Development and validation of novel UPLC-MS/MS method for the analysis of macitentan in pharmaceutical formulations

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Abstract

Macitentan is an endothelin receptor antagonist drug used in the treatment of pulmonary arterial hypertension. A new, sensitive, simple, accurate and rapid UPLC-MS/MS method is developed and validated for determination of macitentan in pharmaceutical formulations. Macitentan and bosentan which is used as internal standard (IS) were detected using APCI in positive ion, multiple reaction monitoring mode by monitoring the mass transitions (precursor to product) m/z 589.1→203.3 and 552.6→311.5, respectively. Chromatographic separation was carried out on reverse phase C18 column (5 µm, 4.6 * 150 mm). It was used water containing 0,2 % acetic acid: acetonitrile (90:10, V/V) in isocratic elution as mobile phase. The system was optimized as injection volume of 10 µl, column temperature of 35 °C and flow rate of 1 mL/min. Retention times were 1.97 min. for macitentan and 1.72 min. for IS. The calibration curve with high correlation coefficient (0.9997) was linear range 0.5-500 ng mL^{-1}. The LLOQ and average recovery values were determined as 0.5 ng mL^{-1} and 99.7 %, respectively. The developed novel method has been successfully applied for determination of macitentan in pure form and pharmaceutical formulations.

Keywords: Macitentan, Pulmonary arterial hypertension, UPLC-MS/MS, Validation

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a slowly progressing serious disease of the arteries that connect the lungs to the heart (pulmonary arteries), often diagnosed late and leading to death at an early age. PAH is a chronic and progressive disease that leads to right heart failure [1] and is associated with high morbidity and mortality and death if not treated [2]. Macitentan is an endothelin receptor antagonist commonly used in the treatment of PAH [3]. Macitentan is approved for the long-term treatment of PAH in monotherapy or combination therapy and is effective in reducing vasoconstriction, endothelial disfunction and smooth muscle cell proliferation [4].). Macitentan is taken orally and the daily dose is 10 mg [5]. The maximum concentration in the blood reaches an average of 8 hours after the medication is taken [6]. The bioavailability of the medication is about 74% [7]. The chemical formula for macitentan is $C_{19}H_{20}Br_2N_6O_4S$, the chemical name is N- [5- (4-Bromophenyl) -6- [2 - [(5-bromo-2-pyrimidinyl) oxy] ethoxy] -4-pyrimidinyl] -N'-propylsulfamide. Figure 1 shows the chemical structure of macitentan. Based on a literature review, no studies have been conducted in determining the amount of macitentan from pharmaceutical preparations using the UPLC-MS/MS method. There have been studies that were carried out to determine macitentan from plasma by LC-MS/MS method [6, 8-14] There are analytical studies available using the HPLC method [15-19] to determine the amount of macitentan from pharmaceutical preparations. It was observed that studies conducted with the HPLC method have used gradient separation which is difficult to prepare and apply as opposed to isocratic separation In addition, the sensitivity and retention time of macitentan were found to be advantageous in this study. Proposed UPLC-MS/MS method is more sensitive than HPLC method. In this study, easy to prepare mobile phase and easy to apply isocratic separation were used. The aim of the study was to develop a novel, simple, cheap, accurate, reliable, highly sensitive UPLC-MS/MS method with high recovery and short run-time for the determination of macitentan in pharmaceutical preparation and pure form and to validate this method according to Food and Drug Administration (FDA) guidelines [20].

MATERIALS AND METHODS

Chemicals and Reagents

Pure form of macitentan was purchased from Santa Cruz Biotechnology. Bosentan, used as internal standard (IS), was obtained from Actelion Pharmaceuticals Ltd, Switzerland. Opsumit tablet (containing macitentan 10 mg) was obtained from local pharmacy. LC-MS hypergrade methanol and asotonitrile were purchased from Merck, Germany. Analytical grade acetic acid

and HCl from Sigma-Aldrich, Germany. The vacuum concentrator (Eppendorf Concentrator Plus) was supplied from Hamburg, Germany. The water used in the preparation of the mobile phase was obtained from the ultrapure water device Merck Millipore Simplicity-UV (Merck-Millipore, Darmstadt, Germany).

