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Short communication

APPLICATION OF LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR DETECTING TERLIPRESSIN LEVELS IN RAT PLASMA

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ABSTRACT: This work describes a liquid chromatography– electrospray tandem mass spectrometry method for detection of terlipressin in Wistar rat plasma in the low nano-gram range. Terlipressin is a synthetic analogue of the antidiuretic hormone arginine vasopressin and it might be used when Arginine vasopressin (AVP) is not readily available. For experimental evaluation of the pharmacokinetics of terlipressin, rats can be injected 3.0 µg/kg or 6.0 µg/kg i.v. of terlipressin and blood can be collected for Mass spectrometry characterization of terlipressin which can be performed with a high-resolution Orbitrap-based mass spectrometer.

Keywords: LC-MS, Terlipressin, Rat plasma

INTRODUCTION

In some countries, AVP is not readily available, and thus terlipressin (TP), a synthetic, long-acting vasopressin analogue, is commonly considered as last resort therapy in the late phase of septic shock, when high dosages of catecholamines fail to counteract sepsis-related arterial hypotension (O'Brien et al 2002). Due to its long effective half-life of four to six hours, TP is commonly administered as high-dose bolus infusion (about 1 mg every four to six hours). The potential problem, however, is that TP bolus infusion may contribute to excessive vasoconstriction and a reflectory decrease in cardiac output with a proportional depression in oxygen delivery (Albanese et al 2005, Leone et al 2004). This may be especially problematic in a condition of increased oxygen demand, such as early sepsis (Westphal et al 2003). Notably, preliminary experimental and clinical reports have shown that TP may also be administered as low-dose continuous infusion, thereby mitigating, or even preventing such adverse events (Jolley et al 2003, Lange et al 2007). Liquid chromatography (LC)–mass spectrometry (MS) shows good sensitivity together with a high degree of selectivity and specificity to allow unequivocal confirmation of the presence of a substance on the basis of its molecular weight. Hence, LC-MS methods have become the method of choice for the analysis of peptides (Mihailova et al 2008). Until now, only one study using LC-MS to identify desmopressin has been published (Getie & Neubert, 2004). This study focuses on the possible use of LC-MS for estimation of Terlipressin (Morelli et al 2007).

Sample preparation and extraction:

Aliquot 300 µL of test sample to corresponding wells of a 48 or 96-well deep well plate**.** Add 50.0 µL working internal standard [600 ng/mLTerlipressin]**.** Add 250 µL of extraction buffer and mix well. Transfer samples from the 48-well plate WCX µElution plate which is pre-conditioned with 250 µL MeOH and 200 µL water. Wash with 300 µL 5% NH4OH and 400 µL Water. Elute samples with 2 x 30 µL of elution buffer. Dilute samples with 150 µL of water.

Mass spectrometry

LC/MS method development for detection of Terlipressin in rat plasma can be performed with a TSQ Quantum Discovery triple-stage quadrupole mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an ESI source operating in positive mode The ESI–MS operating variables can be used in the following manner: capillary voltage, 3.5 kV; source temperature, 350 °C; sheath gas pressure, 30 psi; auxiliary gas pressure, 10 psi; tube lens offset, 84 V.

However for MS characterization of terlipressin and related peptides can be performed with an Exactive benchtop Orbitrap-based mass spectrometer (Thermo Scientific, Bremen, Germany) operating in positive high-energy collisional dissociation (HCD) scan mode at 50 eV. The sheath gas must be set to 60 (arbitrary units), the auxiliary gas set to 30 (arbitrary units), and the capillary temperature set to 380 °C. The capillary voltage and spray voltage must be set to 30 V and 3 kV, respectively. The column used is a 4.6 x 250 mm Everest TM-C18 5µm cartridge, with a flow rate of 400uL/min. Initial conditions were 98% solvent A (0.1% formic acid in water)/2% solvent B (0.1% formic acid in acetonitrile) ramped to 25:75 in 2 minutes, returns to initial conditions at 3 minutes, and equilibrated for 2 minutes, for a total run time of 5 minutes. The injection volume should be 20uL. The most intense transition can be chosen for monitoring during sample analysis.The instrument was operated in full-scan mode from m/z 60 to m/z 1,200 at 100,000 resolving power. The data acquisition rate was 1 Hz.

DISCUSSION

The use of mass spectrometry has recently been reported for the detection of very low concentrations of oxytocin and vasopressin (Omar et al 2012, Thevis & Schanzer 2007). The use of liquid chromatography with mass spectrometry provides additional selectivity and sensitivity thereby which eliminating the need for time consuming sample preparation methods that are usually required for concentrating dilute samples (Figure 1). The separation and characterization of vasopressin and oxytocin and other peptides using reversed-phase liquid chromatography – mass spectrometry has been performed successfully, indicating the potential usefulness of this technique for the detection of oxytocin (Zhang et al 2011) Furthermore, the degradation of oxytocin/vasopressin and other peptides has also been studied (Mihailova et al 2008) using mass spectrometry and shows the application of this method to monitor oxytocin levels in pharmaceutical dosage forms. The fragmentation patterns of pituitary peptides were used to identify their degradation products. Karbiwnyk et al., (2008) used LC-MS to determine the concentration of oxytocin in dilute intravenous solutions. An LC-MS ion trap instrument with an electrospray ionization interface in a positive ion mode was used for the analysis. The isocratic method used an Agilent Zorbax® SB C_{18}, 5µm, 150 mm x 2.1 mm i.d., stationary phase with a mobile phase of 50% acetonitrile (v/v) and water containing 0.05% formic acid at a flow rate of 0.25 ml/minute. Under these conditions the limits of quantitation and detection were 7 and 2ng/ml, respectively. Terlipressin is a synthetic vasopressin analogue and is a 12 aminoacid peptide acting on V1a receptors and has prominent effects in the splanchnic area and increases mean arterial pressure. It is used in hepatorenal syndrome to improve renal function.In this study an injection of 3 µg/kg or 6 µg/kg i.v. can be done to ascertain the levels of the peptide in the plasma. In conclusion, this study describes a novel technique to ascertain the levels of terlipressin in the rat plasma.

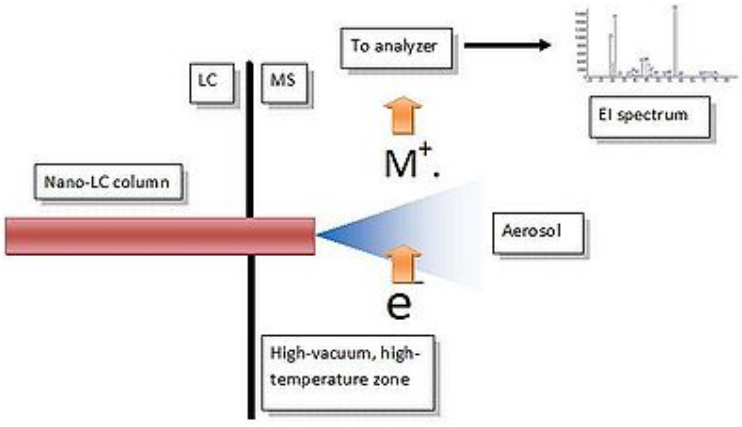

Figure 1: Schematic design of Liquid chromatography-Mass Spectrometry

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