



16.1.9.3 BIOANALYTICAL REPORTS

Determination of 11-Dehydrothromboxane B₂ in Human Urine Samples by LC-MS/MS
(Study AA99071-11)



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**Determination of 11-Dehydrothromboxane B₂ in Human Urine Samples from “A
Randomized, Controlled, Open-label, 3-Arm Parallel Group, Single-Center Study to
Demonstrate Reductions in Exposure to Selected Smoke Constituents in Smoking, Healthy
Subjects Switching to the Tobacco Heating System 2.2 (THS 2.2) or Smoking Abstinence,
Compared to Continuing to Use Conventional Cigarettes, for 5 Days in Confinement” by
LC-MS/MS**

Study: AA99071-11

Bioanalytical Final Report

Philip Morris Products S.A.
Quai Jeanrenaud 5
2000 Neuchâtel, Switzerland

Protocol ZRHR-REXC-03-EU

Report Date: 13-Mar-2015

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11-Dehydrothromboxane B₂ in Human Urine
Celerion Study AA99071-11

STUDY LOCATION

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SPONSOR

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APPROVAL SIGNATURES

TEST SITE

Celerion:

Bioanalytical Principal Investigator

Kirk Newland, B.S.
Technical Director, Tobacco Sciences

13-Mar-2015

Date

Management

Rafiqul Islam, M.S.
Senior Director, Bioanalytical Services

13-Mar-2015

Date



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SPONSOR
Philip Morris Products, S.A.:

Manager Clinical Science

Christelle Haziza, PhD

16.03.2015

Date



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STATEMENT OF COMPLIANCE

The bioanalytical phase of the study was performed according to applicable GLP requirements and in compliance with Standard Operating Procedures (SOPs) in effect in the bioanalytical laboratory of Celerion, Lincoln, Nebraska. The SOPs are written based on the principles and requirements described in United States Food and Drug Administration Title 21 Code of Federal Regulations (CFR) Part 58, the Guidance for Industry – Bioanalytical Method Validation (CDER, May 2001), and Guideline on Bioanalytical Method Validation (European Medicines Agency [EMA/CHMP/EWP/192217/2009], Effective February 2012).

This production study was conducted in accordance with the guidelines documented in the bioanalytical study plan. To ensure the integrity of the reported data, the bioanalytical laboratory verified all results. The Quality Assurance unit of Celerion, Lincoln, Nebraska, audited the study. A Quality Assurance statement was then issued and is included within this document.

The data summaries, results, and conclusions in this bioanalytical report have been reviewed and were found to be consistent and scientifically rational. All deviations from the protocol and/or significant deviations from SOPs documented in this report have been reviewed and are scientifically valid.

I accept responsibility for the scientific integrity of the data included within this bioanalytical report.

Kirk Newland, B.S.
Technical Director, Tobacco Sciences

13-Mar-2015

Date



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QUALITY ASSURANCE STATEMENT

Phase Audited	Audit Date(s)	Date Reported to Study Director/ Bioanalytical Principal Investigator	Date Audit Report Signed by Management
Bioanalytical Study Plan	08-Jul-2013	08-Jul-2013	29-Jul-2013
Critical Phase Inspection	02-Dec-2013	03-Dec-2013	17-Dec-2013
Database	27, 30-Dec-2013	30-Dec-2013	08-Jun-2014
Bioanalytical Report (Final Draft)	07-Apr-2014	07-Apr-2014	23-Apr-2015
Bioanalytical Report (Final)	12-Mar-2015	12-Mar-2015	13-Mar-2015

Celerion Quality Assurance audited various phases of this study as shown above. This statement confirms that the methods, procedures, and results as presented in this report accurately reflect the raw data of the study.

Jennifer Ortiz Torres, B.S., ASQ-CQA
Quality Assurance Auditor

13 Mar 2015

Date



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1. INTRODUCTION

The purpose of this bioanalytical study (hereafter referred to as study) was to determine the concentration of 11-dehydrothromboxane B₂ in human urine samples by LC-MS/MS method. The study samples were collected in the clinical study ZRHR-REXC-03-EU, entitled, "A Randomized, Controlled, Open-label, 3-Arm Parallel Group, Single-Center Study to Demonstrate Reductions in Exposure to Selected Smoke Constituents in Smoking, Healthy Subjects Switching to the Tobacco Heating System 2.2 (THS 2.2) or Smoking Abstinence, Compared to Continuing to Use Conventional Cigarettes, for 5 Days in Confinement" [3]. Sample analysis was conducted between 27-Nov-2013 and 16-Dec-2013.

This report provides the results and supporting documentation from the analysis of study samples and includes an evaluation of assay performance.

2. EXPERIMENTAL

2.1. Test Item

The test items are defined in the clinical study protocol [3].

2.2. Reference Items and Internal Standards

	Analyte	Internal Standard (IS)
ID	11-Dehydrothromboxane B ₂	d ₄ -11-Dehydrothromboxane B ₂
Source	(b) (4)	(b) (4)
Lot No.	0447214-4	4785-18
Purity/Concentration	100.0% (500 µg/mL)	98.0% (100 µg/mL)
Celerion Assigned Correction Factor	1.00	1.00
Expiry Date	30-Apr-2014	24-Dec-2013
Storage Conditions	Freezer (-20 C), protected from light	Freezer (-20 C), protected from light

The certificate(s) of analysis for the reference items and internal standards are presented in [Attachment 6](#).

Reference items and internal standards are retained under the conditions that are specified until they become expired. They will then be removed from the active library or stored for an additional period for the testing of long-term stability.



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2.3. Biological Matrix

Human urine was collected in-house at Celerion in Lincoln, Nebraska. Human urine stored at -20°C may be stored for a period less than 24 months prior to use. Human urine, free of significant interference, was used to prepare quality control samples B - D (QC B - D). Urisub[®], a urine substitute, was purchased from (b) (4), and stored at 5°C for a period less than 24 months prior to use. Urisub[®] was used to prepare calibration standard samples and quality control sample A (QC A), and used as control matrix.

2.4. Test System

2.4.1. Procedure and Instruments

Procedure and Instrumentation	
Extraction Method	Solid-phase extraction
Chromatography system	Waters Acquity [™] UPLC Binary Solvent Manager [^]
MS/MS system	AB SCIEX API 5000 [™] [^]
Regression Type	Linear (1/concentration ²)
Quantitation Method	Peak Area Ratio
Assay Volume	1.00 mL

[^] = Qualified systems

2.4.2. Computer Application Software

Software	
LC-MS/MS software	Applied Biosystems Analyst [®] 1.5.1 [^]
LIMS	Thermo Electron Corporation Watson [™] 7.3 Bioanalytical LIMS 7.3 [^]
LIMS application	Inspector Version 1.1.1 [^]
Laboratory Documentation System	Labnotes [™] Web Client 1.21 [^]
Office applications	Microsoft [®] Office 2007 Package

[^] = Validated systems

2.5. Calibration Standards, Quality Control Samples and Dilution Quality Control Samples

Non-zero calibration standards were prepared fresh daily at the concentration levels of 20.0, 50.0, 100, 200, 500, 1000, 1500, 1800, and 2000 pg/mL from calibration spiking standard solutions which were prepared in bulk on 18-Nov-2013, and stored at -20°C for a period less than 118 days prior to use. The calibration standard spiking solutions were prepared at 10x concentrations. To achieve the required standard concentration, 0.100 mL of standard spiking solution is added to 1.00 mL of artificial urine.



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Quality control (QC) samples at the concentration levels of 60.0 pg/mL (in Urisub[®]), 199 pg/mL (basal level + 120 pg/mL), and 1450 pg/mL (basal level + 1370 pg/mL) were prepared in bulk on 20-Nov-2013, aliquoted and stored at -20°C. QC samples in clear polypropylene tubes were stored under the same conditions as the study samples were stored. The quality control samples were analyzed within the established stability period of 434 days.

Standard calibrators and quality control samples were prepared from separate stock solutions.

2.6. Study Samples

2.6.1. Sample Source and Date of Receipt

Study samples were collected between 12-Jul-2013 and 18-Sep-2013 and were received frozen on dry ice between 22-Jul-2013 and 18-Oct-2013 from Covance Central Laboratories, Meyrin, Switzerland.

2.6.2. Sample Identification

Study samples were identified based on the subject screening number and time point documented on the sample label.

2.6.3. Sample Storage and Stability

Study samples were stored from sample collection in clear polypropylene tubes to the end of sample analysis at a nominal temperature of -20°C for a duration not exceeding 158 days.

Study samples were analyzed without exceeding long-term, short-term, freeze-thaw, or post-preparative stability. The following evaluations have been conducted:

Stability Summary [5]	
Long-term Stability	434 days in polypropylene tubes at -20 C
Short-term Stability	27 hours in polypropylene tubes at ambient temperature under white light
Freeze-thaw Stability	6 cycles in polypropylene tubes at -20 C under white light
Post-preparative Stability	219 hours in injection vials with silanized glass inserts at 5 C
Processed Sample Integrity	151 hours in injection vials with silanized glass inserts at 5 C
Sample Shipping Stability	14 days in polypropylene tubes at -80 C

2.6.4. Sample Summary

The Sponsor's protocol specifies 160 subjects, with 2 sampling times for 24-hour urine collections [3]. In study AA99071, a single subject discontinued from the clinical phase after randomization. The samples from this subject were analyzed and the results reported. Additional information regarding the subject discontinuance is provided in [Section 8.2](#).



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	No. of Samples
Specified in protocol/received	320/328
Analysis not required (subject discontinued from enrollment)	9
Analytical failure	1
Duplicates received	328
Total number of study samples analyzed	318

Following analysis, the study samples were kept frozen at -20°C. After submission of the final bioanalytical report the study samples will be further stored under the same conditions for up to 1 month on-site. Then, upon agreement with the Sponsor, the study samples will be destroyed after the completion of the clinical study report and Sponsor notification.

