements
The authors wish to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

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