Determination of fluvastatin in plasma using the Agilent 6410B Triple Quadrupole LC/MS system coupled with the Agilent 1200 Series Rapid Resolution LC system

Application Note

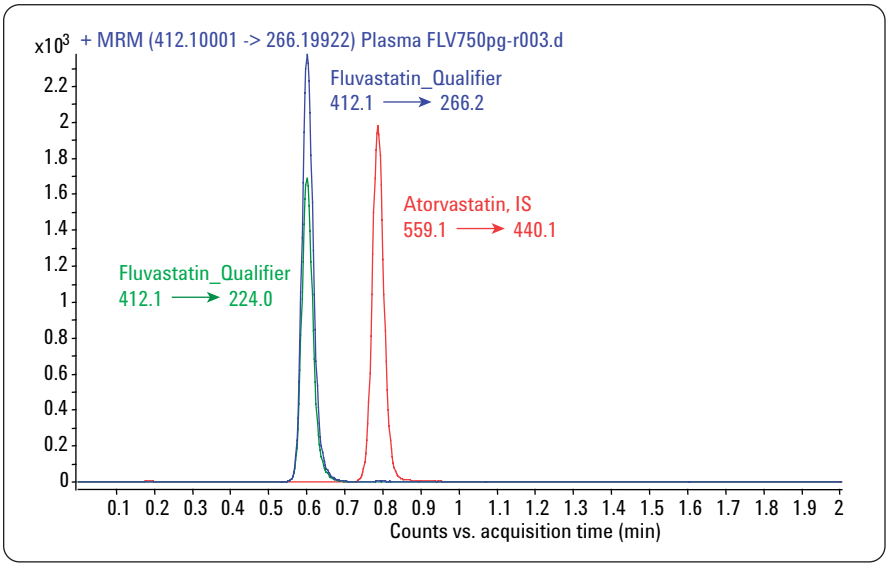
Drug Development

Author

Srividya Kailasam

Agilent Technologies

Bangalore, India



Abstract

This Application Note describes a rapid and sensitive method for quantification of fluvastatin in plasma using the Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system and the Agilent 6410 Triple Quadrupole LC/MS system. Sample preparation was carried out by a simple protein precipitation step. Atorvastatin at 40 ng/mL was used as the internal standard. The developed method had a short run time of two minutes and was found to be linear in the concentration range of 0.5 ng/mL to 200 ng/mL. The limit of detection (LOD) of the method is 0.2 ng/mL, or 1 pg on-column.

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Introduction

During drug development, pharmacokinetic studies are performed to understand the fate of a drug in the body and its therapeutic efficacy. To achieve this objective, the drug under study is administered to select healthy volunteers at an appropriate amount and in suitable dosage form. Subsequently, its concentration is measured in a biological matrix such as plasma, which is collected at regular time intervals. Plasma drug concentrations are plotted as a function of time to determine the various pharmacokinetic parameters, such as maximum concentration of the drug in the body following dosing and the time required to reach the maximum concentration.

Fluvastatin belongs to a class of compounds used in the treatment of hypercholesterolemia. Although this water-soluble statin is known to be readily absorbed by the body, it has low bioavailability due to extensive first-pass metabolism. Hence, very sensitive analytical techniques are required to detect and quantify small amounts of fluvastatin found in plasma during pharmacokinetic studies. Tandem mass spectrometry (MS/MS) is a popular choice for this purpose, as it is not only specific but can achieve high sensitivity.

In the literature, only a few analytical techniques such as electrospray HPLC/MS/MS[1,2] and fluorimetry[3] have been reported for quantification of fluvastatin in plasma. This Application Note describes a rapid and sensitive method with an easy sample preparation step for high-throughput detection and quantification of fluvastatin in plasma using the Agilent 6410B Triple Quadrupole LC/MS system coupled with the Agilent 1200 Series Rapid Resolution LC system. In this method, atorvastatin,[4] which is structurally similar to fluvastatin, was used as the internal standard (IS).