3. SAMPLE ANALYSIS

3.1. Analytical Method

The determination of 11-dehydrothromboxane B₂ in human urine samples was carried out over a calibration range of 20.0 pg/mL to 2000 pg/mL. The analytical procedure was performed at Celerion, Lincoln, Nebraska and is documented in the Method Validation Report for Celerion Study ZZ00102-02 [5]. The analytical method is documented in BAM SOP ZZ00102-02 [6]. See Attachment 7.

An aliquot of human urine containing the analyte and internal standard was extracted using a solid phase extraction procedure. The extracted samples were analyzed by an UPLC equipped with an AB SCIEX API 5000™ mass spectrometer. Negative ions were monitored in the multiple reaction monitoring (MRM) mode. Quantitation was determined using a weighted linear regression analysis ($1/x^2$) of peak area ratios of the analyte and internal standard.

Though listed as a standard, the control blank sample with internal standard (Standard A) was not used to plot the calibration curve.

3.2. Acceptance Criteria

3.2.1. Analytical Run Acceptance Criteria

An analytical run is acceptable if all of the following criteria are met:

- at least 75% of the non-zero calibration standards were within ±15.0% (±20.0% for the lower limit of quantification (LLOQ) calibration standard) of their nominal concentration,
- at least two-thirds of the QC samples and at least 50% at each concentration level were within ±15.0% of their nominal concentration,
- at least 50% of the standard zero samples are free of interference at the retention time of the analyte(s) of interest,



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- at least 50% of the blank samples are free of interference both at the retention time of the analyte(s) of interest and at the retention time of the IS,
- at least two-thirds of all blank and standard zero samples fulfilled the above described interference criteria.

Interference at the retention time of the analyte of interest is defined as a response greater than 20% of the mean analyte response of the LLOQ calibration standard(s).

Interference at the retention time of the IS is defined as a response greater than 5% of the mean IS response of the LLOQ calibration standard(s).

Individual data of QC samples that were out of their acceptance criteria are flagged appropriately in the study file and in the bioanalytical report. QCs will be excluded from statistics only for analytical reasons (see [Attachment 5](#)).

3.2.2. Acceptance Criteria for System Suitability Testing

The system suitability testing performed with each analytical run is designed to assess the sensitivity, reproducibility of response (absence of response drift based on interpolated concentrations), and carry-over.

- Sensitivity assessed at the start and end of each analytical run is performed by evaluating the signal-to-noise ratio (SNR) of extracted system suitability samples spiked at the lower limit of quantitation. The SNR must be greater than 5:1 unless otherwise specified in the method.
- System stability (reproducibility of response) is performed by replicate injections at the start (5) and the end (2) of the analytical run with pooled high concentration system suitability samples. The percent coefficient of variation (% CV) of the calculated concentration must be less than or equal to 6%. The mean of the calculated concentration of the last 2 replicates or middle replicates (if applicable) of high concentration system suitability samples must be within 15% difference of the mean of the calculated concentration of the first 5 high concentration system suitability samples.
- The carryover percentage is assessed at the beginning and end of each analytical run. This test is performed by injecting a blank (reconstitution solution) sample immediately after a high concentration system suitability sample. The area counts of the analyte in the blank injection are divided by the analyte area counts in the high concentration system suitability sample and the result is multiplied by 100.

$$\% \text{ carryover} = \left(\frac{\text{area (blank sample)}}{\text{area (high sys suit)}} \right) * 100$$

Analyte	Carryover criteria (needs to be less than)
11-dehydro Thromboxane B ₂	0.1%



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3.2.3. Acceptance Criteria for ISR

The % difference was calculated for each pair of original and repeat analyses as follows:

$$\% \text{ difference} = 100 * \frac{|\text{repeat value} - \text{original value}|}{(\text{repeat value} + \text{original value}) / 2}$$

If the % difference was less than or equal to 20%, a pair of results was considered a passing match. Any pair with a % difference of more than 67% (indicating that the repeat value is either less than half or more than twice the original concentration) was considered an event and was investigated. The analytical method will be considered reproducible if at least 67% of the result pairs match. If less than 67% of the pairs match, an event investigation was initiated.

4. RESULTS

Due to rounding procedures, recalculations using the results presented in this report may differ slightly from the reported statistics.

A summary of analytical runs performed is presented in [Table 1](#).

4.1. Quality Control Sample Performance

Between-analytical run precision and accuracy results for QC samples prepared at 60.0, 199, 1450 pg/mL are summarized in [Table 2](#).

4.2. Calibration Standard Performance

Back-calculated calibration curve standard concentrations are provided in [Table 3](#).

4.3. Standard Curve Parameters

Standard curve parameters from 7 successful analytical runs are provided in [Table 4](#). A representative calibration curve is illustrated in [Figure 1](#).

4.4. Study Sample Concentrations

Study sample concentrations are provided in [Table 5](#). The column “Split” refers to the “for analysis” or “back-up” sample collected.

Study samples, if any, with no significant peak at the mass transition and retention time of 11-dehydrothromboxane B₂, or with peak area ratios below that of the LLOQ standard, are reported as being below the limit of quantitation (BLQ).

4.5. Reassays

4.5.1. Reassays for Analytical Reasons

Study samples needing re-analysis according to [section 3.2.1](#) are identified in [Table 6](#).



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4.5.2. Reassays for Non-analytical Reasons (Value Requiring Confirmation, VRC)

There were no study samples that were reassayed due to non-analytical reasons.

4.5.3. Sponsor Selected Reassays

There were no Sponsor selected reassays.

4.5.4. Incurred Sample Reproducibility

The method for the determination of 11-dehydrothromboxane B₂ was considered reproducible, 88.9% out of 36 repeat analyses met acceptance criteria as defined in [section 3.2.3](#). Results are presented in [Table 7](#).

5. CHROMATOGRAMS

Representative chromatograms are provided in [Attachment 8](#).

6. DEVIATIONS

There were no protocol and/or significant SOP deviations.

7. EVENTS

There were no unexpected observations or results during the conduct of the study regarded as events which required investigation.

8. ANALYTICAL NOTES

8.1. The following sample did not have reportable data due to repeated unacceptable chromatography (UCR).

<u>Subject</u>	<u>Period</u>	<u>Day Nominal</u>	<u>Sample Number</u>
067	1	1	05112030000151

8.2. During the course of analysis of study AA99077 (ZRHR-REXC-04-JP), it was determined that incomplete documentation of subject consent for further analysis of bioanalytical samples after subject discontinuation existed. A review of the possible impacted studies included ZRHR-REXC-03-EU (AA99071). One subject, 0083, discontinued from the clinical phase post-randomization. Consent for analysis was later confirmed by the Principal Investigator. The results from subject 0083 were included with the final deliverables for this study.



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9. ARCHIVES

At a minimum the following records will be retained:

- Study Plan Bioanalysis (and all amendments, if applicable)
- Raw data
- Study related correspondence
- Bioanalytical report (and all amendments, if applicable)

These documents will be kept in the archives of Celerion for at least ten (10) years, taken from the date of Bioanalytical Principal Investigator's signature on the final bioanalytical report. After this time the Sponsor will be contacted to decide if the records should be retained for a further defined time at Celerion, returned to the Sponsor, or disposed of. Study data and documentation are archived at the Celerion Lincoln facility for 90 days, after which the records may be transferred to:

Iron Mountain
1601 Leavenworth
Omaha, Nebraska 68102

10. CONCLUSION

In this bioanalytical study the concentration was determined in a total of 318 samples for 11-dehydrothromboxane B₂ in human urine samples collected in the Philip Morris International Research and Development clinical study ZRHR-REXC-03-EU using a validated LC-MS/MS method.

The overall performance of the LC-MS/MS method met acceptance criteria and the results obtained were of the required integrity and quality. These data can be used for further interpretation.



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11. REFERENCES

- [1] Guidance for Industry – Bioanalytical Method Validation: US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM) May 2001
- [2] OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1, OECD Publishing, Paris, France (2003).
- [3] Protocol ZRHR-REXC-03-EU: "A Randomized, Controlled, Open-label, 3-Arm Parallel Group, Single-Center Study to Demonstrate Reductions in Exposure to Selected Smoke Constituents in Smoking, Healthy Subjects Switching to the Tobacco Heating System 2.2 (THS 2.2) or Smoking Abstinence, Compared to Continuing to Use Conventional Cigarettes, for 5 Days in Confinement"
- [4] Study Plan Bioanalysis: Determination of 11-Dehydrothromboxane B₂ in Human Urine Samples from "A Randomized, Controlled, Open-label, 3-Arm Parallel Group, Single-Center Study to Demonstrate Reductions in Exposure to Selected Smoke Constituents in Smoking, Healthy Subjects Switching to the Tobacco Heating System 2.2 (THS 2.2) or Smoking Abstinence, Compared to Continuing to Use Conventional Cigarettes, for 5 Days in Confinement" by LC-MS/MS, Celerion Study AA99071-11
- [5] Validation of an LC-MS/MS Method for the Determination of 11-Dehydrothromboxane B₂ in Human Urine, Celerion Study ZZ00102-02
- [6] Bioanalytical Method SOP for the Determination of 11-Dehydrothromboxane B₂ in Human Urine, Celerion Study ZZ00102-02



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RESULT TABLES

Table 1 Summary of Analytical Runs Performed

Run ID	Regression Status	Extraction Date	Assay Date	Description	Comment
1	Accepted	27-Nov-2013	27-Nov-2013	SEE WORKLIST FOR SUBJECT AND TIMEPOINTS	OK
2	Accepted	02-Dec-2013	02-Dec-2013	SEE WORKLIST FOR SUBJECT AND TIMEPOINTS	OK
3	Accepted	03-Dec-2013	03-Dec-2013	SEE WORKLIST FOR SUBJECT AND TIMEPOINTS	OK
4	Accepted	05-Dec-2013	05-Dec-2013	SEE WORKLIST FOR SUBJECT AND TIMEPOINTS	OK
5	Accepted	06-Dec-2013	09-Dec-2013	SEE WORKLIST FOR SUBJECT AND TIMEPOINTS	OK
6	Accepted	12-Dec-2013	12-Dec-2013	ISRs + REASSAYS	OK
7	Accepted	16-Dec-2013	16-Dec-2013	REASSAY	OK

"Regression Status" reflects the status of the run with respect to run acceptance criteria.



