

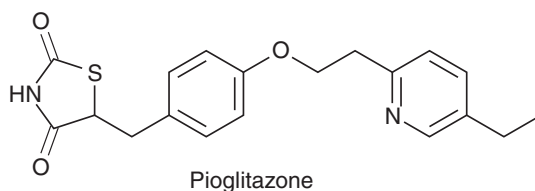


# **Validation of Pioglitazone in Human Serum by Two Sample Introduction Methods: LDTD-APCI/MS/MS and LC-ESI/MS/MS**

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# Overview

- Prior publications have described method development and validation for pioglitazone in human serum using traditional LC-MS/MS methodologies
- We present and compare validation data from two sample introduction methods: LC-ESI/MS/MS and LDTD-APCI/MS/MS
- LDTD-APCI/MS/MS analysis utilizes the same extraction technique as does LC analysis, but decreases instrumental analysis time by approximately an order of magnitude
- Traditional LC-MS/MS - Validation sample (VS) precisions of 1.1% - 3.6%
- LDTD-MS/MS - Validation sample (VS) precisions of 1.3% - 5.9%



## Introduction

Pioglitazone is a prescription drug of the class thiazolidinedione with hypoglycemic (antihyperglycemic, antidiabetic) action and is used for the treatment of diabetes mellitus type 2 in monotherapy. It is usually administered in combination with sulfonylurea, metformin, or insulin. Pioglitazone is typically assayed with traditional LC-MS/MS methodologies. Here we present validation data for pioglitazone assayed on an alternative system, LDTD-MS/MS and the notion that utilization of the LDTD™ source has the potential to reduce cost of analysis and method development while at the same time increasing throughput, analysis turnaround, and efficiency of instrumentation usage.

## Methods

### Sample Preparation

- 100  $\mu$ L sample + 50  $\mu$ L of IS + 600  $\mu$ L of acidified water
- Tomtec automated extraction procedure
- Solid phase extraction with Phenomenex® Strata™ X (30mg/well)
- Pioglitazone-d<sub>4</sub> internal standard
- Evaporated samples reconstituted in 250  $\mu$ L of a water/acetonitrile/acetate buffer mixture
- Method range is 25.0- 2500 ng/mL in human serum

### HPLC

- Column: Imtakt Cadenza CD-C18 (2.0 x 50 mm, 3 micron)
- Isocratic elution with water/acetonitrile/acetate buffer mobile phase
- Injection volume: 10.0  $\mu$ L
- Autosampler cycle time: 4.5 minutes

### LC-ESI/MS/MS

- Sciex API3000™
- Source: Electrospray ionization
- Ion mode: Positive
- Resolution (Q1/Q3): Unit/Unit
- Ions monitored: Pioglitazone m/z 357.0/134.2; Pioglitazone-d<sub>4</sub> m/z 361.0/138.2
- Reconstituted samples injected via HPLC setup noted above

### LDTD-APCI/MS/MS

- Sciex API4000™
- Thermo TSQ Quantum Ultra™
- Source: Phytronix Technologies, Inc LDTD™ ionization source (model S-960) using APCI
- Ion mode: Positive
- Resolution (Q1/Q3): Unit/Unit
- Ions monitored: Pioglitazone m/z 357.0/134.2; Pioglitazone-d<sub>4</sub> m/z 361.0/138.2
- Reconstituted samples (3  $\mu$ L) spotted onto LazWell sample plate, evaporated to dryness at room temperature, and loaded into the Phytronix LDTD source

# Results

**Table 1. LC-ESI/MS/MS (Sciex API3000)**  
VS Precision and Accuracy

Run Date	Curve Number	VS 25.0	VS 50.0	VS 1000	VS 2000
		25.0 ng/mL	50.0 ng/mL	1000 ng/mL	2000 ng/mL
16-Aug-2008	12	26.3	48.6	1020	2010
		25.5	49.1	1010	1980
		25.5	50	1040	2010
		25.5	50	1020	2040
		25.2	49.6	1020	2050
16-Aug-2008	13	25.8	50.5	1010	2030
		27.9	52.3	1010	2010
		27.9	49.8	1020	2030
		26.6	49.3	1030	2020
		26.1	49	1040	2030
17-Aug-2008	14	26	48.7	1000	2030
		25.3	47.6	1020	2080
		28.1	50.8	1010	2030
		26.5	50.7	1000	2040
		27	50.5	1020	2010
20-Aug-2008	5	28.2	50.5	1020	2040
		26.9	48.8	1000	2020
		27.2	48.9	1010	2060
		25.9	51	1010	2020
		25.9	47.7	1020	1990
Mean Concentration Found (ng/mL)		26.3	49.8	1020	2020
Inter-run SD		0.957	1.13	11.8	21.9
Inter-run %CV		3.6	2.3	1.2	1.1
Inter-run %Bias		5.2	-0.4	2	1
n		24	24	24	24

