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Simultaneous determination of metabolic stability and identification of buspirone metabolites using multiple column fast liquid chromatography time-of-flight mass spectrometry

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Abstract

A recent trend in the drug discovery and development process is to shift the starting point of drug metabolism and pharmacokinetic (DMPK) studies to a time as early as possible in the development chain to address potential issues in parallel with the optimization of the drug's lead structure. Therefore, it is necessary to develop assay methods to determine early adsorption, distribution, metabolism and excretion (ADME) parameters like metabolic stability and metabolite identification. For metabolite identification it is of crucial importance to work with fast liquid chromatography/mass spectrometry (LC/MS) systems, which provide the necessary high throughput functionalities to handle a large number of samples in combination with high speed and high resolution chromatography as well as mass accuracy. In this study a fast two-column liquid chromatography (LC) method will be used to simultaneously determine metabolic stability and to identify metabolites of buspirone using highly accurate mass measurement by means of an electrospray time-of-flight (ESI-TOF) mass spectrometer. Whereby, the metabolic stability will be determined on a short sub-two micron column, the main metabolites will be identified in the same experiment by the automated use of a long sub-two micron column, which provides the necessary high resolution.

Keywords: Metabolite identification; Metabolic stability; Buspirone; High resolution LC; Time-of-flight; Mass spectrometry

1. Introduction

In modern pharmaceutical drug development it is of crucial importance to know the adsorption, distribution, metabolism and excretion (ADME) behavior of a new drug substance. The confident identification of metabolites derived from a drug substance even in very low concentrations is highly important because of the potential to cause a toxic reaction in humans. Therefore, a recent trend in the drug discovery and development process is to shift the starting point of drug metabolism and pharmacokinetic (DMPK) studies to a time as early as possible in the development chain to address potential issues in parallel with the optimization of the drug's lead structure [1]. To address this need, it is necessary to develop in vitro frontline assays which focus on potential problems in the development of a new drug substance as early as possible. A typical experiment is the determination of the drug's metabolic stability and identification of the major metabolites. In this type of experiment, the time-dependant declining parent drug concentration is monitored and the metabolites are identified from a highly metabolized sample at the end of the study [2,3].

It is necessary to work with a fast high throughput LC/MS system to handle the large number of samples generated by experiments where the metabolic reaction is stopped at various times in sample aliquots. The LC/MS system must be capable of analyzing samples in the single digit minutes range. To achieve this goal, a system must be able to use multiple columns in a temperature controlled column oven for various analytical methods and high throughput functionalities like overlapped injections. State of the art sub-two micron particle columns are necessary to achieve high speed and resolution. It is possible to determine the metabolic stability semi-quantitatively and to identify the possible metabolites by empirical formula calculation from the measured highly accurate molecular masses with an ESI time-of-flight mass spectrometer [4]. The connected time-of-flight mass spectrometer must be able to scan at high speed in conjunction

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with the fast LC system and to provide the necessary dynamic range to identify low concentrations of compounds with high sensitivity.

The metabolism of the anxiolytic pharmaceutical drug substance buspirone was excessively examined in vivo in a variety of organisms as well as under different in vitro conditions. In phase I metabolism reactions buspirone undergoes a variety of oxidation and hydroxylation reactions at different sides of the molecule and a conjunction to glucuronic acid is the common reaction for phase II. The identified metabolites were well characterized by their MS and MS/MS spectra and the structures of the metabolites were elucidated [5]. Therefore, this drug is very often used in evaluation trial for instruments and methods [6,7] as well as new computer algorithms, which should find their use in metabolite identification applications [8].

In this study a metabolic stability profile of the drug substance buspirone will be acquired by a fast LC/TOF system on a short sub-two micron column. Simultaneously, the major metabolites can be identified from these fast LC/MS runs. Additionally, minor metabolites will be identified by the following automated use of a long sub-two micron column in a high resolution LC/MS run, which provides the necessary high resolution to separate out minor metabolite compounds.

