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Application News

No. AD-0104

Quantitative Bioanalysis / LCMS-8060

LC/MS/MS High Sensitivity Bioanalytical Method: Atorvastatin Calcium in Human Plasma

❑ Introduction

Atorvastatin Calcium is under a drug class known as statins, widely used for the treatment of severe hypercholesterolemia by lowering blood cholesterol and prevention of events related to cardiovascular disease [1]. It works through inhibiting HMG-CoA reductase found in liver tissue which plays a part in production of cholesterol in the body [2,3]. Bioanalyses of atorvastatin in human plasma have been reported in pharmacokinetics and bioequivalence studies, which are carried out mostly on LC/MS/MS with liquid-liquid extraction (LLE) or solid phase extraction (SPE) method for high sensitivity and selectivity [1-3]. The reliability of a bioassay depends on the performance of LC/MS/MS system employed and the method of sample pre-treatment. With rapid progress of interface and triple quadrupole MS techniques, simpler sample pre-treatment without SPE may be adopted to achieve not only reliable quantitative results, but also higher throughput and lower running cost. In this Application News, a simple, fast and highly sensitive method for quantitative determination of atorvastatin in human plasma is described. The LC/MS/MS used is LCMS-8060 with a heated ESI, coupled with UHPLC Nexera X2 (Figure 1). The high sensitivity and robust interface design of the system allow the use of protein-precipitation only in plasma pre-treatment and achieve a LLOQ of 40 pg/mL.

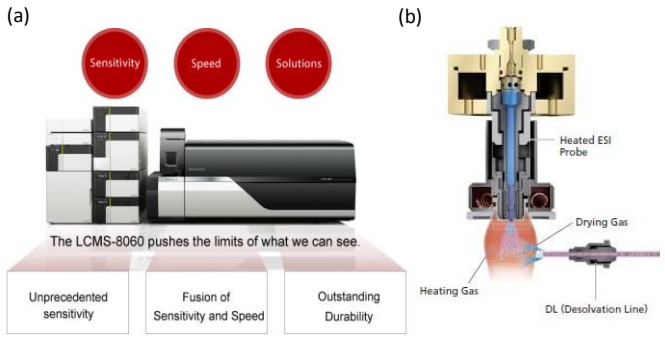

Figure 1: Shimadzu LCMS-8060 system (Left) and Heated ESI Interface (right).

❑ Experimental

Instrumental and analytical conditions

A Nexera X2 UHPLC system coupled with LCMS-8060 system (Shimadzu Corporation) was employed and controlled by LabSolutions workstation. The details of column, mobile phases and gradient programs of LC separations and MS conditions are compiled into Table 1.

Standards and samples

Pooled human plasma was obtained from i-DNA Biotechnology Pte Ltd. Atorvastatin calcium was obtained from Sigma Aldrich. The stock solution of atorvastatin calcium was prepared from high purity solid chemicals in methanol. Calibrants were prepared from the stock solution using a mixed diluent (water/methanol/acetonitrile in ratios of 2:3:3, vol/vol/vol). The sample pre-treatment without SPE or other cleanup method but only simple protein precipitation steps were shown in Figure 2.

Table 1: LC/MS/MS analytical conditions of Atorvastatin on LCMS-8060

UHPLC condition:

Column	Phenomenex Synergi 2.5u Polar-RP (100 mmL. x 2.00mm I.D.)
Flow Rate	0.3 mL/min
Mobile Phase	A: 0.1% formic acid in Water B: 0.1% formic acid in Acetonitrile
Elution Mode	Gradient elution, LC program 8.0 minute: 10% (0.0 to 1.0min) ➔ 95% (4.0 to 5.5min) ➔ 10% (6.0 to 8.0min)
Oven Temperature	40°C
Injection Volume	1.0 µL

MS Interface condition

Interface	ESI
MS mode	Positive
Block Temperature	400°C
Interface Temp.	350°C
DL Temperature	250°C
CID Gas	Ar (270kPa)
Nebulizing Gas Flow	N_2, 2.0L/min
Drying Gas Flow	N_2, 10.0L/min
Heating Gas Flow	Zero Air, 10.0L/min

