Simultaneous Determination of Therapeutic Drug Analytes in Human Plasma using LC/MS/MS

Application Note

Authors

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Abstract

Simultaneous determination of atenolol, amlodipine, clonidine, enalapril, lisinopril, furosemide, hydrochlorothiazide, losartan, valsartan, telmisartan, and canrenone (the active metabolite of spironolactone) in human plasma by liquid chromatography-mass spectrometry (LC/MS/MS) for clinical research has been described in this application note. The mass spectral parameters were optimized within a day using MassHunter Optimizer software, which identifies the most abundant product ions in a mass spectrum along with the most suitable fragmentor voltages and collision energies required for generating these product ions. A simple protein precipitation sample preparation technique was used to extract the eleven drug analytes from 100 µL of plasma. Linear calibration curves were achieved over the concentration range of 5 - 5000 ng/mL for all the target analytes in human plasma with good reproducibility.

Introduction

LC/MS/MS has become the technique of choice for analyzing exogenous substances in biofluids due to its high sensitivity and specificity. This application note describes the simultaneous analysis of 11 therapeutic drugs in human plasma using the Agilent 6410B Triple Quadrupole LC/MS coupled to an Agilent 1260 Infinity LC.

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Experimental

System

The Agilent LC/MS/MS system consisted of the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Degasser (G1379B)
- Agilent 1260 Infinity autosampler (G1367D)
- Agilent 1260 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 6410 Triple Quadrupole LC/MS (G6410B)
- Software
  MassHunter B.03.01 (Acquisition and Qualitative Analysis)
  MassHunter B.04.00 (Quantitative analysis)

Chemicals and reagents

Human plasma (lyophilized) and drug standards including atenolol, amlodipine, clonidine, furosemide, hydrochlorothiazide, enalapril, lisinopirl, and telmisartan were purchased from Sigma-Aldrich (Bangalore, India). Losartan, valsartan and canrenone were purchased from Varda Biotech (Mumbai, India). Acetonitrile, methanol, and formic acid were purchased from Fluka (India).

Preparation of aqueous drug standard mixtures

Stock solutions of each drug standard at 500 µg/mL concentrations were prepared in methanol. The eleven drug standard stock solutions were then combined and diluted in 9:1(v/v) water/methanol to prepare aqueous drug standard mixture solutions at concentrations of 5000 ng/mL and 10,000 ng/mL, respectively. These two drug standard mixture solutions were then serially diluted with 9:1 (v/v) water/methanol to obtain aqueous drug standard mixtures at concentrations of 5, 25, 50, 100, 250, 500, 1000, 2500, and 5000 ng/mL. This concentration range covers any anticipated therapeutic value.

Preparation of plasma calibration standards and blanks

To prepare the plasma calibration standards, 100 µL of human plasma was spiked with 100 µL of aqueous drug standard mixture in a microfuge tube. The concentrations of each analyte in the aqueous mixtures range from 5 - 5000 ng/mL. To prepare the plasma blank, 100 µL of plasma was spiked with 100 µL of 9:1(v/v) water:methanol. All plasma samples were treated with 500 µL of cold acetonitrile and then vortexed for 1 min. From the supernatant solutions, 695 µL was transferred to new microfuge tubes and dried by vacuum concentration. The residues were resuspended in 20 µL of 1:1 (v/v) methanol:water, and then further diluted to 100 µL with water.

Chromatographic conditions

Mobile phases	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	
Flow rate	0.35 mL/min	
Gradient	**Time (min)**	**% B**
	0.0	6
	2.0	18
	7.0	98
	7.5	98
	8.0	6
	9.0	6
	Stop time: 9.0 min	
	Post time: 1.0 min	
Injection volume	10 µL	
Needle wash	10s wash port with 1 : 1 methanol:water mix containing 0.1% formic acid	
Column	Agilent ZORBAX RRHT Eclipse Plus - C18 2.1 X 50 mm, 1.8 µm (P.N. 959741-902)	
Column temperature	40 °C on both sides	

Mass spectrometric conditions

Source parameters:

Ionization mode	ESI (+ /-); polarity switching
Gas temperature	325 °C
Gas flow	10 L/min
Nebulizer pressure	40 psi
Capillary voltage	4000 V (+) and 3500 V (-)
Delta EMV	300 V (+) and 300 V (-)

MRM transitions:

Precursor ion (*m/z*)/ (Ionization Mode)	Product ion (*m/z*)	Fragmentor voltage (V)	Collision energy (eV)	Dwell time (ms)
Telmisartan 515.2/ (+)	497.3 (Qual)	160	40	20
	276.1 (Quant)	155	50	20
Valsartan 436.2/ (+)	235 (Qual)	98	14	20
	291.1 (Quant)	98	14	20
Losartan 423.2/ (+)	206.6 (Qual)	120	38	20
	180 (Quant)	120	42	20
Amlodipine 409.2/ (+)	294.1 (Qual)	90	9	20
	238.2 (Quant)	95	9	20
Lisinopril 406.2/ (+)	245.9 (Qual)	136	20	20
	84.1 (Quant)	136	30	20
Enalapril 377.2/ (+)	234.1 (Qual)	106	6	20
	303.1 (Quant)	106	14	20
Canrenone 341.2/ (+)	107.1 (Quant)	150	30	20
Atenolol 267.2/ (+)	190 (Qual)	120	14	20
	145 (Quant)	120	26	20
Clonidine 230/ (+)	213.2 (Qual)	125	25	20
	44.1 (Quant)	130	30	20
Hydrochlorothiazide 296/ (-)	268.9 (Quant)	130	10	20
	204.7 (Qual)	130	15	20
Furosemide 329.3/ (-)	205 (Quant)	105	10	20

Results

Chromatographic separation was optimized for the target compounds. Selectivity and sensitivity was achieved by measuring each analyte in multiple reaction monitoring (MRM) mode. MassHunter Optimizer was used to identify the most abundant MRM transitions for all the target compounds. The software also provided two important MS parameters associated with the selected MRM transitions – the fragmentor voltages and collision energies. Thus, mass spectral parameters could be readily optimized within a day. A common set of electrospray ioniziation (ESI) source parameters were used for all of the target compounds. It was found that nine out of the eleven analytes showed good signal responses in the ESI positive ion mode, while the other two analytes, hydrochlorothiazide and furosemide, showed improved signal responses in the ESI negative ion mode. Compounds ionizing in different modes can be analyzed in a single run by using the fast polarity switching feature of the Agilent 6410B Triple Quadrupole LC/MS.

A simple protein precipitation method was used to extract plasma samples. This method is quick and amenable to clinical research use. Optimal separation of the selected compounds was achieved within a run time of less than 10 minutes (**Figure 1**).

All calibration curves were generated with linear curve fitting and were weighted (1/x). A linear dynamic range of 5 - 5000 ng/mL was achieved for all analytes with an R^2 value greater than 0.9. **Figure 2** shows four representative calibration curves. Since spironolactone is converted to its active metabolite, canrenone, in solution, canrenone was used for the development of calibration curves.

