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Highly sensitive quantitative analysis of Felodipine and Hydrochlorothiazide from plasma using LC/MS/MS

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Introduction

Felodipine is a calcium antagonist (calcium channel blocker), used as a drug to control hypertension[1]. Hydrochlorothiazide is a diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water. It is, therefore, frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis and the prevention of kidney stones[2].
Efforts have been made here to develop high sensitive methods of quantitation for these two drugs using LCMS-8050 system from Shimadzu Corporation, Japan. Presence of heated Electro Spray Ionization (ESI) probe in LCMS-8050 ensured good quantitation and repeatability even in the presence of a complex matrix like plasma. Ultra high sensitivity of LCMS-8050 enabled development quantitation method at low ppt level for both Felodipine and Hydrochlorthiazide.

Felodipine

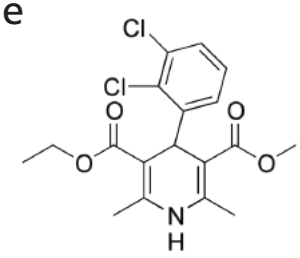

Figure 1. Structure of Felodipine

Felodipine is a calcium antagonist (calcium channel blocker). Felodipine is a dihydropyridine derivative that is chemically described as ± ethyl methyl 4-(2,3-dichlorophenyl)1,4-dihydro-2,6-dimethyl-3,5-pyridin edicarboxylate. Its empirical formula is $C_{18}H_{19}Cl_2NO_4$ and its structure is shown in Figure 1.

Hydrochlorothiazide

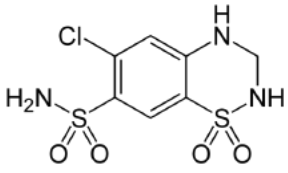

Figure 2. Structure of Hydrochlorothiazide

Hydrochlorothiazide, abbreviated HCTZ (or HCT, HZT), is a diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water.
Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide.Its empirical formula is $C_7H_8ClN_3O_4S_2$ and its structure is shown in Figure 2.

Method of Analysis

Preparation of matrix matched plasma by protein precipitation method using cold acetonitrile

To 100 µL of plasma, 500 µL of cold acetonitrile was added for protein precipitation then put in rotary shaker at 20 rpm for 15 minutes for uniform mixing. It was centrifuged at 12000 rpm for 15 minutes. Supernatant was collected and evaporated to dryness at 70 °C and finally reconstituted in 200 µL Methanol.

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Preparation of matrix matched plasma by liquid-liquid extraction method using diethyl ether and hexane mixture (1:1 v/v)

To 500 µL plasma, 100 µL sodium carbonate (1.00 mol/L) and 5 mL of diethyl ether : hexane (1:1 v/v) was added. It was placed in rotary shaker at 20 rpm for 15 minutes for uniform mixing and centrifuged at 12000 rpm for 15 minutes. Supernatant was collected and evaporated to dryness at 60 °C. It was finally reconstitute in 1000 µL Methanol.

Preparation of calibration standards in matrix matched plasma

Response of Felodipine and Hydrochlorothiazide were checked in both above mentioned matrices. It was found that cold acetonitrile treated plasma and diethyl ether: hexane (1:1 v/v) treated plasma were suitable for Felodipine and Hydrochlorothiazide molecules respectively. Calibration standards were thus prepared in respective matrix matched plasma.

- Felodipine Calibration Std : 5 ppt, 10 ppt, 50 ppt, 100 ppt, 500 ppt, 1 ppb and 10 ppb
- HCTZ Calibration Std : 2 ppt, 5 ppt, 10 ppt, 50 ppt, 100 ppt, and 500 ppt

Figure 3. LCMS-8050 triple quadrupole mass spectrometer by Shimadzu

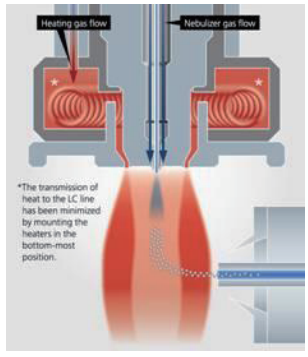

Figure 4. Heated ESI probe

LCMS-8050 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 3), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), Ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msecs (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 4) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

LC/MS/MS analysis

Compounds were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8050 triple quadrupole system (Shimadzu Corporation, Japan), The details of analytical conditions are given in Table 1 and Table 2.