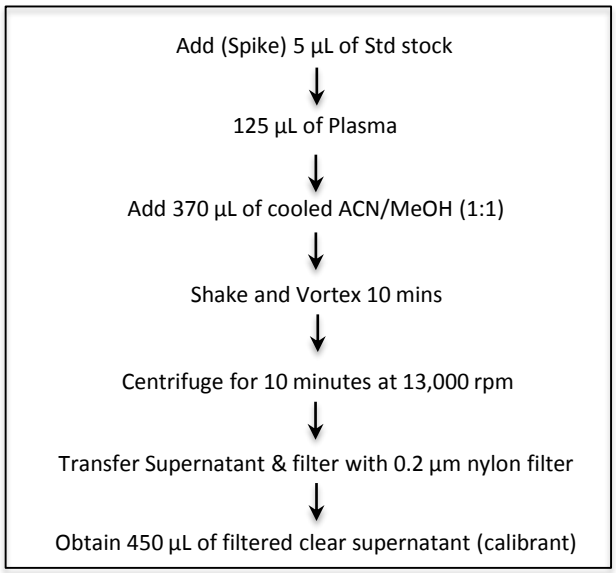

Figure 2: Flow chart of sample pre-treatment of human plasma with pre-spiked atorvastatin. Post-spiked samples are prepared in similar procedure by adding stock standard at last step.

❑ Results and Discussion

Development of LC/MS/MS method

A MRM method was established on LCMS-8060 with optimized MRM parameters as shown in Table 2. Atorvastatin was eluted as sharp peaks under the gradient conditions as shown in Figure 3(a) and 3(b). The first MRM transition was used to establish calibration method. The second and third MRMs were used as reference ions for identification.

Table 2: MRMs and CID parameters of Atorvastatin on LCMS-8060

Name	RT (min)	MRM Transition (m/z)		CID Voltage (V)		
		Precursor $[M+H]^+$	Product	Q1 Pre Bias	CE	Q3 Pre Bias
Atorvastatin	4.398	559.25	440.25	-20	-24	-30
			250.30	-20	-42	-28
			276.20	-20	-44	-19

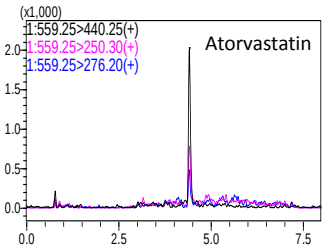

Figure 3a: MRM chromatograms of atorvastatin (50 pg/mL) post-spiked in plasma

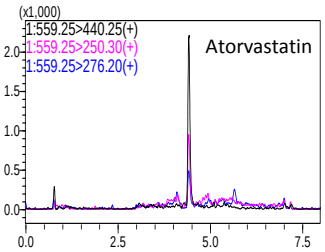

Figure 3b: MRM chromatograms of atorvastatin (50 pg/mL) pre-spiked in plasma

Calibration curve, linearity and accuracy

A quantification method for atorvastatin in plasma was set up based on the first MRM transition of atorvastatin in Table 2. Linear calibration curves were established by external standard calibration method for plasma samples prepared by post-spiked procedures displayed in Figure 4. Excellent linearity was obtained with an R^2 coefficient greater than 0.999 across the range from 10.0 pg/mL to 5000 pg/mL. The details of the calibrant series, RSD% and accuracy in triplicate measurements are shown in table 3.

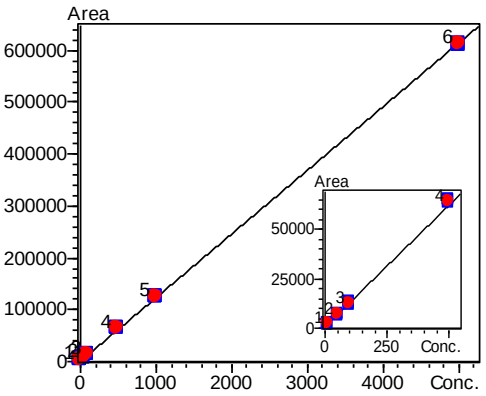

Figure 4: Calibration curve of atorvastatin by post-spiked procedure in human plasma, ranging from 10 pg/mL ~ 5000 pg/mL.

Table 3: Calibrant series and performance of calibration method for quantitation of atorvastatin in plasma (post spiked)

Level[1]	Conc. Cal. (pg/mL)	Conc. Mea. (pg/mL)	RSD% (n=3)	Average Acc. (%)
1	10	9.9	10.6	99.5
2	50	49.6	6.8	99.2
3	100	97.3	6.2	97.3
4	500	517.3	1.4	103.5
5	1000	1010.1	1.0	101.0
6	5000	4975.7	0.4	99.5

1. Triplicate injections of each level

Evaluation of method performance

The LLOQ could be measured as the sample blank is clean as shown in Figure 5. The LLOQ of the method was determined to be 10.0 pg/mL (S/N~10) with post-spiked plasma sample (Figure 6). It can be confirmed that the LLOQ of the method is at 10 pg/mL in plasma solution and 40 pg/mL in plasma before adding three volumes of acetonitrile for protein precipitation (dilution factor of sample preparation: 4.0).