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Table 2 Quality Control Sample Data (Between-Analytical Run Precision and Accuracy)

Assay Date	Run ID	QC A	QC B	QC C
		60.0 pg/mL	199 pg/mL	1450 pg/mL
27-Nov-2013	1	63.1	196	1440
		63.6	201	1510
02-Dec-2013	2	60.2	209	1480
		60.9	209	1370
03-Dec-2013	3	64.9	187	1520
		62.4	201	1400
05-Dec-2013	4	66.3	204	1410
		64.6	199	1540
09-Dec-2013	5	56.4	193	1390
		59.4	200	1430
12-Dec-2013	6	62.9	194	1290
		56.8	194	1270
16-Dec-2013	7	57.9	204	1360
		59.2	187	1450
Mean		61.3	198	1420
S.D.		3.12	7.00	80.6
%CV		5.1	3.5	5.7
%Theoretical		102.2	99.5	97.9
%Bias		2.2	-0.5	-2.1
n		14	14	14



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Table 3 Back-calculated Calibration Standard Concentrations

Assay Date	Run ID	STD B 20.0 pg/mL	STD C 50.0 pg/mL	STD D 100 pg/mL	STD E 200 pg/mL	STD F 500 pg/mL	STD G 1000 pg/mL	STD H 1500 pg/mL	STD I 1800 pg/mL	STD J 2000 pg/mL
27-Nov-2013	1	19.6	51.1	104	215	500	961	1490	1720	1960
02-Dec-2013	2	19.6	53.3	97.0	197	505	996	1510	1810	1960
03-Dec-2013	3	19.4	52.8	101	210	485	995	1470	1780	1950
05-Dec-2013	4	19.5	52.5	*120	207	507	1020	1490	1750	1880
09-Dec-2013	5	19.1	54.1	106	196	517	971	1490	1820	1830
12-Dec-2013	6	19.4	53.2	104	201	467	994	1440	1760	2120
16-Dec-2013	7	20.1	47.8	103	216	510	986	1500	1690	1970
Mean		19.5	52.1	103	206	499	989	1480	1760	1950
S.D.		0.304	2.11	3.15	8.21	17.2	19.1	23.0	46.7	90.1
%CV		1.6	4.0	3.1	4.0	3.4	1.9	1.6	2.7	4.6
%Bias		-2.5	4.2	3.0	3.0	-0.2	-1.1	-1.3	-2.2	-2.5
n		7	7	6	7	7	7	7	7	7

Reason Deactivated

* Rejected



11-Dehydrothromboxane B₂ in Human Urine
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Table 4 Standard Curve Parameters

Assay Date	Run ID	Slope	Intercept	R-Squared
27-Nov-2013	1	0.00510215656	0.000487320093	0.9982
02-Dec-2013	2	0.00518673488	0.0111784216	0.9990
03-Dec-2013	3	0.00515122492	0.0127717592	0.9986
05-Dec-2013	4	0.00467825138	0.00704007825	0.9984
09-Dec-2013	5	0.00493294123	0.0125733958	0.9965
12-Dec-2013	6	0.00510916429	0.00245294454	0.9974
16-Dec-2013	7	0.00523767033	0.00276091163	0.9979
Mean		0.00505687766	0.00703783302	0.9980
S.D.		0.000192333244	0.00521036342	0.0008
%CV		3.8	74.0	0.1
n		7	7	7



11-Dehydrothromboxane B₂ in Human Urine
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Table 5 Study Sample Concentrations

Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000001	1	0010	0	1	137	1	OK		11-DTXB2
05112030000002	1	0010	5	6	285	1	OK		11-DTXB2
05112030000003	1	0011	0	1	612	1	OK		11-DTXB2
05112030000004	1	0011	5	6	366	1	OK		11-DTXB2
05112030000005	1	0014	0	1	465	1	OK		11-DTXB2
05112030000006	1	0014	5	6	263	1	OK		11-DTXB2
05112030000007	1	0015	0	1	354	1	OK		11-DTXB2
05112030000008	1	0015	5	6	299	1	OK		11-DTXB2
05112030000009	1	0016	0	1	152	1	OK		11-DTXB2
05112030000010	1	0016	5	6	297	1	OK		11-DTXB2
05112030000011	1	0017	0	1	245	1	OK		11-DTXB2
05112030000012	1	0017	5	6	422	1	OK		11-DTXB2
05112030000013	1	0020	0	1	449	1	OK		11-DTXB2
05112030000014	1	0020	5	6	210	1	OK		11-DTXB2
05112030000015	1	0022	0	1	576	1	OK		11-DTXB2
05112030000016	1	0022	5	6	492	1	OK		11-DTXB2
05112030000017	1	0023	0	1	179	1	OK		11-DTXB2
05112030000018	1	0023	5	6	171	1	OK		11-DTXB2
05112030000019	1	0025	0	1	300	1	OK		11-DTXB2
05112030000020	1	0025	5	6	186	1	OK		11-DTXB2
05112030000021	1	0028	0	1	465	1	OK		11-DTXB2
05112030000022	1	0028	5	6	327	1	OK		11-DTXB2
05112030000023	1	0029	0	1	61.1	1	OK		11-DTXB2
05112030000024	1	0029	5	6	216	1	OK		11-DTXB2
05112030000025	1	0031	0	1	517	1	OK		11-DTXB2



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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000026	1	0031	5	6	327	1	OK		11-DTXB2
05112030000027	1	0038	0	1	259	1	OK		11-DTXB2
05112030000028	1	0038	5	6	277	1	OK		11-DTXB2
05112030000029	1	0039	0	1	305	1	OK		11-DTXB2
05112030000030	1	0039	5	6	289	1	OK		11-DTXB2
05112030000031	1	0044	0	1	384	1	OK		11-DTXB2
05112030000032	1	0044	5	6	346	1	OK		11-DTXB2
05112030000033	1	0049	0	1	79.2	1	OK		11-DTXB2
05112030000034	1	0049	5	6	155	1	OK		11-DTXB2
05112030000035	1	0052	0	1	204	1	OK		11-DTXB2
05112030000036	1	0052	5	6	291	1	OK		11-DTXB2
05112030000037	1	0053	0	1	309	1	OK		11-DTXB2
05112030000038	1	0053	5	6	404	1	OK		11-DTXB2
05112030000039	1	0057	0	1	248	1	OK		11-DTXB2
05112030000040	1	0057	5	6	225	1	OK		11-DTXB2
05112030000041	1	0060	0	1	349	1	OK		11-DTXB2
05112030000042	1	0060	5	6	277	1	OK		11-DTXB2
05112030000043	2	0062	0	1	353	1	OK		11-DTXB2
05112030000044	2	0062	5	6	407	1	OK		11-DTXB2
05112030000045	1	0030	0	1	349	1	OK		11-DTXB2
05112030000046	1	0030	5	6	225	1	OK		11-DTXB2
05112030000047	1	0034	0	1	131	1	OK		11-DTXB2
05112030000048	1	0034	5	6	140	1	OK		11-DTXB2
05112030000049	1	0055	0	1	294	1	OK		11-DTXB2
05112030000050	1	0055	5	6	442	1	OK		11-DTXB2
05112030000051	2	0064	0	1	458	1	OK		11-DTXB2
05112030000052	2	0064	5	6	564	1	OK		11-DTXB2
05112030000107	1	0008	0	1	541	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000108	1	0008	5	6	335	1	OK		11-DTXB2
05112030000111	1	0035	0	1	133	1	OK		11-DTXB2
05112030000112	1	0035	5	6	329	1	OK		11-DTXB2
05112030000115	1	0001	0	1	75.8	1	OK		11-DTXB2
05112030000116	1	0001	5	6	228	1	OK		11-DTXB2
05112030000119	1	0004	0	1	668	1	OK		11-DTXB2
05112030000120	1	0004	5	6	132	1	OK		11-DTXB2
05112030000123	1	0013	0	1	315	1	OK		11-DTXB2
05112030000124	1	0013	5	6	165	1	OK		11-DTXB2
05112030000127	1	0021	0	1	200	1	OK		11-DTXB2
05112030000128	1	0021	5	6	205	1	OK		11-DTXB2
05112030000131	1	0037	0	1	869	1	OK		11-DTXB2
05112030000132	1	0037	5	6	709	1	OK		11-DTXB2
05112030000135	1	0042	0	1	549	1	OK		11-DTXB2
05112030000136	1	0042	5	6	510	1	OK		11-DTXB2
05112030000139	1	0051	0	1	508	1	OK		11-DTXB2
05112030000140	1	0051	5	6	171	1	OK		11-DTXB2
05112030000143	2	0063	0	1	403	1	OK		11-DTXB2
05112030000144	2	0063	5	6	259	1	OK		11-DTXB2
05112030000147	2	0066	0	1	276	1	OK		11-DTXB2
05112030000148	2	0066	5	6	129	1	OK		11-DTXB2
05112030000149		0067	0	1	-	2	Other	Not Reportable-Repeat UCR	11-DTXB2
05112030000151		0067	0	1	-	1	Other	Not Reportable-Repeat UCR	11-DTXB2
05112030000152	6	0067	5	6	103	1	OK		11-DTXB2
05112030000155	2	0069	0	1	476	1	OK		11-DTXB2
05112030000156	2	0069	5	6	441	1	OK		11-DTXB2
05112030000159	2	0071	0	1	317	1	OK		11-DTXB2
05112030000160	2	0071	5	6	469	1	OK		11-DTXB2



