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Direct injection human plasma analysis for the quantification of antihypertensive drugs for therapeutic drug monitoring using hydrophilic interaction liquid chromatography/electrospray ionization mass spectrometry

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ABSTRACT

The concept of personalized medicine is related to the development of new sensitive, precise and accurate analytical methods for therapeutic drug monitoring. In this article a rapid, sensitive and specific method was developed for the quantification of aliskiren, losartan, valsartan and hydrochlorothiazide in human plasma. Sample preparation was performed by protein precipitation with acetonitrile followed by filtration. All analytes and the internal standard (tiamulin) were separated by hydrophilic interaction liquid chromatography using an X-Bridge-HILIC analytical column (150.0 × 2.1 mm i.d., particle size 3.5 μm) under isocratic elution. The mobile phase was composed of a 10% 5 mM ammonium formate water solution pH 4.5, adjusted with formic acid, in acetonitrile and pumped at a flow rate of 0.25 mL min^{-1}. The assay was linear over the concentration range of 5–500 ng mL^{-1} for all the analytes. Intermediate precision was less than 5.2% over the tested concentration ranges. The method is the first reported application of HILIC in the analysis antihypertensives in human plasma. With a small sample size (50 μL human plasma) and a run time less than 6.0 min for each sample the method can be used to support a wide range of clinical studies and therapeutic drug monitoring.

1. Introduction

Hypertension increases the risk for a variety of cardiovascular diseases, including stroke, coronary artery disease, heart failure and peripheral vascular disease [1]. In the antihypertensive treatment clinical trials document that achieving blood pressure targets is usually not possible with a single agent. The majority of patients with hypertension require two or more agents, which either interfere with different pressure mechanisms or effectively block counter regulatory responses, so as to lower blood pressure effectively [2].

Aliskiren [3,4] is the first in a new class of orally active direct renin inhibitors that has been approved since 2007 for use in the treatment of hypertension. It is administered alone [5] or in combination with other antihypertensive agents, including thiazide diuretics like hydrochlorothiazide [6] or angiotensin receptor blockers like valsartan or losartan [7,8]. Hydrochlorothiazide is used in many cases as initial antihypertensive treatment. However, the addition of an agent acting on the renin-angiotensin system is a commonly used therapeutic strategy for patients with an unsatisfactory blood pressure response to hydrochlorothiazide [9].

The concept of personalized medicine along with the introduction of new drug combinations for therapy is related to the development of new sensitive, precise and accurate analytical methods for therapeutic drug monitoring. The purpose of this study was to develop a new method for the detection and identification of some of the most commonly prescribed antihypertensive drugs in human plasma.

Literature survey revealed only a few methods that have been applied to the determination of aliskiren in combination with other antihypertensive drugs in biofluids. These methods include,

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the determination of aliskiren alone by high performance liquid chromatography after derivatization with 1-naphthyl isocyanate using UV detection [10], a liquid chromatography-tandem mass spectrometric assay for aliskiren in human plasma [11] and in saliva [12]. Recently an ultra-performance liquid chromatographic tandem mass spectrometric method has been developed for the determination of aliskiren with prasugrel and rivaroxaban in human urine [13] Valsartan in combination with nebivolol has been determined in human plasma using a liquid chromatography-tandem mass spectrometric assay [14]. High performance liquid chromatography with UV detection [15] and liquid chromatography-tandem mass spectrometry [16] have been used for the determination of losartan in human plasma. Hydrochlorothiazide has been determined alone or in combination with other drugs in biofluids using various methods among which are hyphenated techniques such as liquid chromatography mass spectrometry [17,18] and liquid chromatography tandem mass spectrometry [19,20].

To the best of our knowledge, no bioanalytical assay has previously been developed for the simultaneous quantification of aliskiren, valsartan, losartan and hydrochlorothiazide in human plasma. In this paper, a direct injection LC-MS method suitable for therapeutic drug monitoring of these antihypertensive drugs in human plasma is presented using hydrophilic interaction liquid chromatography (HILIC) in combination with electrospray ionization mass spectrometry. HILIC chromatography was selected as an alternative chromatographic technique using polar stationary phases and low aqueous/high organic mobile phases in order to achieve effective retention of the polar analytes [21]. In HILIC the order of elution is reversed relative to reversed-phase chromatography, with hydrophilic compounds being retained longer than hydrophobic compounds [22,23]. The proposed method has been successfully validated and applied to real samples and it is suitable for therapeutic drug monitoring of these drugs.