Experimental

Chemicals

Fluvastatin from Calbiochem, atorvastatin from Molekula UK Ltd., and human plasma sample from Sigma-Aldrich were used in the study. Double-distilled water was obtained from a Millipore Milli-Q water purification system. HPLC-grade acetonitrile, methanol, and ammonium acetate were purchased from Merck.

LC/MS/MS instrumentation

The LC/MS/MS system used in this work incorporated an Agilent 1200 Series Rapid Resolution LC system consisting of:

- Agilent 1200 Series vacuum degasser
- Agilent 1200 Series binary pump SL
- Agilent 1200 Series high performance autosampler SL with thermostat
- Agilent 1200 Series thermostatted column compartment

The system also included an Agilent 6410B Triple Quadrupole LC/MS system equipped with an electrospray (ESI) source. The Agilent MassHunter Workstation software version B.01.03 was used for data acquisition and qualitative analysis, and version B.01.04 was used for quantitative analysis.

LC/MS/MS method details

The LC conditions are given in table 1, while the MS conditions are given in table 2. Table 3 lists the MS parameters for the analysis of the target compound and the internal standard by multiple reaction monitoring (MRM).

Sample preparation

Fluvastatin and the internal standard atorvastatin were spiked into 250 µL plasma. The spiked sample was treated with 750 µL of acetonitrile and vortexed for 30 seconds to precipitate the proteins. After centrifugation at 15000 rpm for 15 minutes, the supernatant solution was transferred to a clean Eppendorf tube and evaporated to dryness using a SpeedVac concentrator. The residue was reconstituted in a mix of 70 % mobile phase A and 30 % mobile phase B. To remove suspended particulates, the solution was once again centrifuged at 15000 rpm for 10 minutes. The supernatant was carefully transferred to an HPLC vial for analysis. The internal standard concentration was maintained at 40 ng/mL.

Results and discussion

LC conditions were optimized to obtain a short run time and adequate resolution between fluvastatin and the internal standard atorvastatin. MS parameters, such as fragmentor voltage, colli-

Parameters	Detail
Column	Agilent ZORBAX Eclipse XDB-C18, 2.1 x 30 mm, 1.8 µm particle size (p/n 924700-902)
Column temperature	40 °C
Mobile phase	A = 10 mM ammonium acetate in water B = 90 % acetonitrile and 10 % ammonium acetate (10 mM)
Flow rate	0.5 mL/min
Gradient conditions	at 0 min, 40 %B; at 0.3 min, 95 %B; from 0.3 min to 0.6 min, 95 %B; from 0.61 min to 2 min, 40 %B
Run time	2 min
Injection volume	5 µL
Needle wash	10 s in wash port with 1:1 methanol:water mix

Table 1
LC conditions.

Parameters	Detail
Ionization mode	ESI – positive
Drying gas temperature	300 °C
Drying gas flow	12 L/min
Nebulizer	60 psig
Capillary voltage	5500 V
Delta EMV	500 V
Q1 resolution	Unit
Q2 resolution	Wide

Table 2
MS conditions.

	Transition	Fragmentor voltage (V)	Collision energy (V)	Dwell time (ms)
Fluvastatin_Quantifier	412.1 → 266.2	135	15	50
Fluvastatin_Qualifier	412.1 → 224.0	135	20	50
Atorvastatin (IS)	559.1 → 440.1	180	20	50

Table 3
Parameters used for acquiring MRM transitions.

sion energy, dwell time, drying gas temperature and flow rate, nebulization pressure, and capillary voltage were optimized to obtain maximum sensitivity. Figure 1 shows good baseline separation between the two peaks within a short run time of two minutes, which includes column equilibration time. The absence of fluvastatin or atorvastatin peaks in the total ion chromatograms (TICs) of blank plasma samples (as can be seen in figure 2) establishes the specificity of the method.

The developed method was then applied to evaluate linearity in plasma. Standard solutions of fluvastatin and atorvastatin were spiked into 250 µL of plasma to obtain final concentrations in the linear range of 0.2 to 200 ng/mL. The following samples were prepared along with these calibration standards:

- A double-blank plasma sample containing neither fluvastatin nor the IS
- A blank plasma sample containing only IS
- Three quality control (QC) samples at 1.6, 16, and 160 ng/mL fluvastatin in plasma

The concentration of the IS was maintained at 40 ng/mL for all samples. The target analyte and IS were extracted from plasma by protein precipitation as described in the Experimental section of this Application Note. Five injections were made for each blank, calibration standard, and QC sample.