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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000163	2	0072	0	1	199	1	OK		11-DTXB2
05112030000164	2	0072	5	6	223	1	OK		11-DTXB2
05112030000167	2	0074	0	1	209	1	OK		11-DTXB2
05112030000168	2	0074	5	6	258	1	OK		11-DTXB2
05112030000171	2	0076	0	1	189	1	OK		11-DTXB2
05112030000172	2	0076	5	6	265	1	OK		11-DTXB2
05112030000175	2	0080	0	1	305	1	OK		11-DTXB2
05112030000176	2	0080	5	6	296	1	OK		11-DTXB2
05112030000179	2	0083	0	1	369	1	OK		11-DTXB2
05112030000180	2	0083	5	6	238	1	OK		11-DTXB2
05112030000183	2	0085	0	1	348	1	OK		11-DTXB2
05112030000187	2	0086	0	1	606	1	OK		11-DTXB2
05112030000188	2	0086	5	6	537	1	OK		11-DTXB2
05112030000191	2	0087	0	1	549	1	OK		11-DTXB2
05112030000192	2	0087	5	6	173	1	OK		11-DTXB2
05112030000195	2	0088	0	1	585	1	OK		11-DTXB2
05112030000196	2	0088	5	6	340	1	OK		11-DTXB2
05112030000199	2	0090	0	1	293	1	OK		11-DTXB2
05112030000200	2	0090	5	6	393	1	OK		11-DTXB2
05112030000203	2	0093	0	1	242	1	OK		11-DTXB2
05112030000204	2	0093	5	6	181	1	OK		11-DTXB2
05112030000207	2	0104	0	1	253	1	OK		11-DTXB2
05112030000208	2	0104	5	6	205	1	OK		11-DTXB2
05112030000211	2	0105	0	1	430	1	OK		11-DTXB2
05112030000212	2	0105	5	6	182	1	OK		11-DTXB2
05112030000215	2	0106	0	1	296	1	OK		11-DTXB2
05112030000216	2	0106	5	6	169	1	OK		11-DTXB2
05112030000219	2	0107	0	1	261	1	OK		11-DTXB2



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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000220	2	0107	5	6	142	1	OK		11-DTXB2
05112030000223	2	0110	0	1	453	1	OK		11-DTXB2
05112030000224	2	0110	5	6	459	1	OK		11-DTXB2
05112030000227	2	0112	0	1	297	1	OK		11-DTXB2
05112030000228	2	0112	5	6	297	1	OK		11-DTXB2
05112030000231	2	0114	0	1	549	1	OK		11-DTXB2
05112030000232	2	0114	5	6	506	1	OK		11-DTXB2
05112030000235	2	0117	0	1	436	1	OK		11-DTXB2
05112030000236	2	0117	5	6	195	1	OK		11-DTXB2
05112030000239	2	0118	0	1	602	1	OK		11-DTXB2
05112030000240	2	0118	5	6	531	1	OK		11-DTXB2
05112030000243	2	0121	0	1	383	1	OK		11-DTXB2
05112030000244	2	0121	5	6	233	1	OK		11-DTXB2
05112030000247	2	0122	0	1	314	1	OK		11-DTXB2
05112030000248	2	0122	5	6	200	1	OK		11-DTXB2
05112030000251	2	0123	0	1	248	1	OK		11-DTXB2
05112030000252	2	0123	5	6	212	1	OK		11-DTXB2
05112030000255	2	0126	0	1	331	1	OK		11-DTXB2
05112030000256	2	0126	5	6	266	1	OK		11-DTXB2
05112030000259	2	0127	0	1	341	1	OK		11-DTXB2
05112030000260	2	0127	5	6	402	1	OK		11-DTXB2
05112030000263	2	0128	0	1	657	1	OK		11-DTXB2
05112030000264	2	0128	5	6	404	1	OK		11-DTXB2
05112030000267	3	0129	0	1	426	1	OK		11-DTXB2
05112030000268	3	0129	5	6	232	1	OK		11-DTXB2
05112030000271	3	0130	0	1	428	1	OK		11-DTXB2
05112030000272	3	0130	5	6	219	1	OK		11-DTXB2
05112030000275	3	0133	0	1	420	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000276	3	0133	5	6	233	1	OK		11-DTXB2
05112030000279	3	0134	0	1	408	1	OK		11-DTXB2
05112030000280	3	0134	5	6	308	1	OK		11-DTXB2
05112030000283	3	0136	0	1	544	1	OK		11-DTXB2
05112030000284	3	0136	5	6	325	1	OK		11-DTXB2
05112030000287	3	0137	0	1	374	1	OK		11-DTXB2
05112030000288	3	0137	5	6	269	1	OK		11-DTXB2
05112030000291	3	0139	0	1	490	1	OK		11-DTXB2
05112030000292	3	0139	5	6	307	1	OK		11-DTXB2
05112030000295	3	0140	0	1	260	1	OK		11-DTXB2
05112030000296	3	0140	5	6	228	1	OK		11-DTXB2
05112030000299	3	0145	0	1	405	1	OK		11-DTXB2
05112030000300	3	0145	5	6	305	1	OK		11-DTXB2
05112030000303	3	0147	0	1	331	1	OK		11-DTXB2
05112030000304	3	0147	5	6	242	1	OK		11-DTXB2
05112030000307	3	0148	0	1	471	1	OK		11-DTXB2
05112030000308	3	0148	5	6	356	1	OK		11-DTXB2
05112030000311	3	0149	0	1	220	1	OK		11-DTXB2
05112030000312	3	0149	5	6	246	1	OK		11-DTXB2
05112030000315	3	0150	0	1	375	1	OK		11-DTXB2
05112030000316	3	0150	5	6	247	1	OK		11-DTXB2
05112030000319	3	0152	0	1	111	1	OK		11-DTXB2
05112030000320	3	0152	5	6	85.1	1	OK		11-DTXB2
05112030000323	6	0153	0	1	219	1	OK		11-DTXB2
05112030000324	3	0153	5	6	136	1	OK		11-DTXB2
05112030000327	3	0155	0	1	240	1	OK		11-DTXB2
05112030000328	3	0155	5	6	195	1	OK		11-DTXB2
05112030000331	3	0156	0	1	273	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000332	3	0156	5	6	203	1	OK		11-DTXB2
05112030000335	3	0160	0	1	251	1	OK		11-DTXB2
05112030000336	3	0160	5	6	172	1	OK		11-DTXB2
05112030000339	3	0162	0	1	506	1	OK		11-DTXB2
05112030000340	3	0162	5	6	401	1	OK		11-DTXB2
05112030000343	3	0167	0	1	514	1	OK		11-DTXB2
05112030000344	3	0167	5	6	309	1	OK		11-DTXB2
05112030000347	3	0169	0	1	113	1	OK		11-DTXB2
05112030000348	3	0169	5	6	132	1	OK		11-DTXB2
05112030000351	3	0170	0	1	292	1	OK		11-DTXB2
05112030000352	3	0170	5	6	214	1	OK		11-DTXB2
05112030000355	3	0177	0	1	375	1	OK		11-DTXB2
05112030000356	3	0177	5	6	341	1	OK		11-DTXB2
05112030000359	3	0181	0	1	514	1	OK		11-DTXB2
05112030000360	3	0181	5	6	221	1	OK		11-DTXB2
05112030000363	3	0183	0	1	290	1	OK		11-DTXB2
05112030000364	3	0183	5	6	254	1	OK		11-DTXB2
05112030000367	3	0185	0	1	513	1	OK		11-DTXB2
05112030000368	3	0185	5	6	560	1	OK		11-DTXB2
05112030000371	3	0187	0	1	252	1	OK		11-DTXB2
05112030000372	3	0187	5	6	594	1	OK		11-DTXB2
05112030000375	3	0189	0	1	283	1	OK		11-DTXB2
05112030000376	3	0189	5	6	306	1	OK		11-DTXB2
05112030000379	3	0190	0	1	208	1	OK		11-DTXB2
05112030000380	3	0190	5	6	228	1	OK		11-DTXB2
05112030000383	3	0191	0	1	289	1	OK		11-DTXB2
05112030000384	3	0191	5	6	238	1	OK		11-DTXB2
05112030000387	3	0192	0	1	410	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000388	3	0192	5	6	364	1	OK		11-DTXB2
05112030000391	4	0198	0	1	452	1	OK		11-DTXB2
05112030000392	4	0198	5	6	376	1	OK		11-DTXB2
05112030000395	3	0193	0	1	788	1	OK		11-DTXB2
05112030000396	3	0193	5	6	387	1	OK		11-DTXB2
05112030000399	3	0195	0	1	291	1	OK		11-DTXB2
05112030000400	3	0195	5	6	124	1	OK		11-DTXB2
05112030000403	4	0196	0	1	272	1	OK		11-DTXB2
05112030000404	4	0196	5	6	101	1	OK		11-DTXB2
05112030000407	4	0197	0	1	503	1	OK		11-DTXB2
05112030000408	4	0197	5	6	305	1	OK		11-DTXB2
05112030000411	4	0200	0	1	222	1	OK		11-DTXB2
05112030000412	4	0200	5	6	119	1	OK		11-DTXB2
05112030000415	4	0202	0	1	849	1	OK		11-DTXB2
05112030000416	4	0202	5	6	239	1	OK		11-DTXB2
05112030000419	4	0203	0	1	213	1	OK		11-DTXB2
05112030000420	4	0203	5	6	117	1	OK		11-DTXB2
05112030000423	4	0204	0	1	209	1	OK		11-DTXB2
05112030000424	4	0204	5	6	203	1	OK		11-DTXB2
05112030000427	4	0206	0	1	450	1	OK		11-DTXB2
05112030000428	4	0206	5	6	341	1	OK		11-DTXB2
05112030000431	4	0210	0	1	242	1	OK		11-DTXB2
05112030000432	4	0210	5	6	117	1	OK		11-DTXB2
05112030000433		0211	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000435		0211	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000439	4	0216	0	1	411	1	OK		11-DTXB2
05112030000440	4	0216	5	6	216	1	OK		11-DTXB2
05112030000443	4	0218	0	1	513	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000444	4	0218	5	6	250	1	OK		11-DTXB2
05112030000447	4	0220	0	1	214	1	OK		11-DTXB2
05112030000448	4	0220	5	6	69.5	1	OK		11-DTXB2
05112030000451	4	0224	0	1	501	1	OK		11-DTXB2
05112030000452	4	0224	5	6	385	1	OK		11-DTXB2
05112030000455	4	0228	0	1	570	1	OK		11-DTXB2
05112030000456	4	0228	5	6	134	1	OK		11-DTXB2
05112030000459	4	0229	0	1	455	1	OK		11-DTXB2
05112030000460	4	0229	5	6	374	1	OK		11-DTXB2
05112030000463	4	0230	0	1	561	1	OK		11-DTXB2
05112030000464	4	0230	5	6	307	1	OK		11-DTXB2
05112030000467	4	0232	0	1	214	1	OK		11-DTXB2
05112030000468	4	0232	5	6	120	1	OK		11-DTXB2
05112030000471	4	0240	0	1	216	1	OK		11-DTXB2
05112030000472	4	0240	5	6	147	1	OK		11-DTXB2
05112030000475	4	0241	0	1	302	1	OK		11-DTXB2
05112030000476	4	0241	5	6	223	1	OK		11-DTXB2
05112030000477		0242	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000479		0242	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000483	6	0244	0	1	337	1	OK		11-DTXB2
05112030000484	4	0244	5	6	262	1	OK		11-DTXB2
05112030000485		0245	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000487		0245	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000489		0247	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000491		0247	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000495	4	0249	0	1	429	1	OK		11-DTXB2
05112030000496	4	0249	5	6	452	1	OK		11-DTXB2
05112030000499	4	0251	0	1	222	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000500	4	0251	5	6	154	1	OK		11-DTXB2
05112030000503	4	0252	0	1	519	1	OK		11-DTXB2
05112030000504	4	0252	5	6	328	1	OK		11-DTXB2
05112030000507	4	0255	0	1	454	1	OK		11-DTXB2
05112030000508	4	0255	5	6	379	1	OK		11-DTXB2
05112030000511	4	0256	0	1	395	1	OK		11-DTXB2
05112030000512	4	0256	5	6	233	1	OK		11-DTXB2
05112030000515	4	0262	0	1	476	1	OK		11-DTXB2
05112030000516	4	0262	5	6	298	1	OK		11-DTXB2
05112030000519	4	0264	0	1	307	1	OK		11-DTXB2
05112030000520	4	0264	5	6	118	1	OK		11-DTXB2
05112030000523	4	0265	0	1	403	1	OK		11-DTXB2
05112030000524	4	0265	5	6	301	1	OK		11-DTXB2
05112030000527	4	0234	0	1	544	1	OK		11-DTXB2
05112030000528	4	0234	5	6	316	1	OK		11-DTXB2
05112030000535	4	0266	0	1	245	1	OK		11-DTXB2
05112030000536	4	0266	5	6	236	1	OK		11-DTXB2
05112030000539	4	0272	0	1	789	1	OK		11-DTXB2
05112030000540	4	0272	5	6	520	1	OK		11-DTXB2
05112030000543	4	0273	0	1	296	1	OK		11-DTXB2
05112030000544	4	0273	5	6	223	1	OK		11-DTXB2
05112030000547	4	0276	0	1	356	1	OK		11-DTXB2
05112030000548	4	0276	5	6	314	1	OK		11-DTXB2
05112030000551	5	0277	0	1	509	1	OK		11-DTXB2
05112030000552	5	0277	5	6	274	1	OK		11-DTXB2
05112030000555	5	0278	0	1	97.7	1	OK		11-DTXB2
05112030000556	5	0278	5	6	137	1	OK		11-DTXB2
05112030000559	5	0279	0	1	423	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000560	5	0279	5	6	479	1	OK		11-DTXB2
05112030000563	5	0281	0	1	1090	1	OK		11-DTXB2
05112030000564	5	0281	5	6	803	1	OK		11-DTXB2
05112030000567	5	0282	0	1	548	1	OK		11-DTXB2
05112030000568	5	0282	5	6	260	1	OK		11-DTXB2
05112030000571	5	0283	0	1	273	1	OK		11-DTXB2
05112030000572	5	0283	5	6	172	1	OK		11-DTXB2
05112030000575	5	0285	0	1	485	1	OK		11-DTXB2
05112030000576	5	0285	5	6	420	1	OK		11-DTXB2
05112030000579	5	0287	0	1	516	1	OK		11-DTXB2
05112030000580	5	0287	5	6	442	1	OK		11-DTXB2
05112030000581		0288	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000583		0288	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000587	5	0289	0	1	375	1	OK		11-DTXB2
05112030000588	5	0289	5	6	229	1	OK		11-DTXB2
05112030000591	5	0291	0	1	524	1	OK		11-DTXB2
05112030000592	5	0291	5	6	259	1	OK		11-DTXB2
05112030000595	5	0292	0	1	404	1	OK		11-DTXB2
05112030000596	5	0292	5	6	287	1	OK		11-DTXB2
05112030000599	5	0296	0	1	428	1	OK		11-DTXB2
05112030000600	5	0296	5	6	478	1	OK		11-DTXB2
05112030000603	5	0298	0	1	488	1	OK		11-DTXB2
05112030000604	5	0298	5	6	468	1	OK		11-DTXB2
05112030000605		0299	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000607		0299	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000611	5	0300	0	1	244	1	OK		11-DTXB2
05112030000612	5	0300	5	6	211	1	OK		11-DTXB2
05112030000615	5	0301	0	1	447	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000616	5	0301	5	6	311	1	OK		11-DTXB2
05112030000619	5	0306	0	1	136	1	OK		11-DTXB2
05112030000620	5	0306	5	6	148	1	OK		11-DTXB2
05112030000623	5	0307	0	1	365	1	OK		11-DTXB2
05112030000624	5	0307	5	6	257	1	OK		11-DTXB2
05112030000627	5	0308	0	1	693	1	OK		11-DTXB2
05112030000628	5	0308	5	6	527	1	OK		11-DTXB2
05112030000629		0309	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000631		0309	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000633		0312	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000635		0312	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000639	5	0313	0	1	367	1	OK		11-DTXB2
05112030000640	5	0313	5	6	282	1	OK		11-DTXB2
05112030000643	5	0315	0	1	301	1	OK		11-DTXB2
05112030000644	5	0315	5	6	330	1	OK		11-DTXB2
05112030000647	5	0316	0	1	702	1	OK		11-DTXB2
05112030000648	5	0316	5	6	318	1	OK		11-DTXB2
05112030000651	5	0317	0	1	263	1	OK		11-DTXB2
05112030000652	5	0317	5	6	232	1	OK		11-DTXB2
05112030000655	5	0318	0	1	410	1	OK		11-DTXB2
05112030000656	5	0318	5	6	557	1	OK		11-DTXB2
05112030000659	5	0320	0	1	845	1	OK		11-DTXB2
05112030000660	5	0320	5	6	631	1	OK		11-DTXB2
05112030000663	5	0321	0	1	483	1	OK		11-DTXB2
05112030000664	5	0321	5	6	537	1	OK		11-DTXB2
05112030000667	5	0322	0	1	499	1	OK		11-DTXB2
05112030000668	5	0322	5	6	203	1	OK		11-DTXB2
05112030000671	5	0325	0	1	307	1	OK		11-DTXB2