2. Experimental

2.1. Chemical and reagents

Aliskiren hemifumarate and hydrochlorothiazide were kindly provided from Novartis Pharma AG (Basel, Switzerland). Losartan, valsartan and the internal standard (tiamulin) were obtained from Sigma–Aldrich, Germany. All solvents were of HPLC grade and were purchased from Merck, Darmstadt, Germany. Ammonium formate was obtained from Acros Organics (New Jersey, USA). Acrodisk® GHP membrane syringe filters (13 mm, pore size 0.45 μm) were obtained from Pall Life Sciences (Ann Arbor, MI, USA). Pooled drug-free human plasma was obtained from General Hospital "Korgialenio-Benakio National Red Cross, Athens, Greece.

2.2. Equipment

Experiments were performed using a Finnigan AQA single quadrupole mass spectrometer (Thermo-Quest, Manchester, UK) equipped with an electrospray ionization interface and a SpectraSeries P100 LC pump (SP ThermoSeparation, UK). A Nitrox-N2, model UPLC-MS12E, nitrogen generator, Domnick hunter (Gateshead, England) was used to provide highly pure nitrogen. Data acquisition and analysis were performed using Xcalibur software (v.1.2, ThermoQuest, Manchester,UK).

2.3. Chromatographic conditions

Hydrophilic interaction liquid chromatography was performed using an Xbridge HILIC analytical column (150.0 × 2.1 mm i.d., particle size 3.5 μm, 135 Å) under isocratic elution, (Merck-Se Quant, Darmstadt, Germany). A XBridge HILIC guard cartridge (20 × 2.1 mm, 3.5 μm) was used to prolong column lifetime. The mobile phase was consisted of 10% 5 mM ammonium formate water solution pH 4.5, adjusted with formic acid, in acetonitrile and pumped at a flow rate of 0.25 mL min^{-1}. Chromatography was performed at 25 ± 2 °C with a chromatographic run time of 6 min, 60-μL aliquots of samples were injected into a 20-μL loop.

2.4. Mass spectrometric conditions

The detection of aliskiren, losartan, valsartan and tiamulin (internal standard) was performed in electrospray positive ion mode, while hydrochlorothiazide was detected in electrospray negative ion mode. The ESI probe temperature was set at 260 °C, the cone voltage AQAmax was set at 20.0 V and the capillary voltage was set at 3.7 kV for both ESI positive and ESI negative ion mode. Data acquisition and analysis were performed using the Xcalibur (v.1.2) software. The SIM mode was chosen for the quantitative determination of the analytes. Different time windows presented in Table 1 were used to monitor the dominant mass peaks of the analytes.

2.5. Stock and working standard solutions

Stock standard solutions of aliskiren, valsartan, losartan and hydrochlorothiazide were prepared at 400 μg mL^{-1} in acetonitrile. The stock standard solutions were further diluted in acetonitrile to prepare a series of mixed working standard solutions of the analytes over the concentration range 50–5000 ng mL^{-1}. Stock standard solution of tiamulin (ISTD) was prepared at 500 μg mL^{-1} in acetonitrile. This solution was further diluted in the same solvent to prepare a working standard solution containing 250 ng mL^{-1} of tiamulin (ISTD). Tiamulin is a veterinary antibiotic showing similar chromatographic behavior with the analytes. This compound is not administered in humans thus it cannot be found in patient's plasma samples.

The stock standard solutions were stored in amber bottles at −20 °C and were found to be stable for at least one month. The working standard solutions were freshly prepared every week and stored at 4 °C.

2.6. Calibration spiked plasma standards and quality control samples

Aliquots of the mixed working standard solutions were further diluted in blank human plasma to obtain calibration spiked plasma

Table 1
Instrument method in SIM mode for the quantitative determination of aliskiren, losartan, valsartan and hydrochlorothiazide by HILIC-ESI/MS.

Time window (min)	Analyte	Polarity	Cone voltage (V)/capillary voltage (kV)	Selected ions (m/z)	Mass span
1.00–2.85	Hydrochlorothiazide	−ve	20/3.7	$[M-H]^-$:295.9	0.20
2.86–9.00	Losartan	+ve	20/3.7	$[M+H]^+$, $[M+K]^+$:423.2, 461.1	0.20
–	Valsartan	+ve	20/3.7	$[M+H]^+$, $[M+Na]^+$:436.5, 458.2	0.20
–	Tiamulin	+ve	20/3.7	$[M+H]^+$:494.3	0.20
–	Aliskiren	+ve	20/3.7	$[M+H]^+$:552.3	0.20