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Table 1. LC/MS/MS conditions for Felodipine

• Column	: Shim-pack XR-ODS (75 mm L x 3 mm I.D.; 2.2 µm)
• Flow rate	: 0.3 mL/min
• Oven temperature	: 40 °C
• Mobile phase	: A: 10 mM ammonium acetate in water B: methanol
• Gradient program (%B)	: 0.0 – 3.0 min → 90 (%); 3.0 – 3.1 min → 90 – 100 (%); 3.1 – 4.0 min → 100 (%); 4.0– 4.1 min → 100 – 90 (%) 4.1 – 6.5 min → 90 (%)
• Injection volume	: 10 µL
• MS interface	: ESI
• Nitrogen gas flow	: Nebulizing gas 1.5 L/min; Drying gas 10 L/min;
• Zero air flow	: Heating gas 10 L/min
• MS temperature	: Desolvation line 200 °C; Heating block 400 °C Interface 200 °C

Table 2. LC/MS/MS conditions for Hydrochlorothiazide

• Column	: Shim-pack XR-ODS (100 mm L x 3 mm I.D.; 2.2 µm)
• Flow rate	: 0.2 mL/min
• Oven temperature	: 40 °C
• Mobile phase	: A: 0.1% formic acid in water B: acetonitrile
• Gradient program (%B)	: 0.0 – 1.0 min → 80 (%); 1.0 – 3.5 min → 40 – 100 (%); 3.5 – 4.5 min → 100 (%); 4.5– 4.51min → 100 – 80 (%) 4.51 – 8.0 min → 90 (%)
• Injection volume	: 25 µL
• MS interface	: ESI
• Nitrogen gas flow	: Nebulizing gas 2.0 L/min; Drying gas 10 L/min;
• Zero air flow	: Heating gas 15 L/min
• MS temperature	: Desolvation line 250 °C; Heating block 500 °C Interface 300 °C

Results

LC/MS/MS analysis results of Felodipine

LC/MS/MS method for Felodipine was developed on ESI positive ionization mode and 383.90>338.25 MRM transition was optimized for it. Checked matrix matched plasma standards for highest (10 ppb) as well as lowest concentrations (5 ppt) as seen in Figure 5 and Figure 6 respectively. Calibration curves as mentioned with R^2 = 0.998 were plotted (shown in Figure 7). Also as seen in Table 3, % Accuracy was studied to confirm the reliability of method. Also, LOD as 2 ppt and LOQ as 5 ppt was obtained.

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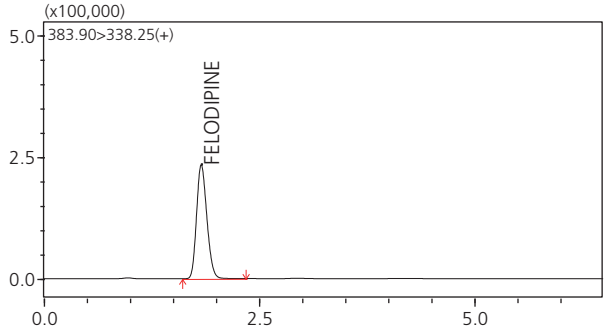