**Table 2. LDTD-APCI/MS/MS (Sciex API4000)**  
VS Precision and Accuracy

Run Date	Curve Number	VS 25.0	VS 50.0	VS 1000	VS 2000
		25.0 ng/mL	50.0 ng/mL	1000 ng/mL	2000 ng/mL
14-Jan-2009	28	24.7	51.9	1040	1870
		26.2	53.1	985	2080
		25	50.9	1000	1910
		25.4	52.2	966	2060
		25.6	50.8	965	1980
14-Jan-2009	29	26.3	52	960	1900
		25.3	51.9	929	2120
		25.3	51.5	925	2100
		25.5	51.5	1010	1840
		25.5	53.4	956	2040
14-Jan-2009	30	25.8	52.6	944	1670
		25.5	52.6	1020	1950
		25.7	51.6	951	1980
		25.8	51.6	992	1960
		25.5	52.3	907	2100
16-Jan-2009	33	25.7	51.2	986	2120
		25.6	51.8	975	1900
		25.9	*39.1	1020	*1550
		25.4	50.9	1050	1890
		24.4	51.6	991	2190
Mean Concentration Found (ng/mL)		25.4	51.8	976	1980
Inter-run SD		0.445	0.699	35.7	116
Inter-run %CV		1.8	1.3	3.7	5.9
Inter-run %Bias		1.6	3.6	-2.4	-1
n		24	23	24	23