2. Experimental

2.1. Equipment

The liquid chromatography system used was an Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies R&D and Marketing GmbH & Co. KG, Waldbronn, Germany) together with an Agilent 6210 ESI/TOF mass spectrometer (Agilent Technologies Inc., Santa Clara, California, CA, USA). The columns used were: (1) ZORBAX SB C18, 2.1 mm × 50 mm, 1.8 μm particle size (Agilent Technologies Inc., Wilmington, Delaware, DE, USA) and (2) ZORBAX SB C18 2.1 mm × 150 mm, 1.8 μm particle size (Agilent Technologies Inc.).

All chemicals, bio-reagents and solvents were purchased from Sigma/Aldrich (Taufkirchen, Germany).

2.2. System description

To perform the whole analysis both columns, the LC pump/autosampler as well as the diode array detector (DAD) and the ESI-TOF MS are connected to a 2-position/10-port valve, which is located in the column oven (Fig. 1). After injection of the sample onto the selected column the depending fast or high resolving gradient (method 1 or method 2) is applied to elute the compounds of the sample from the column. The contained parent drug and metabolites are detected with the DAD and the MS. For the fast LC method the next sample is drawn while the column regeneration is performed. To switch over to the other column and perform the other type of analysis the respective method is chosen. The complete analysis on both columns was performed automatically in a sequence of runs. The data were examined after the complete analysis was performed. The major metabolites can be identified from the fast LC separations used for the determination of the metabolic stability. The minor metabolites are determined from the high resolution LC run, which is applied to avoid coelution by better separation performance of the longer column.

2.3. Methods

(1) Fast LC method: solvent A—water + 0.025% TFA; solvent B, ACN + 0.025% TFA. Flow rate: 1 mL/min. Gradient: 0 min 15% B, 0.2 min 15% B, 1.2 min 95% B, 1.5 min 95% B. Stop time: 1.5 min. Post-time: 1 min. Sample injection volume 5 μL with needle wash and the samples were cooled to 4 °C in the injector. The automated delay volume reduction function of the autosampler was used to switch the injector loop volume out of the main flow path after sample injection. The overlapped injection function of the autosampler was used to draw the next sample while the current runs was in progress to save time between end of the run and start of the following. The DAD was used at 220 nm (±4), Ref. 360 nm (±4) with a 2 μL (10 mm path) flow cell. The column oven was operated at 50 °C and the 2-position/10-port valve (located in the oven) was set in the position for column 1.

(2) High resolution LC method: solvent A—water + 0.025% TFA; solvent B, ACN + 0.025% TFA. Flow rate: 0.5 mL/min. Gradient: 0 min 5% B, 0.2 min 5% B, 17 min 85% B, 17.1 min 95% B, 20 min 95% B. Stop time: 20 min. Post-time: 10 min. Sample injection volume 10 μL with needle wash and the samples were cooled to 4 °C in the injector. Automated delay volume reduction was used (see above). The DAD was used at 220 nm (±4), Ref. 360 nm

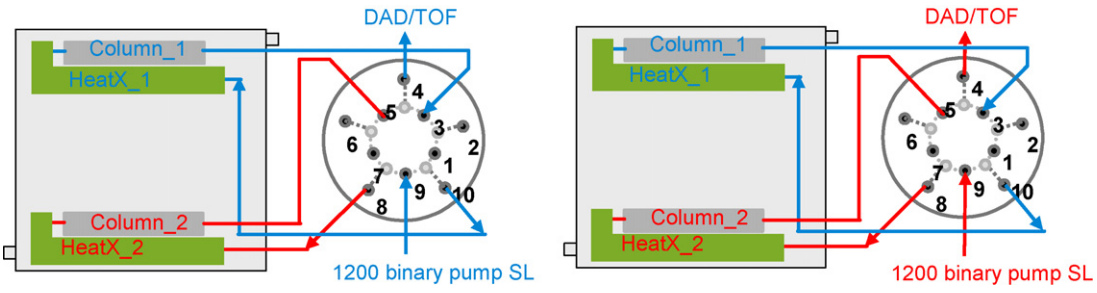

Fig. 1. Column switching with the 2-position/10-port valve in the column oven for fast LC/TOF with column 1 [blue] (2.1 mm × 50 mm, 1.8 μm particle size) and high resolution LC/TOF with column 2 [red] (2.1 mm × 150 mm, 1.8 μm particle size). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)