Instrumentation and UPLC–MS/MS Conditions

UHPLC-MS / MS system consists of 6460 series sequential mass system (Agilent 6460 Triple Quadropol mass spectrometer) connected to the Agilent 1200 series HPLC system equipped with dual mixing pump, vacuum membrane degasser, thermostatic column chamber and automatic sampler. The chromatographic separation was performed in the reverse phase C18 column (5 µm, 4.6 * 150 mm) at 35 ° C column temperature. It was used water containing containing 0.2% acetic acid : acetonitrile (10:90, v/v) in isocratic separation as mobile phase. Injection volume was adjusted to 10 µl and flow rate was 1 ml/min. Retention times were 1.97 min. for macitentan, 1.72 min. for bosentan. The total analysis time was approximately 3 minutes. In the mass spectrometry (MS) system, atmospheric pressure chemical ionization (APCI) was used as the ion source and 4500 V capillary voltage was performed in the positive ion mode. Quantitative analyzes were carried out using mass transitions mass/charge (m/z) ratio 589.1→203.3 for macitentan, m/z ratio 552.6→311.5 for bosentan in multiple reaction monitoring (MRM) mode. The MRM parameters were optimized as fragmentor voltage 150 V for macitentan, 230 V for IS, 10 V for collision energy and 7 V for cell accelerator voltage. Nitrogen was used for nebulization and collision gas. Ion source parameters was optimized as nebulizer 20 psi, 325 °C gas temperature, 350 °C evaporator and gas flow rate was 4 L/min.

Preparation of Stock, Standard and Sample Solution

An accurately weighed quantity of 5 mg pure macitentan was transferred into 10 ml polyethylene tube and dissolved in 1 ml methanol. 5 mg/ml stock of macitentan solution was prepared. The mixture was vortexed for 5 min. and sonicated for 10 min. From this stock solution was prepared 100 µg/ml and 1 µg/ml stock solutions by appropriate dilution with acetonitrile. The nine standard solutions (0.5, 2, 4, 5, 10, 25, 100, 250 and 500 ng mL^{-1}) and three quality control (QC) samples (1, 200 and 480 ng mL^{-1}) were prepared by doing required dilutions with acetonitrile from stock solutions. Five different Opsumit tablets, each containing 10 mg macitentan, were accurately weighed and grinded to form powder. The weight of obtained powder is just equal to one tablet was dissolved in 10 ml methanol and sonicated for 10 min. 1 mg/ml stock of macitentan solution was prepared. The drug stock solution was filtered via 0.45 µm membrane filter. From this stock solution was prepared 20

ng mL^{-1} and 5 ng mL^{-1} drug solutions by appropriate dilution with acetonitrile. All of the stock solutions were stored at -20 °C. 250 ng mL^{-1} IS was added to each standard, sample and QC solutions. Standard, sample and QC solutions were vortexed for 3 min. and all the samples (10 µl) was injected into the UPLC-MS/MS system for analysis 3 times. Also, QC solutions were analyzed 3 times on different days.

Method Validation

The developed analytical method was validated for linearity, specificity, accuracy, precision, sensitivity (The Lower Limit of Quantitation, LLOQ), stability and recovery according to FDA guidelines. Validation runs were conducted on three consecutive days. Each validation run consisted of one set of calibration standards and three replicates of QC samples.

RESULTS AND DISCUSSION

UPLC–MS/MS Method Development and Optimization

In the optimization studies, acid solutions of different concentrations (0.1%, 0.2%, 0.3% acetic acid and (0.1%, 0.2%, 0.3% formic acid), different column and column temperatures (30, 35, 40 °C), mobile phases of different proportions (water containing 0.2% acetic acid: acetonitrile; 50:50, 30:70, 20:80, 10:90) and different flow rates (0,5, 0, 7, 0.8, 1 ml / min) and different injection volumes (5, 8, 10, 20 ınl) were performed for a method short run time with good resolution and good peak shape. For UPLC–MS/MS optimization, positive and negative ionization modes of ESI and APCI were investigated. The better optimization was obtained by APCI ionization in MRM and positive ion mode. The mass spectra obtained using mass transitions m/z ratio 589.1→203.3 for macitentan and m/z ratio 552.6→311.5 for IS under the determined conditions were given in Figure 2 and Figure 3, respectively.

Validation Parameters

Specificity and Linearity

The specificity of developed UPLC-MS/MS method was confirmed by evaluating the peak purity in presence of impurities, excipients and endogenous substances. No interference was observed impurity, excipients and endogenous substances coming from standard and pharmaceutical preparation. Figure 4 shows typical chromatograms of macitentan and IS . The linearity was determined at nine different concentrations of macitentan standard solutions (0.5, 2, 4, 5, 10, 25, 100, 250 and 500 ng mL^{-1}) with three replicates analysis. Peak area ratios of macitentan to IS were plotted against analyte concentrations. Standard calibration curve

shows an excellent correlation between different concentrations and respective peak areas. The linear regression equation was obtained as y=43,647901x +0,748477. The calibration curve (figure 5) with high correlation coefficient (r=0.9997) was linear range 0.5-500 ng mL^{-1}.