11-Dehydrothromboxane B₂ in Human Urine
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Custom ID	Run ID	Subject	Start Day Nominal	Day Nominal	Concentration (pg/mL)	Split	Sample Condition	Sample Comments	Analyte
05112030000672	5	0325	5	6	371	1	OK		11-DTXB2
05112030000673		0269	0	1	.	2	Other	Analysis not required	11-DTXB2
05112030000675		0269	0	1	.	1	Other	Analysis not required	11-DTXB2
05112030000679	5	0328	0	1	251	1	OK		11-DTXB2
05112030000680	5	0328	5	6	242	1	OK		11-DTXB2



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Table 6 Summary of Reassay for Analytical Reasons

Run ID	Reason	Sample Name
2	UCR	AA99071-11 05112030000151 0067 N/A P1 Day 1 URN-1
2	UCR	AA99071-11 05112030000152 0067 N/A P1 Day 6 URN-1
3	UCR	AA99071-11 05112030000323 0153 N/A P1 Day 1 URN-1
4	UCR	AA99071-11 05112030000483 0244 N/A P1 Day 1 URN-1
6	UCR	AA99071-11 05112030000151 0067 N/A P1 Day 1 URN-1



11-Dehydrothromboxane B₂ in Human Urine
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Table 7. Incurred Sample Reproducibility Assessment