Figure 5. Felodipine at 10 ppb in matrix matched plasma

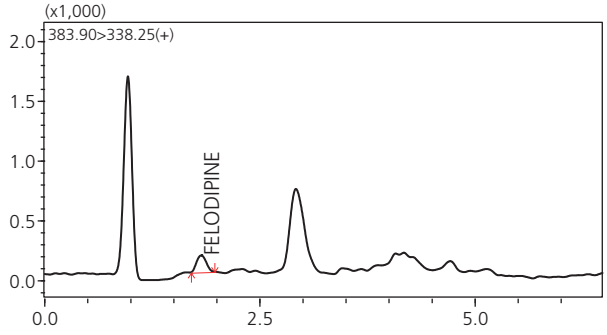

Figure 6. Felodipine at 5 ppt in matrix matched plasma

Table 3: Results of Felodipine calibration curve

Sr. No.	Standard	Nominal Concentration (ppb)	Measured Concentration (ppb)	% Accuracy (n=3)	% RSD for area counts (n=3)
1	STD-FEL-01	0.005	0.005	97.43	9.87
2	STD-FEL-02	0.01	0.010	103.80	8.76
3	STD-FEL-03	0.05	0.053	104.47	2.24
4	STD-FEL-04	0.1	0.103	103.13	1.23
5	STD-FEL-05	0.5	0.469	94.88	1.33
6	STD-FEL-06	1	0.977	97.33	0.95
7	STD-FEL-07	10	10.023	100.90	0.60

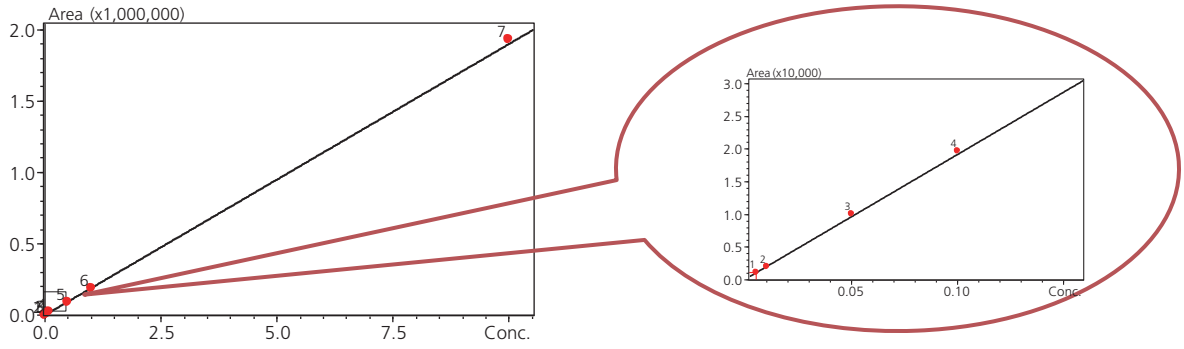

Figure 7. Calibration curve of Felodipine

LC/MS/MS analysis results of Hydrochlorothiazide

LC/MS/MS method for Hydrochlorothiazide was developed on ESI negative ionization mode and 296.10>204.90 MRM transition was optimized for it. Checked matrix matched plasma standards for highest (500 ppt) as well as lowest (2 ppt) concentrations as seen in Figures 8 and 9 respectively.

Calibration curves as mentioned with R^2=0.998 were plotted (shown in Figure 10). Also as seen in Table 4, % Accuracy was studied to confirm the reliability of method. Also, LOD as 1 ppt and LOQ as 2 ppt were obtained.

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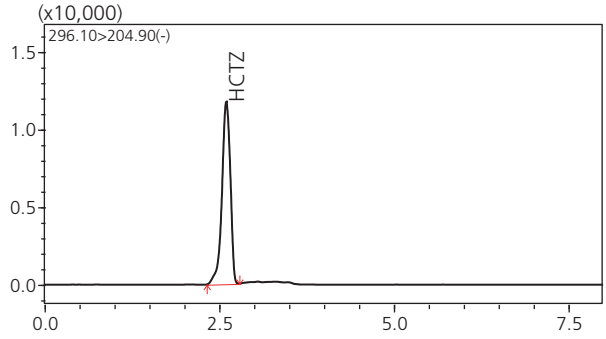