For plasma samples, the method achieved a limit of detection (LOD) of 0.2 ng/mL and a limit of quantification (LOQ) of 0.5 ng/mL. Linearity was demonstrated up to 200 ng/mL. An example of a standard curve in plasma is shown in figure 3. The method was evaluated for linearity on three different days with freshly prepared plasma samples and was found to be linear for the three independent trials with an r^2 value greater than 0.995.

The Agilent MassHunter Workstation Quantitative Analysis software back-calculated the concentrations of calibration standards using the linear

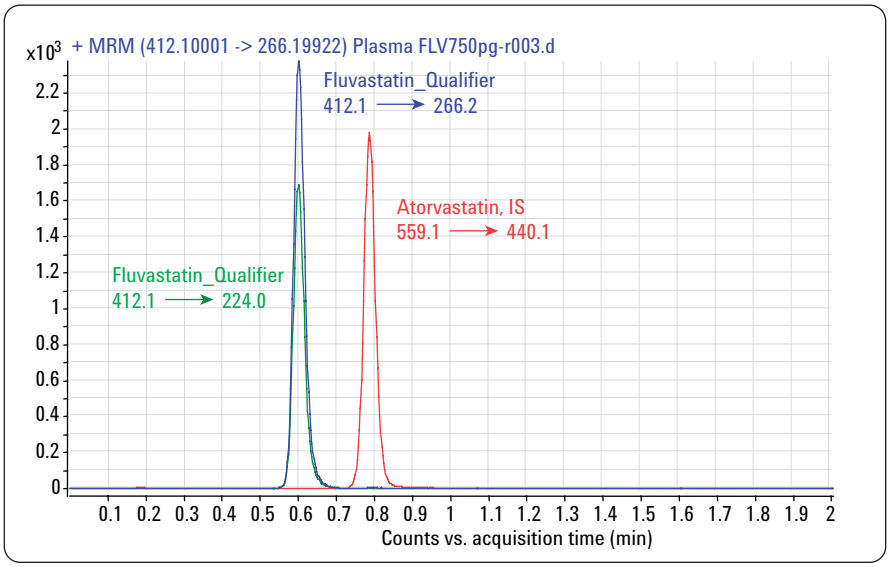

Figure 1
Overlay of MRM transitions of fluvastatin (150 ng/mL, quantifier and qualifier) and atorvastatin IS (40 ng/mL) in plasma.

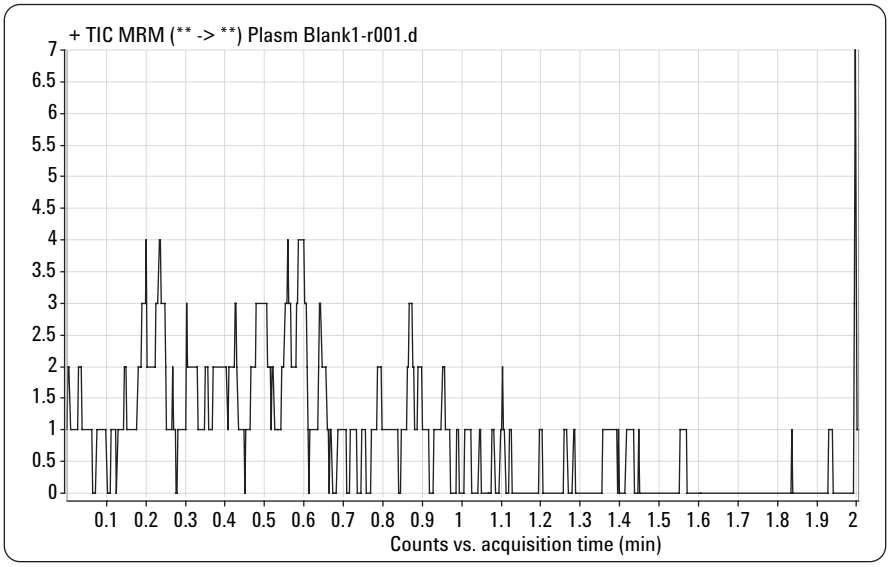

Figure 2
TIC of a double-blank plasma sample.