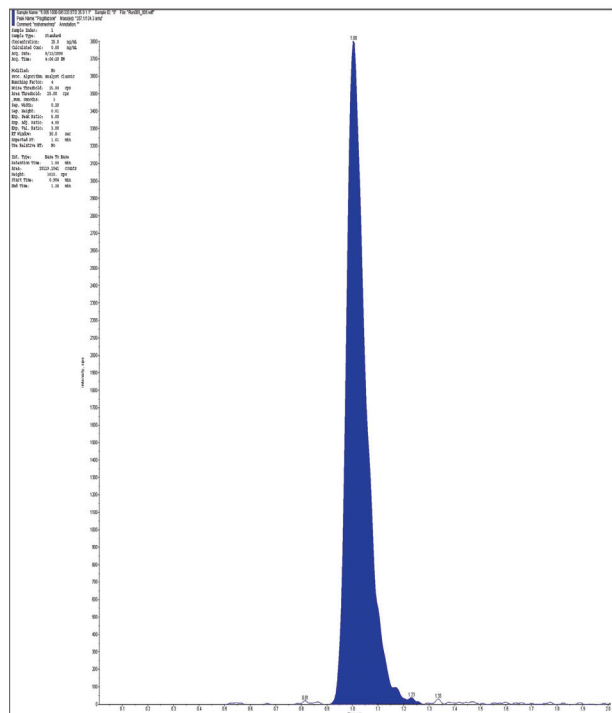
\*Deactivated due to preparation error

**Table 3. LDTD-APCI/MS/MS (Thermo TSQ Quantum Ultra)** VS Precision and Accuracy

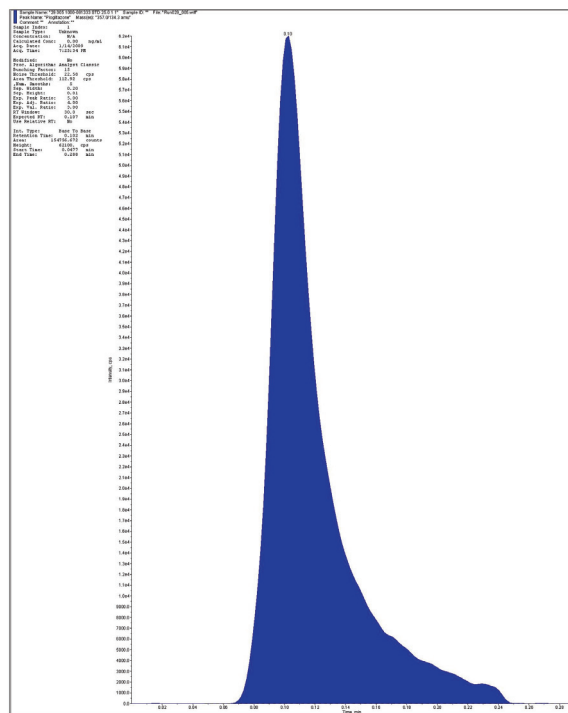
Run Date	Curve Number	VS 25.0	VS 50.0	VS 1000	VS 2000
		25.0 ng/mL	50.0 ng/mL	1000 ng/mL	2000 ng/mL
14-Aug-2008	10	24.7	53.8	924	2090
		26.3	45	942	2130
		25.8	57.7	937	2330
		29.8	53.1	921	1910
		22.8	45.8	763	2150
20-Aug-2008	19	24.7	47.7	977	1700
		24.2	48	927	1910
		26	46.3	903	1810
		25	44	1040	2040
		24.2	49.3	923	1810
20-Aug-2008	21	23.4	44.1	955	2220
		23.7	46.8	1030	2020
		27.4	56.4	917	1740
		25.1	43.9	1010	2170
		23	54.3	1050	1720
20-Aug-2008	22	23.2	42.6	1010	2050
		22.4	44.7	1090	2250
		28.4	53	1110	2210
		27.4	45.4	1120	1820
		28.7	56.6	940	2160
Mean Concentration Found (ng/mL)		25.7	49.3	989	2010
Inter-run SD		2.1	4.69	86	192
Inter-run %CV		8.2	9.5	8.7	9.6
Inter-run %Bias		2.8	-1.4	-1.1	0.5
n		24	24	24	24

**Table 4. Validation Performance – LDTD versus HPLC**  
sample introduction methods

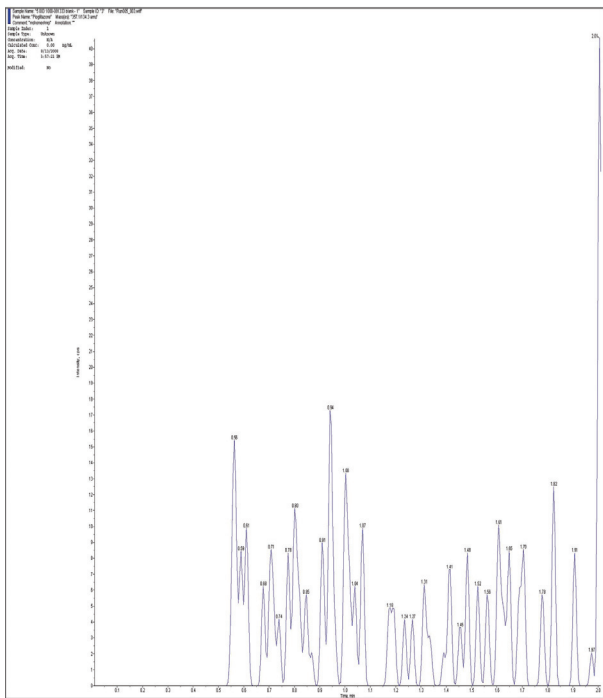
Result	LC/MS/MS		LDTD-MS/MS	
	% CV	% Bias	% CV	% Bias
169 hours extract stability	2.7	4.3	6.9	5.9
Spiked Selectivity	2.1	4.1	4.9	3.0
Selectivity % mean LLOQ response	0%		<10%	
96-well plate run time	450 minutes		37 minutes	



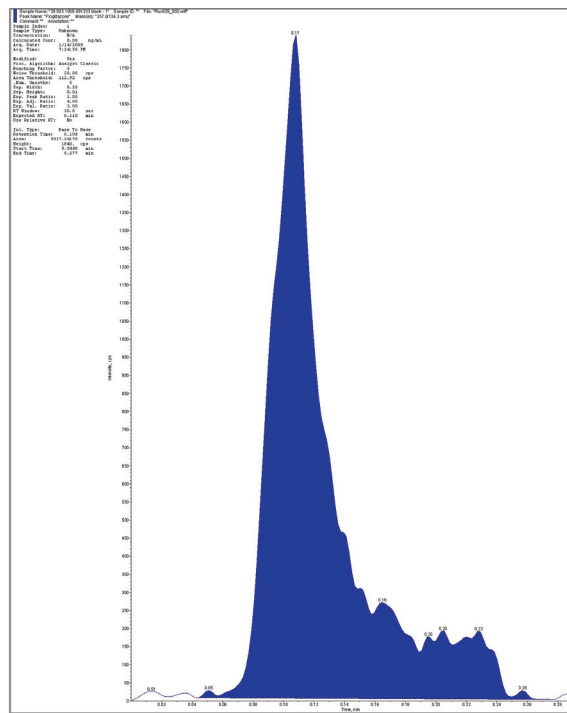
**Figure 1. Representative chromatogram- Pioglitazone LLOQ**  
extract via LC-ESI/MS/MS