Subject	Period	Time Point	Analyte	Units	Original Value	Reassay Value	Mean Value	% Difference	Reproducible?	Event?	% of Passing ISR Samples
0010	1	Day 1	11-DTXB2	pg/mL	137	131	134	4.48	Pass	No	88.9
0011	1	Day 1	11-DTXB2	pg/mL	612	546	579	11.40	Pass	No	
0029	1	Day 1	11-DTXB2	pg/mL	61.1	62.6	61.9	2.42	Pass	No	
0049	1	Day 1	11-DTXB2	pg/mL	79.2	66.1	72.7	18.02	Pass	No	
0034	1	Day 1	11-DTXB2	pg/mL	131	130	131	0.76	Pass	No	
0064	1	Day 6	11-DTXB2	pg/mL	564	482	523	15.68	Pass	No	
0035	1	Day 1	11-DTXB2	pg/mL	133	107	120	21.67	Fail	No	
0001	1	Day 1	11-DTXB2	pg/mL	75.8	88.4	82.1	15.35	Pass	No	
0004	1	Day 1	11-DTXB2	pg/mL	668	629	649	6.01	Pass	No	
0066	1	Day 6	11-DTXB2	pg/mL	129	102	116	23.28	Fail	No	
0086	1	Day 1	11-DTXB2	pg/mL	606	517	562	15.84	Pass	No	
0088	1	Day 1	11-DTXB2	pg/mL	585	488	537	18.06	Pass	No	
0107	1	Day 6	11-DTXB2	pg/mL	142	144	143	1.40	Pass	No	
0118	1	Day 1	11-DTXB2	pg/mL	602	584	593	3.04	Pass	No	
0128	1	Day 1	11-DTXB2	pg/mL	657	542	600	19.17	Pass	No	
0152	1	Day 1	11-DTXB2	pg/mL	111	106	109	4.59	Pass	No	
0153	1	Day 6	11-DTXB2	pg/mL	136	126	131	7.63	Pass	No	
0169	1	Day 1	11-DTXB2	pg/mL	113	116	115	2.61	Pass	No	
0187	1	Day 6	11-DTXB2	pg/mL	594	533	564	10.82	Pass	No	
0193	1	Day 1	11-DTXB2	pg/mL	788	768	778	2.57	Pass	No	
0195	1	Day 6	11-DTXB2	pg/mL	124	108	116	13.79	Pass	No	
0200	1	Day 6	11-DTXB2	pg/mL	119	104	112	13.39	Pass	No	
0202	1	Day 1	11-DTXB2	pg/mL	849	824	837	2.99	Pass	No	
0203	1	Day 6	11-DTXB2	pg/mL	117	124	121	5.79	Pass	No	



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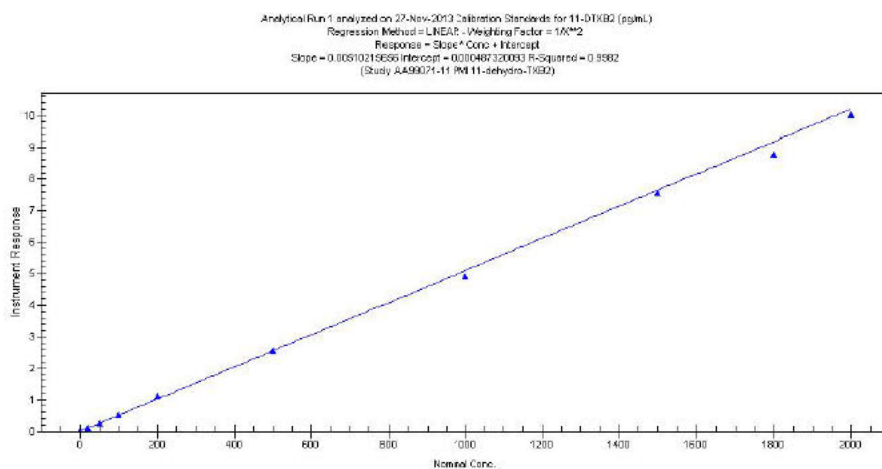
Subject	Period	Time Point	Analyte	Units	Original Value	Reassay Value	Mean Value	% Difference	Reproducible?	Event?	% of Passing ISR Samples
0220	1	Day 6	11-DTXB2	pg/mL	69.5	68.0	68.8	2.18	Pass	No	
0228	1	Day 1	11-DTXB2	pg/mL	570	493	532	14.47	Pass	No	
0232	1	Day 6	11-DTXB2	pg/mL	120	114	117	5.13	Pass	No	
0240	1	Day 6	11-DTXB2	pg/mL	147	136	142	7.75	Pass	No	
0264	1	Day 6	11-DTXB2	pg/mL	118	89.3	104	27.60	Fail	No	
0272	1	Day 1	11-DTXB2	pg/mL	789	723	756	8.73	Pass	No	
0278	1	Day 1	11-DTXB2	pg/mL	97.7	83.5	90.6	15.67	Pass	No	
0281	1	Day 1	11-DTXB2	pg/mL	1090	1010	1,050	7.62	Pass	No	
0306	1	Day 1	11-DTXB2	pg/mL	136	113	125	18.40	Pass	No	
0308	1	Day 1	11-DTXB2	pg/mL	693	559	626	21.41	Fail	No	
0316	1	Day 1	11-DTXB2	pg/mL	702	591	647	17.16	Pass	No	
0320	1	Day 1	11-DTXB2	pg/mL	845	843	844	0.24	Pass	No	



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FIGURES

Figure 1 Calibration Curve for 11-Dehydrothromboxane B₂ in Control Matrix, Watson Run ID 1¹



¹ Note: Though included on the figure above, the Standard 0 (blank sample extracted with internal standard) was not used as a standard to calculate the calibration curve parameters.



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ATTACHMENTS

Attachment 1 General List of Abbreviations used at Celerion

Abbreviations are used in this document as applicable.

Abbreviation	Description
°C	Degree Celsius (centigrade)
µg	Microgram
AAR	Above the acceptable range
AB	Applied Biosystems
API	Atmospheric pressure ionization
ASCII	American standard code for information interchange
BAM	Bioanalytical method
BLK	Blank
BLQ	Below limit of quantification
CC	Conventional Cigarette
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
CRO	Contract research organisation
CV	Coefficient of variation
Da	Dalton
DCU	Diluted concentration unreliable
DFNR	Dilution factor not reliable
DQC	Dilution quality control sample
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylenediaminetetraacetic acid
EQB	Exceeding quadratic bounds
EXT	Extraction
fg	Femtogram
g	Gram
GLP	Good laboratory practices
h	Hour
HDPE	High density polyethylene
HPLC	High performance liquid chromatography

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Abbreviation	Description
HSR	High standard removed
ID	Identifier
INC	Incongruous
INS	Instrumentation
IS	Internal standard
ISA	Insufficient volume for full analysis
ISP	Incomplete sample processing
ISR	Incurred sample reproducibility
ISV	Insufficient volume
IVR	Insufficient volume to reassay
L	Litre, liter
LLOQ	Lower limit of quantitation
LNK	Celerion, Lincoln site
M	Molar
mg	Milligram
mL	Millilitre, milliliter
mol	Mole
MS	Mass spectrometry
MW	Molecular weight
n	Number of data points
N/AP	Not applicable
N/AV	Not available
NFV	Not full volume
ng	Nanogram
No	Number
NU	Not used
OECD	Organization for Economic Cooperation and Development
PD	Period
pg	Picogram
QC	Quality control
QCs	Quality control samples
R E	Relative error
REF	Reference



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Abbreviation	Description
RI	Reinjection
RIA	Rarioimmunoassay
RT	Room temperature
RR	Reanalysis
RVL	Remaining volume low
S A	Smoking Abstinence
S D	Standard deviation
SOP	Standard operating procedure
SPE	Solid-phase extraction
SST	System suitability test
STD	Standard
Sub	Subject
SVD	Sample volume depleted
TBD	To be determined
Temp	Temperature
THS	Tobacco Heating System
UCR	Unacceptable chromatography
UISR	Unacceptable internal standard response
ULOQ	Upper limit of quantitation
U S FDA	United States Food and Drug Administration
USP	US pharmacopeia
\bar{x}	Mean



11-Dehydrothromboxane B₂ in Human Urine
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Attachment 2 Temperature Definitions at Celerion

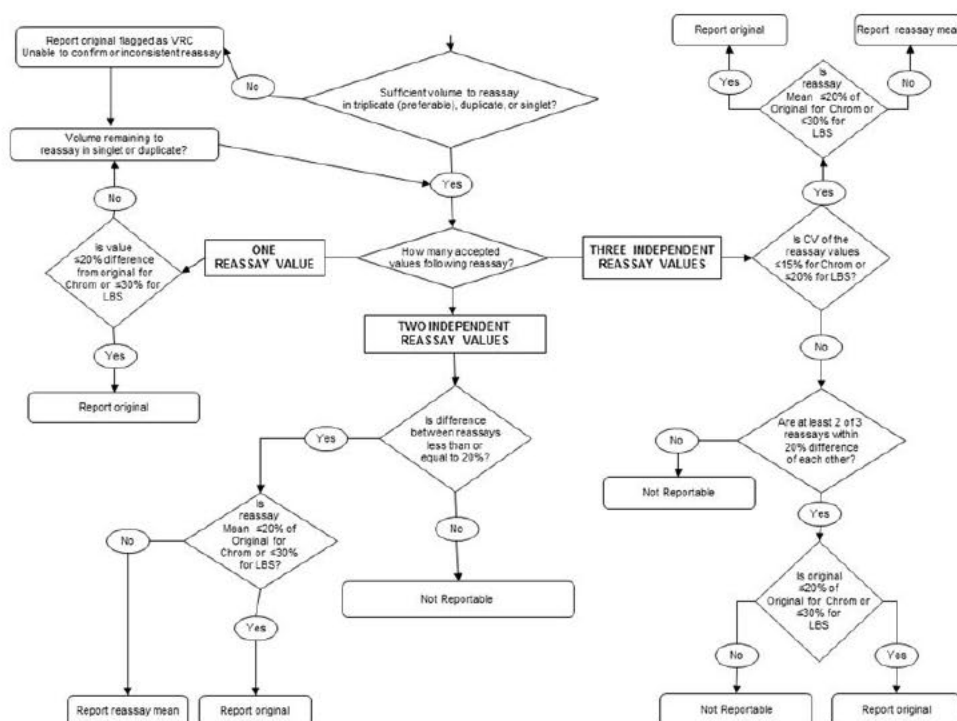
Values for temperatures are nominal temperatures representing the following temperature ranges:

Nominal temperature	Temperature Range
-80 C	-65 C to -90 C
-20 C	-10 C to -30 C
5 C	2 C to 8 C
Room temperature	15 C to 25 C
24 C	22 C to 26 C



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Attachment 3 Procedure for VRC and SSR Reassays and Reporting of Reassay Results



To compare reassays:

$$\frac{|\text{Re assay Value 1} - \text{Re assay Value 2}|}{\text{Mean of Re assay Value 1 and 2}} * 100\%$$

To compare to original:

$$\frac{|\text{Mean of Re assays} - \text{Original Value}|}{\text{Original Value}} * 100\%$$

An LC-MS/MS value as outlined in the decision tree is obtained from a single determination

If BLQ is obtained for a value, the nominal concentration of the LLOQ is used when comparing reassays in this decision tree.