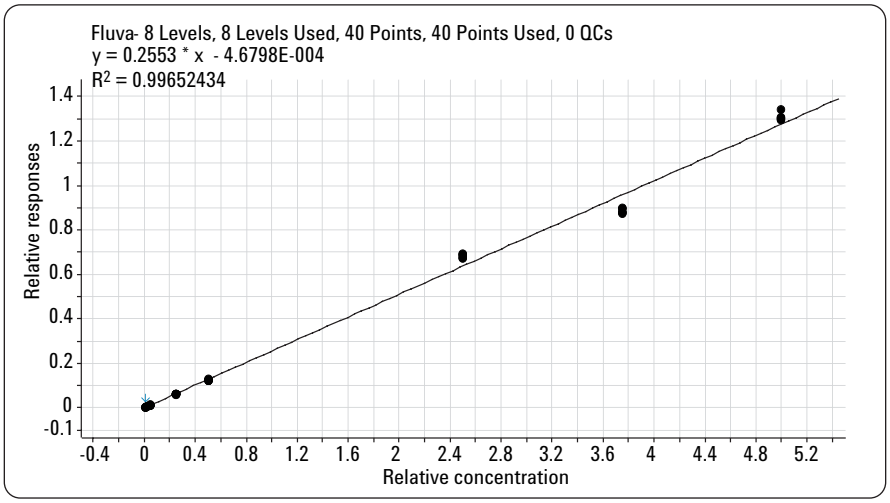

Figure 3
Standard curve in plasma.

regression of peak area ratios versus analyte-to-IS concentration ratios. The day-to-day variations in calculated concentrations were determined by three independent trials on three consecutive days (presented in table 4). Results were compared with guidelines set by the United States Food and Drug Administration (US-FDA) for bioanalytical method development.[5] It was observed that the accuracy at 0.2 ng/mL was insufficient to meet the FDA-approved limit of ± 20 % deviation at the lower limit of quantification (LLOQ). Hence, this concentration of 0.2 ng/mL was set as the LOD of the method. Accuracy at all other levels and for QC standards was found to be within the prescribed limit of ± 15 %. Precision at 0.5 ng/mL was within the ± 20% limit, which is acceptable at the LLOQ according to the US-FDA guidelines.

Fluvastatin concentration (ng/mL)	Day 1 (ng/mL)	Day 2 (ng/mL)	Day 3 (ng/mL)	Average (ng/mL)	CV* (%)	Accuracy (%)
0.2	0.27	0.33	0.30	0.30	10.35	149.40
0.5	0.46	0.60	0.45	0.50	16.75	100.51
1	0.99	1.01	0.85	0.95	9.12	95.42
2	1.63	1.68	1.80	1.70	4.99	85.23
10	9.32	9.63	9.51	9.49	1.62	94.86
20	19.52	18.76	19.46	19.25	2.20	96.23
100	102.95	96.71	107.12	102.26	5.12	102.26
150	145.40	147.28	138.94	143.87	3.04	95.91
200	203.16	206.58	205.28	205.01	0.84	102.50
1.6 (QC)	1.58	1.52	1.39	1.50	6.41	93.51
16 (QC)	13.63	15.83	17.52	15.66	12.46	97.85
160 (QC)	166.53	154.92	160.10	160.52	3.63	100.32

Table 4
Day to day variability in plasma.

*CV = coefficient of variation

Conclusion

An LC/MS/MS method was developed for detection and quantification of fluvastatin in plasma. A simple protein precipitation technique was used for sample preparation. The method has a short run time of two minutes, making it suitable for high-throughput applications. Excellent resolution was observed between the target analyte fluvastatin and the IS atorvastatin. In plasma, the method exhibits linearity over nearly four orders of magnitude with an LOQ of 0.5 ng/mL. Precision and accuracy at all calibration levels were found to be within the FDA-recommended limit for bioanalytical methods.

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