Figure 8. Hydrochlorothiazide at 500 ppt in matrix matched plasma

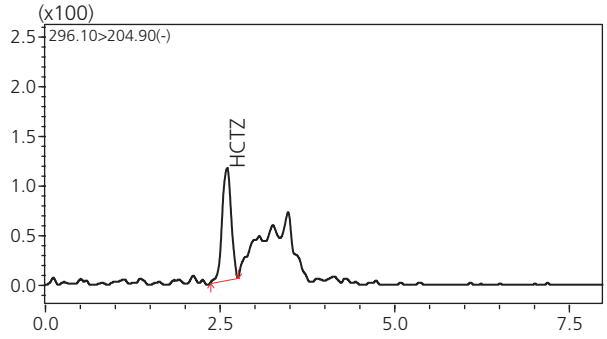

Figure 9. Hydrochlorothiazide at 2 ppt in matrix matched plasma

Table 4. Results of Hydrochlorothiazide calibration curve

Sr. No.	Standard	Nominal Concentration (ppb)	Measured Concentration (ppb)	% Accuracy (n=3)	% RSD for area counts (n=3)
1	STD-HCTZ-01	0.002	0.0020	102.03	6.65
2	STD-HCTZ-02	0.005	0.0048	95.50	3.53
3	STD-HCTZ-03	0.01	0.0099	100.07	3.80
4	STD-HCTZ-04	0.05	0.0512	102.67	1.60
5	STD-HCTZ-05	0.1	0.1019	102.11	3.58
6	STD-HCTZ-06	0.5	0.4944	102.13	1.68

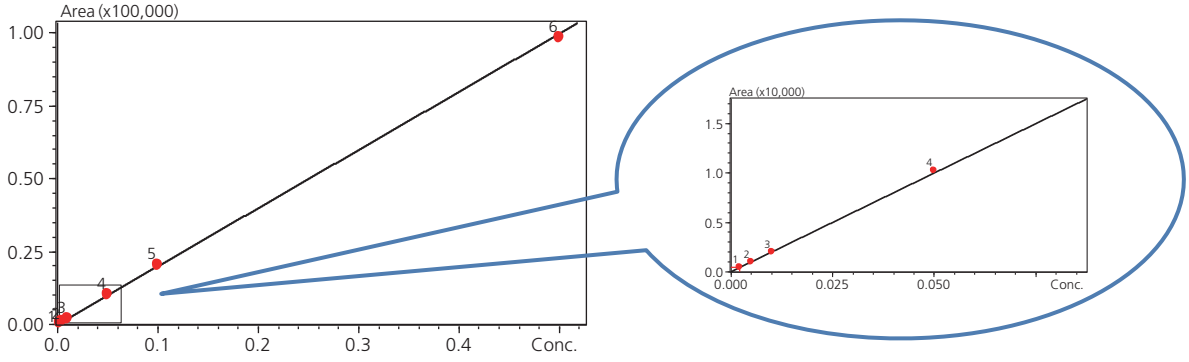

Figure 10. Calibration curve of Hydrochlorothiazide

Conclusion

- Highly sensitive LC/MS/MS method for Felodipine and Hydrochlorothiazide was developed on LCMS-8050 system.
- LOD of 2 ppt and LOQ of 5 ppt was achieved for Felodipine and LOD of 1 ppt and LOQ of 2 ppt was achieved for Hydrochlorothiazide by matrix matched methods.
- Heated ESI probe of LCMS-8050 system enables drastic augment in sensitivity with considerable reduction in background. Hence, LCMS-8050 system from Shimadzu is an all rounder solution for bioanalysis.

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References

[1] YU Peng; CHENG Hang, Chinese Journal of Pharmaceutical Analysis, Volume 32, Number 1, (2012), 35-39(5).
[2] Hiten Janardan Shah, Naresh B. Kataria, Chromatographia, Volume 69, Issue 9-10, (2009), 1055-1060.

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