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# Simultaneous determination of macitentan and its active metabolite in human plasma by liquid chromatography-tandem mass spectrometry

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ABSTRACT

Macitentan is a newly approved endothelin receptor antagonist (ERA) for the long-term treatment of PAH with superior receptor-binding properties and a longer duration of action compared to other available ERAs. However, analytical methods for simultaneous determination of macitentan and its active metabolite, ACT-132577, in human plasma have not been fully reported in the literature. In this work, a fast, sensitive, and reliable high-performance liquid chromatography-tandem mass spectrometry method (HPLC–MS/MS) was firstly developed and completely validated for simultaneous determination of macitentan and its active metabolite in human plasma. Plasma samples were processed with a protein precipitation using acetonitrile, followed by chromatographic separation using an Inertsil ODS-SP column (100 × 2.1 mm, 3.5 μm) under isocratic elution with a mobile phase consisting of acetonitrile and 0.2% formic acid at a flow rate of 0.3 mL/min. Quantification was operated in multiple reaction monitoring (MRM) mode using the transitions $m/z$ 547.1 → 201.0 for macitentan, $m/z$ 589.0 → 203.0 for ACT-132577, and $m/z$ 380.5 → 243.3 for the IS (donepezil). The assay exhibited a linear range of 1–500 ng/mL for both macitentan and ACT-132577. The accuracy and the intra- and inter-precisions were within acceptable ranges and no significant matrix effect was observed during the method validation. The developed method was successfully utilized to a human pharmacokinetic study of macitentan as well as ACT-132577 after oral administration of 10 mg macitentan tablet in healthy Chinese volunteers.



## 1. Introduction

Pulmonary arterial hypertension (PAH) is an incurable, life-threatening disease, which is characterized by the progressive increase in pulmonary vascular resistance potentially leading to eventual right ventricular failure and ultimately death. Conventional therapy for PAH includes diuretics, anticoagulant and calcium channel blockers, which are only effective in a small percentage of patients [1,2], and the median survival in idiopathic PAH is less than 3 years after diagnosis, which results from not only the progress of disease, but also the adverse drug reactions on cardiac repolarization [3]. Therefore, it is important that therapies developed against PAH should not further disturb the cardiac repolarization. Recent improvement in the understanding of the pathobiological pathways involved in the development of PAH has led to the development of new specific medical therapies targeting these pathways [4]. Endothelin receptor antagonists (ERA) with more specific targets, enhanced potency, and improved patient survival status have been proposed for the treatments of PAH [5].

Macitentan, a novel orally active, non-peptide dual ERA [6,7], which can reduce the risk of morbidity and mortality by delaying the progression of PAH [8], has been approved for the long-term treatment of PAH by the United States Food and Drug Administration, Health Canada's Therapeutic Products Directorate, and the European Commission [9,10]. Macitentan exhibited superior receptor-binding properties and a longer duration of action allowing for once-daily dosing compared to other available ERAs [11].

Macitentan is metabolized by oxidative depropylation into ACT-132577 and by oxidative cleavage into ACT-373898 (Fig. 1) in humans [12,13]. Of the two metabolites, only ACT-132577 which

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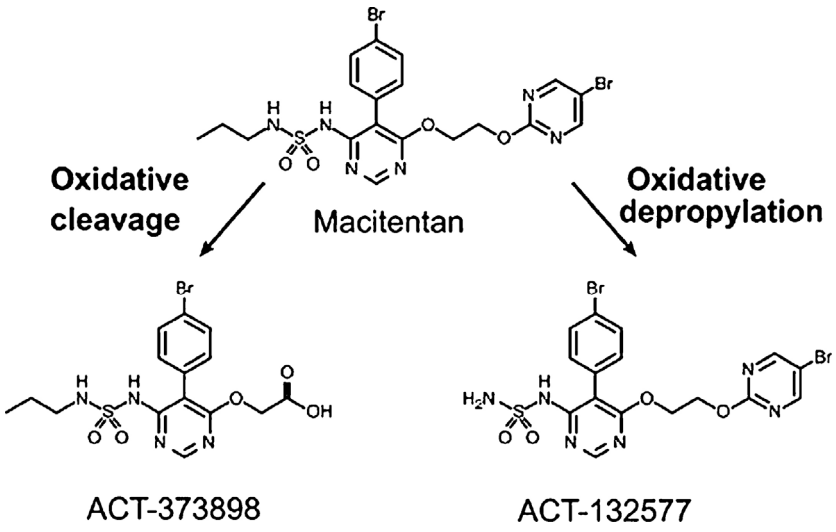