The accuracy and precision of the method were evaluated with the QC1 (low), QC2 (med) and QC3 (high) samples. The results are compiled into Table 4. The accuracy and RSD% obtained for the QCs are within the acceptable range of ±20% and <15% respectively. The six MRM chromatograms of consecutive injections of QC1 are plotted into Figure 7, which shows impressively the excellent reproducibility of the analysis on LCMS-8060.

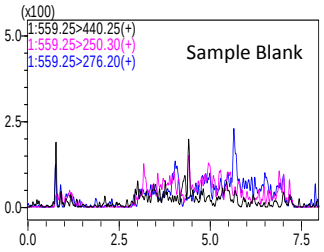

Figure 5: MRM chromatograms of blank sample (plasma)

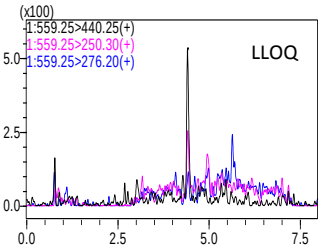

Figure 6: MRM chromatograms of atorvastatin (10 pg/mL, S/N ~10) post-spiked in plasma

Table 4: Performance evaluation of quantitation method for Atorvastatin in plasma with QC samples (n=6)

Sample	Conc. Cal. (pg/mL)	Ave. Conc. (pg/mL)	Accuracy (%)	RSD (%)
QC1 (low)	10	8.7	87	8.5
QC2 (med)	100	103.7	103.7	5.4
QC3 (high)	5000	5270.0	105.4	6.5

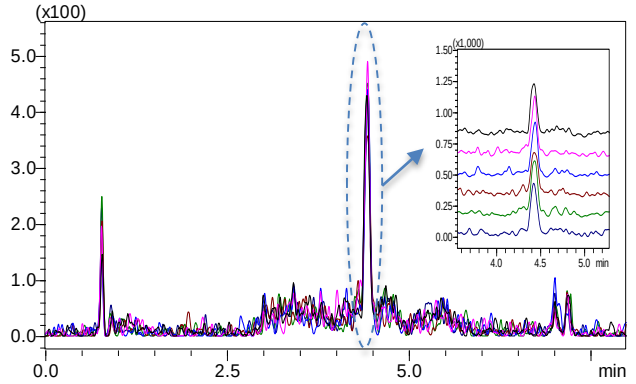

Figure 7: Overlay display of six chromatograms of QC1 (10.0 pg/mL atorvastatin post-spiked in plasma)

Table 5: Performance evaluation of quantitation method for Atorvastatin in plasma, recovery and matrix effect

Concentration (pg/mL)	10	50	100	500	1000	5000
Recovery %	109.6	116.2	115.6	109.0	111.9	112.3
Matrix effect %	46.8	34.8	33.3	34.6	35.8	35.7

The recovery efficiency and matrix effect were investigated. The results were tabulated in Table 5. The recovery percentage was within the ±20%. However, there was ion suppression found in the matrix with an average matrix effect of 36.8%. It is worth to note that the sample pre-treatment of current includes only protein precipitation without any further clean-up. The high sensitivity and good performance of the method are due to the excellent performance of the LCMS-8060.

❑ Conclusions

A high sensitivity bioanalytical method for determination of atorvastatin in human plasma was established on LCMS-8060 with a heated ESI. The method allows the use of simple sample pre-treatment, achieving high sensitivity and reliable performance required for bioanalysis. The LLOQ of the method in spiked plasma samples was 10 pg/mL.

❑ References

[1] Jiang Wang et al, "Liquid chromatography–tandem mass spectrometry assay for the simultaneous quantification of simvastatin, lovastatin, atorvastatin, and their major metabolites in human plasma" Journal of Chromatography B, 983–984 (2015) 18–25

[2] Ying Zhou et al, "Development and validation of a liquid chromatography–tandem mass spectrometry method for simultaneous determination of amlodipine, atorvastatin and its metabolites ortho-hydroxy atorvastatin and para-hydroxyatorvastatin in human plasma and its application in a bioequivalence study" Journal of Pharmaceutical and Biomedical Analysis 83 (2013) 101– 107

[3] Pankaj Partani et al, "Simultaneous quantitation of atorvastatin and its two active metabolites in human plasma by liquid chromatography/(–) electrospray tandem mass spectrometry" Journal of Pharmaceutical Analysis 2014;4(1):26–36

Note: The method and data shown in this application notes are for research use only. Not for Use in Diagnostic Procedures.

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