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Attachment 4 General List of Calculation Formulae

Mean:

$$x_{\text{Mean}} = \frac{1}{n} \sum_{i=1}^n x_i$$

Standard Deviation (SD):

$$SD = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - x_{\text{Mean}})^2}$$

Precision (RSD, CV):

$$CV \% = \left(\frac{SD}{x_{\text{Mean}}} \right) * 100$$

Accuracy (% Theoretical):

$$\text{Accuracy \%} = \left(\frac{x}{x_{\text{Nominal}}} \right) * 100$$

$$\text{Accuracy of Mean \%} = \left(\frac{x_{\text{Mean}}}{x_{\text{Nominal}}} \right) * 100$$

Inaccuracy (% Bias, % RE):

$$\text{Bias \%} = \left(\frac{(x - x_{\text{Nominal}})}{x_{\text{Nominal}}} \right) * 100$$

$$\text{Bias of Mean \%} = \left(\frac{(x_{\text{Mean}} - x_{\text{Nominal}})}{x_{\text{Nominal}}} \right) * 100$$

x = value (e.g. analyte concentration, OD value, cpm value, peak signal)

n = number of values

$$\text{Potency} = \frac{100 - \left(\frac{\% \text{ Salts}}{\text{Determined By Assay}} + \frac{\% \text{ Water}}{\text{Content}} + \frac{\% \text{ Residual}}{\text{Solvent}} + \frac{\% \text{ Other}}{\text{Impurity}} \right)}{100} * \frac{\% \text{ Chromatographic Purity}}{100} * \frac{\% \text{ Chiral Purity}}{100} * \frac{\% \text{ Isotopic Purity}}{100} * \frac{\% \text{ Other Purity}}{100} * \frac{\text{MW Free Base}}{\text{MW Salt}}$$

$$\% \text{ Difference} = \left[\frac{|\text{Re assay} - \text{Original}|}{\left(\frac{\text{Re assay} + \text{Original}}{2} \right)} \right] * 100$$

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Attachment 5 Reassay Descriptions

Analytical Reason (Code)	Description
Above the Accepted Range (AAR)	Identifies a study sample whose calculated concentration is greater than the upper limit of quantitation (ULOQ). This study sample will be diluted before being reassayed.
Diluted Concentration Unreliable (DCU)	Identifies a study sample that has been diluted and determined to have a concentration below LLOQ (BLQ, below limit of quantification) before correction for the final dilution factor.
Dilution Factor Not Reliable (DFNR)	Identifies a study sample that has been diluted, and determined to have a measurable concentration, however >50% of the dilution QC samples (having the same dilution factor) did not meet their acceptance criteria. Identifies a dilution QC sample that does not fulfil the acceptance criterion and is excluded from the DQC statistics.
Highest / Lowest Standard Removed (HSR / LSR)	If the working range of the method is truncated as a result of - the ULOQ calibration standard being rejected or unavailable (e.g. incomplete sample processing or incomplete instrument analysis, unacceptable chromatography), all study samples with concentrations greater than the highest acceptable standard are identified as 'highest standard removed' (HSR). - the calibration standard at the LLOQ being rejected or unavailable (e.g. incomplete sample processing or incomplete instrument analysis, unacceptable chromatography), all study samples with concentrations below the lowest acceptable standard are identified as 'lowest standard removed' (LSR).
Incomplete Sample Processing (ISP)	Identifies a study sample, calibration standard, or QC sample for which data could not be obtained due to processing problems that occurred during the extraction or assay documented by the analyst prior to instrumental analysis.
Insufficient Volume for Reassay (IVR)	Identified a study sample that has insufficient sample volume for reanalysis (including all received splits)
Incomplete Instrument Analysis (IIA)	Identifies a study sample, calibration standard, or QC sample for which data could not be obtained due to processing problems that occurred during HPLC injection or instrumental analysis and were documented by the analyst.
Unacceptable Chromatography (UCR)	Identifies a study sample, calibration standard, or QC sample judged to demonstrate unacceptable chromatography according to the applicable Celerion procedures (e.g. split peak, poor peak symmetry, unseparated interference).



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Attachment 6 Certificates of Analysis



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11-Dehydrothromboxane B₂ in Human Urine
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Attachment 7 Bioanalytical Method Summary



11-Dehydrothromboxane B₂ in Human Urine
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BIOANALYTICAL METHOD SUMMARY (BMS)

Doc No: FOR_QM000496 – CR204A2

Version N°: 2.0

Page 1 of 2

Biomarker: 11-dehydro Thromboxane B ₂		Matrix: Urine
MVR/SOP no. & date: ZZ00102-02 / 03-Nov-2014		CRO/Laboratory: Celerion-Lincoln
LLOQ: 20.0 pg/mL		ULOQ: 2000 pg/mL
Validation	<input checked="" type="checkbox"/> Full <input type="checkbox"/> Partial <input type="checkbox"/> Cross Comments (required for Partial/Cross):	
Assay:	<input checked="" type="checkbox"/> Chromatographic <input type="checkbox"/> Ligand binding <input type="checkbox"/> Enzymatic <input type="checkbox"/> Other describe: <input type="checkbox"/> LC/MS <input checked="" type="checkbox"/> LC/MS/MS <input type="checkbox"/> GC/MS <input type="checkbox"/> GC/MS/MS <input type="checkbox"/> ELISA	
Equipment and short description of extraction and analysis: An aliquot of human urine containing the analyte and internal standard was extracted using a solid-phase extraction procedure. The extracted samples were analyzed by an UPLC equipped with an AB SCIEX API 5000 TM mass spectrometer. Negative ions were monitored in the multiple reaction monitoring (MRM) mode. Quantitation was determined using a weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and internal standard.		
Selectivity/Sensitivity/Matrix effect:	No significant matrix effect was observed in any 17 of the 18 human urine lots that were spiked near the concentration of the LLOQ and in any of the 18 human urine lots that were spiked near the concentration of the high QC sample	
Accuracy:	Intra-batch: -9.0 to 8.7% R.E. Inter-batch: -3.8 to 2.9% R.E.	
Precision:	Intra-batch: 2.3 to 11.2% C.V. Inter-batch: 4.6 to 9.1% C.V.	
Recovery:	83.1% at 50.0 pg/mL in human urine 83.9% at 200 pg/mL in human urine 82.1% at 1500 pg/mL in human urine	
Freeze and thaw stability:	6 cycles in polypropylene tubes at -20°C under white light	
Short-term temperature stability:	27 hours in polypropylene tubes at ambient temperature under white light	
Long-term stability:	76 days in polypropylene tubes at -20°C (LTS A) and 434 days in polypropylene tubes at -20°C (LTS B, C and D); 14 days in polypropylene tubes at -80°C	
Stock solution stability:	126 days at 100 µg/mL in ethanol in a polypropylene container at -20°C	
Post-preparative stability:	219 hours in injection vials with silanized glass inserts at 5°C	
Stability of Analyte During Sample Collection and Handling:	Up to 96 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C and 20°C under UV-shielded light, up to 24 hours in smoker and non-smoker human urine in high-density polyethylene containers at 40°C under UV-shielded light	



11-Dehydrothromboxane B₂ in Human Urine
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PMI RESEARCH & DEVELOPMENT

BIOANALYTICAL METHOD SUMMARY (BMS)

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Version NF: 2.0

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Accreditation/ GLP compliance/ QA statements:	GLP Compliance as Assay Validation conforms to Celerion Standard Operating Procedures which were written in compliance with FDA: Guidance to Industry "Bioanalytical Method Validation"	
BMS completed by:		
Name: Erica Nachi	Date: 03-Nov-2014	Signature:



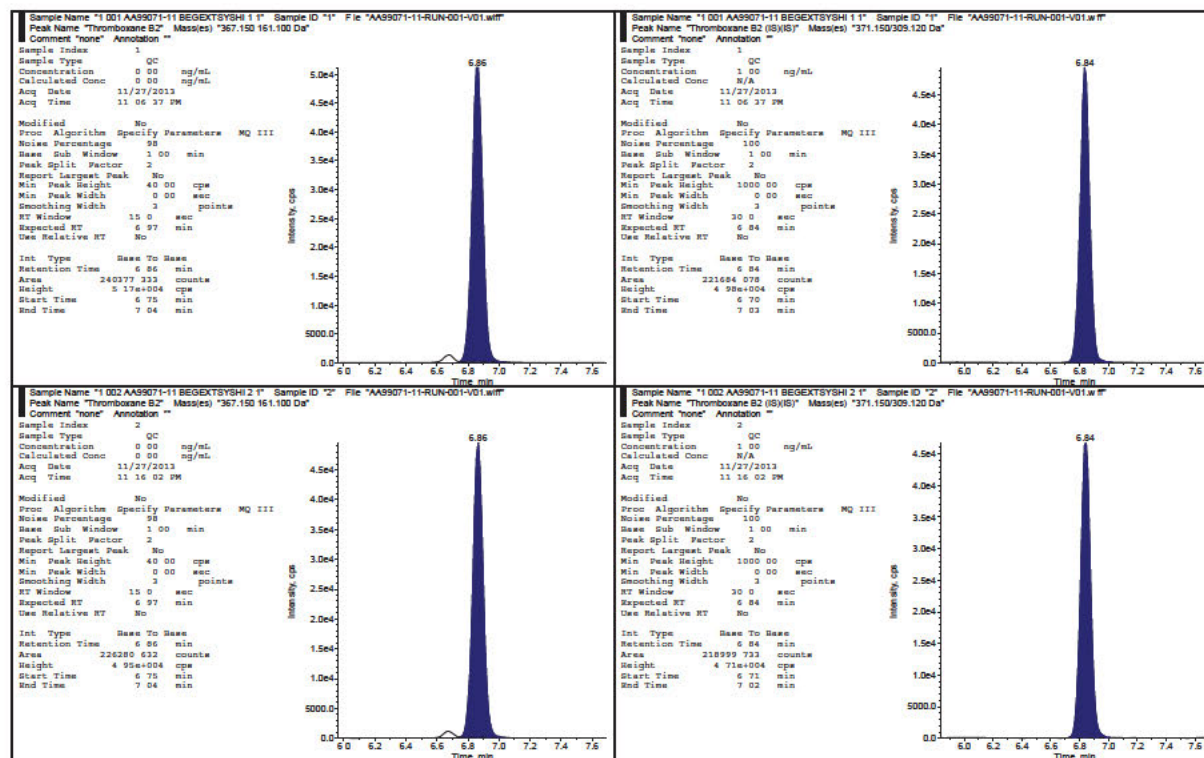
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Attachment 8 Chromatograms