Accuracy and Precision

The accuracy and precision values was determined with percent relative error (% RE) and percent relative standard deviations (% RSD) for QC solutions (1, 200 and 480 ng mL^{-1}) over on the same day (intra-day, n=3) and three consecutive days (inter-day), respectively. The accuracy and precision results were given in Table 1. The intra-day and inter-day values for accuracy and precision were within the acceptable limits in all cases. The method showed a good accuracy and precision with % RE (lower than % 5.03) and % RSD (lower than % 6.67) values.

Recovery and Sensitivity

Analytical recovery was calculated by performing recovery studies following standard addition method by spiking the known quantities of standard macitentan QC solutions to 5 ng mL^{-1} drug solution, and these solutions were analyzed in three times. The results are given in table 2. The average recovery value was determined as 99.7 %. LLOQ was defined as the lowest concentration (0.5 ng mL^{-1}) on the calibration curves at S/N ratios of 10 and with RSD <20 %.

Application of The Method

The prepared 20 ng mL^{-1} drug solution was analyzed three times by UPLC-MS/MS method. The amount of macitentan present in tablet formulation (Opsumit) was determined by comparing the peak area ratio from the standard solutions. The results was given in Table 3. The obtained results demonstrated that the proposed UPLC-MS/MS method is capable for the determination of macitentan in pharmaceutical formulations.

Stability

Standard macitentan QC solutions containing 250 ng mL^{-1} IS were kept at room temperature, +4 ° C and -20 ° C for 12, 24 and 36 hours. Then, the all samples were analyzed three times by UPLC-MS/MS. The obtained results were compared with the freshly prepared and analyzed macitentan solutions. The results was given as % recovery in Table 4. According to the results, standard macitentan QC solutions which were only kept at room temperature for

24 and 32 hours were not stable (not within the range of ± 15 according to the FDA guidelines). All solutions which were kept in all other field were stable.

CONCLUSION

The present method is the first study developed and validated for the determination of macitentan from the pharmaceutical preparations and pure form by UPLC-MS/MS method in the literature. The obtained results demonstrated that the proposed the UPLC-MS/MS method is a new, simple, precise, rapid, accurate and inexpensive. Moreover, this method has a very short analysis time and high sensitivity. In conclusion, this method can be used successfully for the determination of macitentan in pure form and pharmaceutical formulations, routine analysis, quality control laboratories of pharmaceutical industries and monitor the stability.

ACKNOWLEDGEMENT

The author is grateful to Ataturk University Scientific Research Projects Coordination Unit for the financial support of this work (Project No: THD-2018-6467).

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LIST OF TABLES

Table 1. Intra-day and inter-day accuracy and precision data for macitentan

Material	Added (μg mL^{-1})	Intra-day			Inter-day		
		Found±SD (μg mL^{-1})	Accuracy (% RE)	Precision (% RSD)	Found±SD (μg mL^{-1})	Accuracy (% RE)	Precision (% RSD)
Macitentan	1	1,02±0.05	2,04	4,91	1,05±0.07	5.03	6,67
	200	196,8±2.38	-1,63	1.24	194,9±3.34	-2,55	1.71
	480	476,6±3.04	-0.71	0.64	473.4±3.11	-1.38	0.66

SD: Standard deviation

Table 2. Analytical recovery values of macitentan

Commercial Preparation	Concentration of macitentan in formulation (ng mL^{-1})	Concentration of pure macitentan added (ng mL^{-1})	Total concentration of macitentan found ± SD (ng mL^{-1})	% Analytical recovery
Opsumit® Tablet	5	1	5,94±0,17	99,0
		200	198,9±2,43	99,5
		480	483,2±2,78	100,7

Table 3. Assay of macitentan in Commercial preparation

Commercial Preparation	Declared concentration (ng mL^{-1})	Concentration Found±SD (ng mL^{-1})	% Recovery
Opsumit® Tablet (10 mg)	20	19,89±1,05	99.5

Table 4. Stability study of standard macitentan solutions

QC Solutions (ng mL^{-1})	12 hour	24 hour	36 hour
-20 °C (% Recovery ±SD)			
1	99,3±0,17	99.2±0,14	99,5±0,19
200	99,5±3,01	99,4±3,13	99,3±3,55
480	100,3±3,18	99,6±3,29	99,4±3,27
+4°C (% Recovery ±SD)			
1	99,1±0,11	99,4±0,16	99,1±0,18
200	99,4±3,42	99,6±3,67	99,1±3,94
480	98,7±3,46	97,6±3,53	97,1±3,82
Room temperature (% Recovery ±SD)			
1	89,5±0,17	70,3±0,19	76,5±0,32
200	92,2±3,69	73,5±5,47	73,3±4,81
480	93.1±4,86	71,3±4,61	70,4±4,97