**Fig. 1.** Macitentan and its two metabolites: ACT-132577 (active) and ACT-373898 (inactive).

is the main metabolite detectable in human plasma, is pharmacologically active and has a terminal half-life about 46 h. Although ACT-132577 is less potent than macitentan in blocking endothelin receptors, it has a 3-fold higher systemic exposure in humans with a tendency of accumulation [6]. However, the method for simultaneous quantification of macitentan and ACT-132577 in human plasma has not been fully reported in the literature although there were a lot of reports related to pharmacokinetics of macitentan and its metabolites [14–17]. Therefore, simultaneous assay of macitentan and its active metabolite is essential to study their pharmacokinetics and bioavailability. A novel, simple and sensitive LC–MS/MS method for the simultaneous determination of macitentan and its active metabolite in human plasma was established and fully validated in this study, and also applied to the study of pharmacokinetics of macitentan and its active metabolite in healthy Chinese volunteers after oral administration of 10 mg macitentan tablet.

## 2. Experimental

### 2.1. Reagents and materials

Macitentan (99.8% purity) and ACT-132577 (98.5% purity) were provided by Jiangsu Hengrui Medicine Co., Ltd. (Lianyungang, PR China). Donepezil (IS, 99.8% purity) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile, methanol, and formic acid of HPLC grade were purchased from Merck (Darmstadt, Germany). Ultrapure water (Chengdu Ultra Technology Co., Ltd, Chengdu, PR China) was used throughout the study. Blank healthy human plasma was provided by Union Hospital, Tongji Medical College, Huazhong University of Science & Technology (Wuhan, PR China). Other reagents and chemicals were of the analytical grades and obtained commercially.

### 2.2. Instrumentation and LC–MS/MS conditions

An Agilent 1200 liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an autosampler and a column oven was used. Chromatographic separation was achieved on an Inertsil ODS-SP column (100 mm × 2.1 mm, 3.5 μm, GL Science, Japan) using a mobile phase consisting of acetonitrile and 0.2% formic acid (65: 35, v/v) at a rate of 0.3 mL/min under isocratic elution. The autosampler was conditioned at +4 °C and the column oven was conditioned at +30 °C. The injection volume was 5 μL.

The HPLC system was coupled with an API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) equipped with an ESI Turbo ionspray. The mass spectrometer was operated in positive ion mode with the capillary voltage and source temperature set at 5500 V and 500 °C, respectively. Collision activated dissociation gas (CAD) was set at 8, the curtain gas (CUR) at 25 and nebulizer and heater gas (GS1 and GS2) were at 30 and 35, respectively. Quantification was operated in multiple reaction monitoring (MRM) mode using the transitions $m/z$ 547.1 → 201.0 for macitentan, $m/z$589.0 → 203.0 for ACT-132577, and $m/z$ 380.5 → 243.3 for the IS (donepezil), respectively. Data were acquired and processed using Analyst 1.5.1 software (AB Sciex, Framingham, MA, USA).

### 2.3. Preparation of standard solutions

Standard stock solutions of macitentan and ACT-132577 were prepared by dissolving the appropriate amount of each in methanol to obtain a respective concentration of 217.6 and 208.9 μg/mL. The mixture standard stock solution of macitentan and ACT-132577 was prepared in methanol at concentration of 10 μg/mL. The working solutions for the seven calibration standards were prepared by dilution of the mixed stock solution with methanol to obtain the following concentrations: 10, 30, 100, 300, 1000, 2000, and 5000 ng/mL for both analytes. The working solutions for the quality control (QC) samples were prepared at four concentrations of 10, 20, 500, and 4000 ng/mL in the same way. The internal standards (IS) was weighed and dissolved in methanol to achieve the stock solution at the concentration of 1 mg/mL which was further diluted to the working solution with a concentration of 300 ng/mL. All solutions described above were preserved at +4 °C.

### 2.4. Sample preparation

An aliquot of 0.2 mL human plasma sample was mixed with 20 μL of IS working solution (300 ng/mL) and then 0.6 mL of acetonitrile was added into the mixture. After vortex-mixing for about 1 min, the mixture was centrifuged at 14,500 rpm for 5 min under 4 °C. And then 80 μL of the supernatant was transferred into autosampler vails for sample analysis.