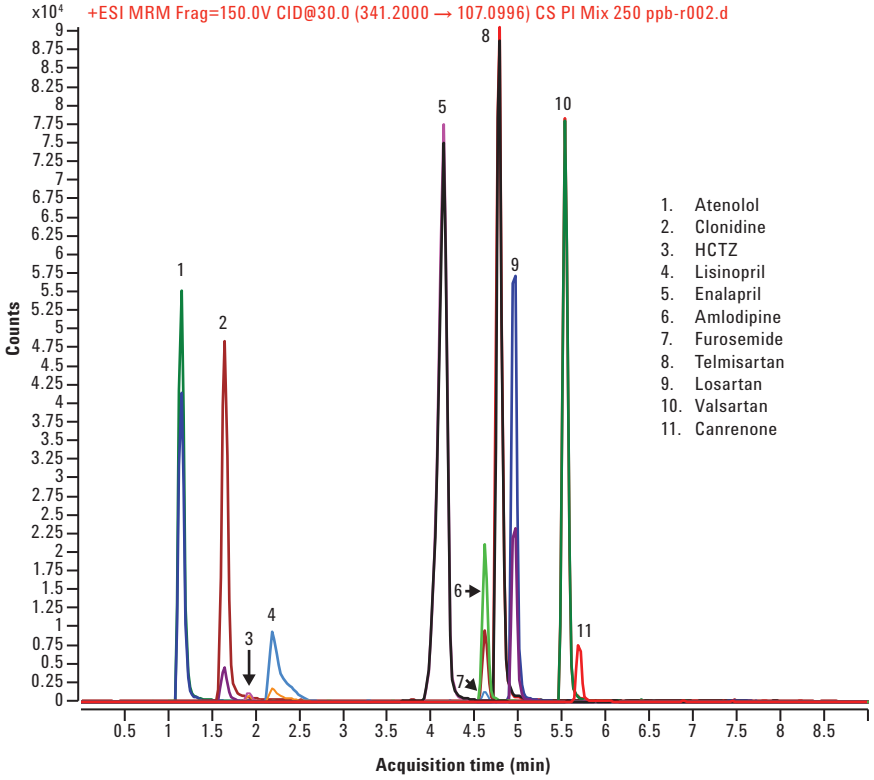

Figure 1. MRM chromatogram of 250 ng/mL calibration standard.

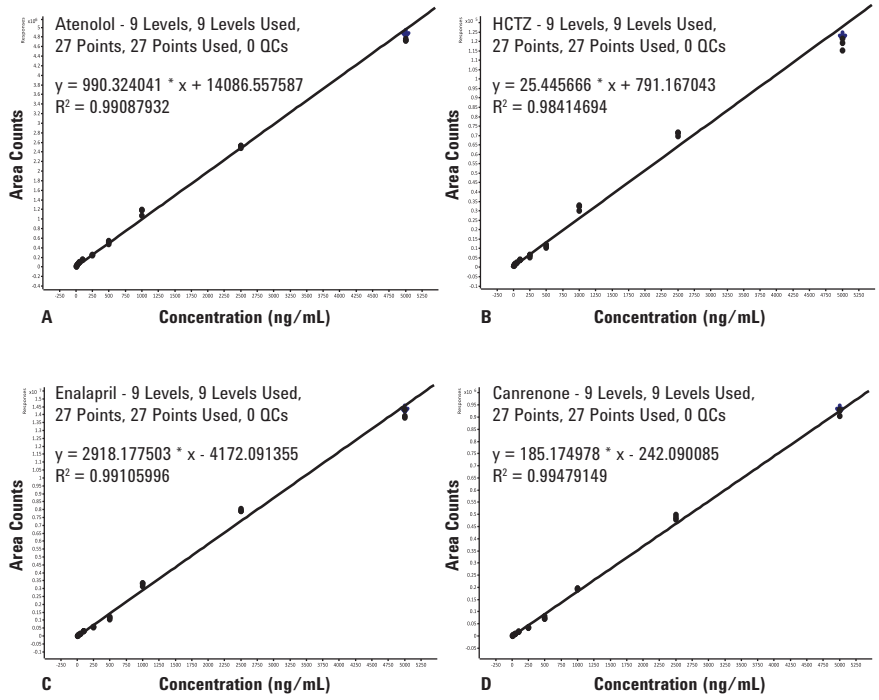

Figure 2. Calibration curves of a) atenolol, b) hydrochlorothiazide, c) enalapril and d) canrenone

Conclusions

In this application note, we have developed a sensitive and selective LC/MS/MS method for the determination of certain therapeutic drug analytes in human plasma using the Agilent 6410B Triple Quadrupole LC/MS coupled to an Agilent 1260 Infinity LC. Rapid LC/MS/MS method development was achieved by using MassHunter Optimizer software, which identified the most abundant MRM transitions, as well as the associated fragmentor voltages and the collision energies. The fast polarity switching feature of the mass spectrometer enables the analysis of compounds ionizing in either positive or negative ion mode in a single run. Good reproducibility for each analyte and broad linear dynamic ranges over 5-5000 ng/mL were demonstrated for all compounds.

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