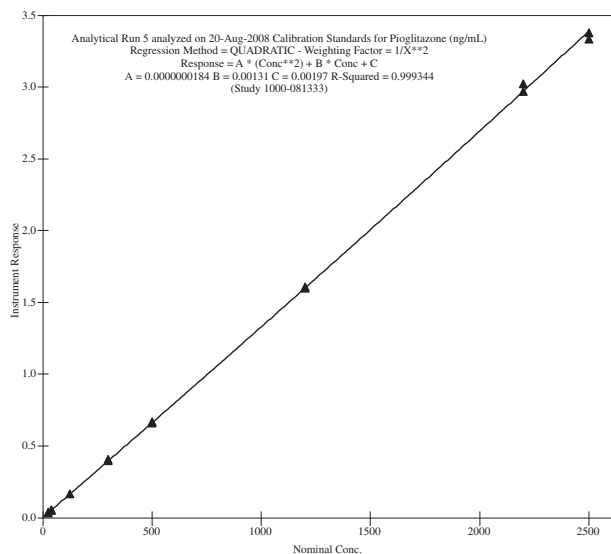
**Figure 2. Representative desorption peak- Pioglitazone LLOQ**  
extract via LDTD-APCI/MS/MS



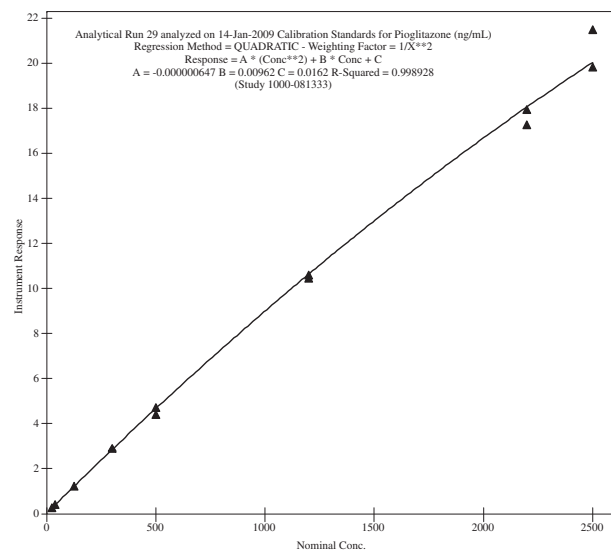
**Figure 3.** Representative chromatogram- Pioglitazone blank serum extract via LC-ESI/MS/MS



**Figure 4.** Representative desorption peak- Pioglitazone blank Serum extract via LDTD-APCI/MS/MS



**Figure 5.** Representative calibration curve for LC-ESI/MS/MS



**Figure 6.** Representative calibration curve for LDTD-APCI/MS/MS

## Conclusion

The comparison of two sample introduction methods for a model bioanalytical system demonstrates that accuracy, precision, and selectivity comparable to those achieved by traditional LC-MS can be obtained in complex biological matrices with the LDTD™ source. Validation results that were well within the recommended criteria for bioanalytical applications allude to promise for use of this system in daily bioanalytical applications. While the LDTD™ source eliminates chromatographic development with no loss in the quality of the results for selected applications, each compound must first be evaluated for compatibility with the LDTD™ source before this system should be deployed.

Although this sample introduction method is not appropriate for all test systems, the advantages offered for those cases where it is appropriate are significant. A potential decrease in the amount of method development time while significantly increasing sample analysis throughput, reduction in solvent use and disposal costs, front-end setup time, and instrument plus peripheral equipment maintenance and verifications are appealing to the end user as well as to clients who may reap the benefits of the rapid analysis turnaround times. Methods able to utilize the LDTD™ source will allow more analysis with less equipment and has the potential to help streamline resource efficiency while reducing costs and improving analysis turnaround times.