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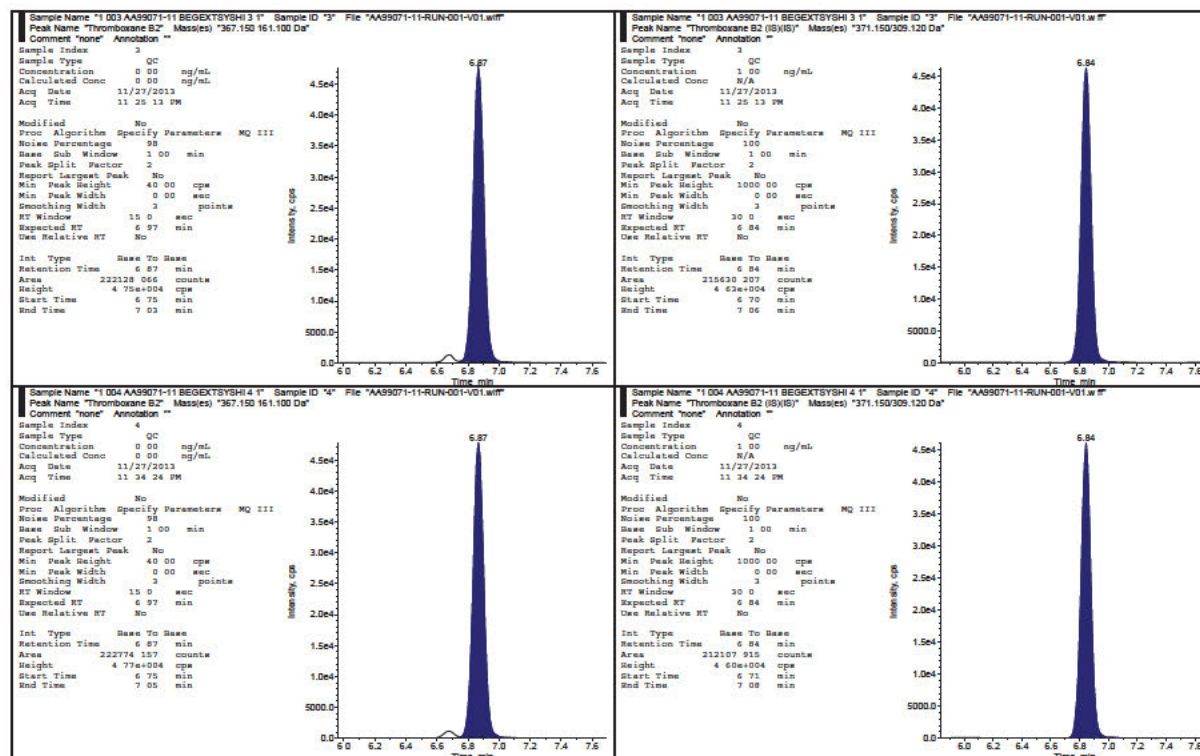


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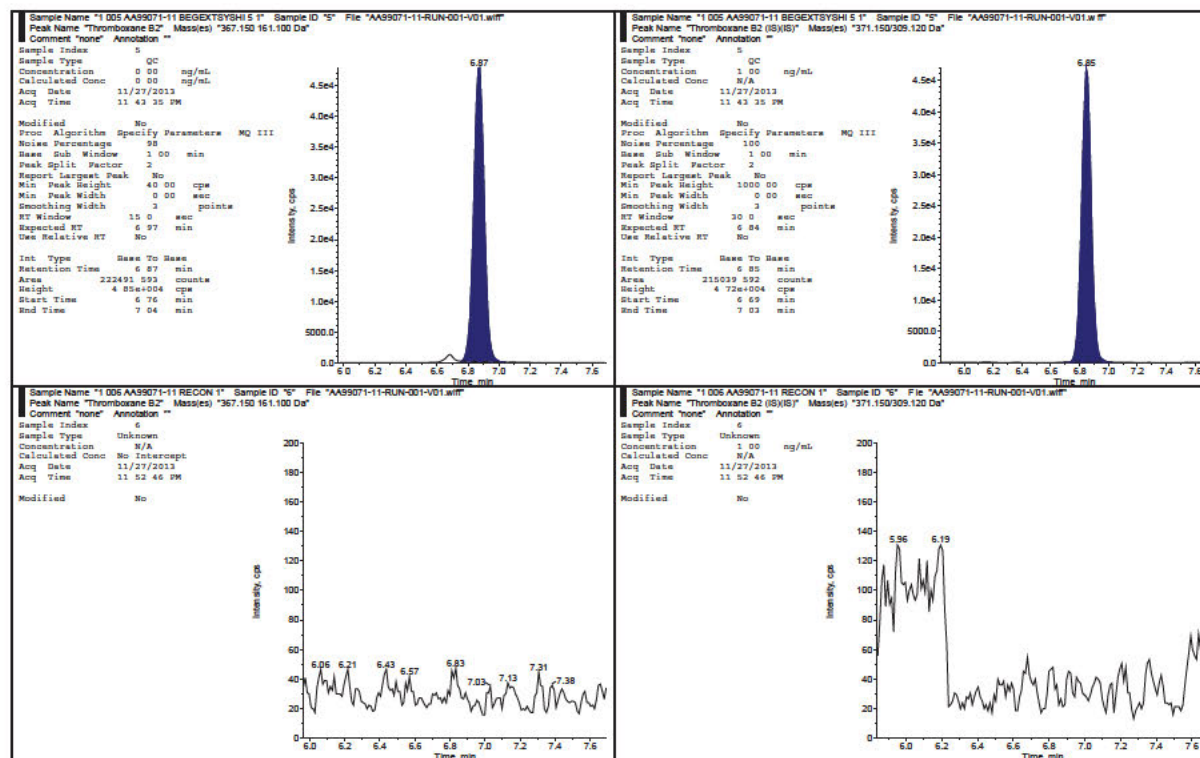


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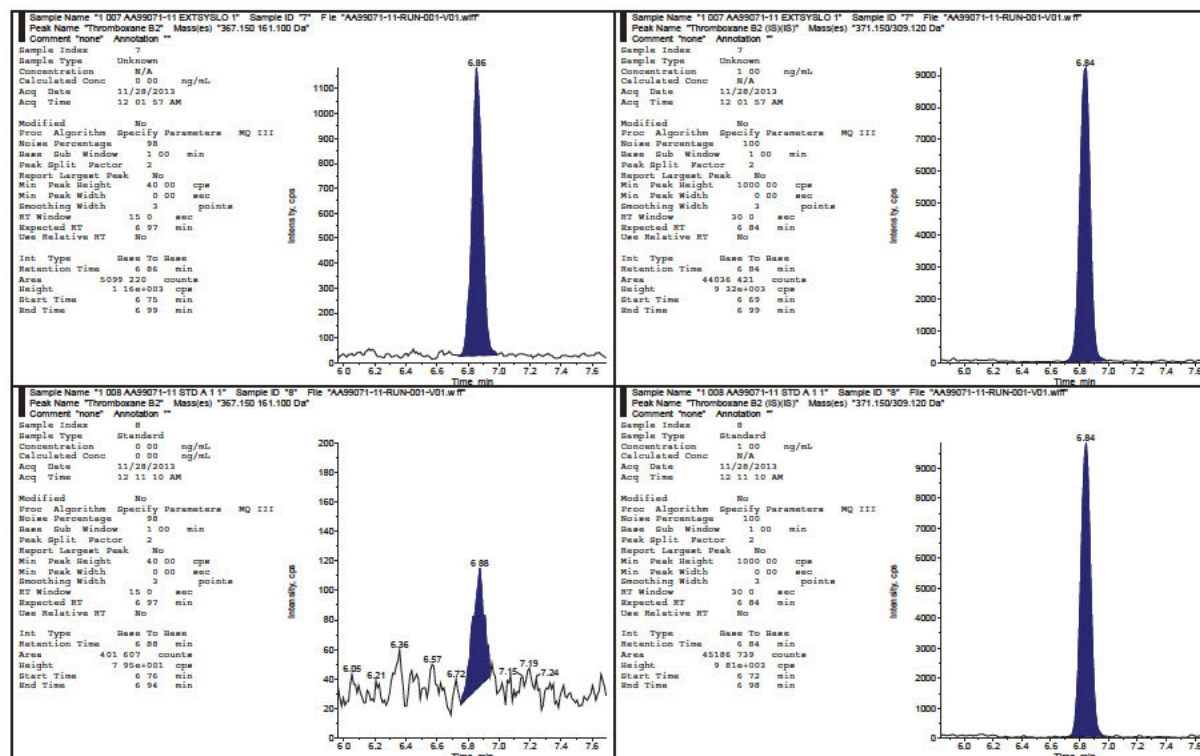


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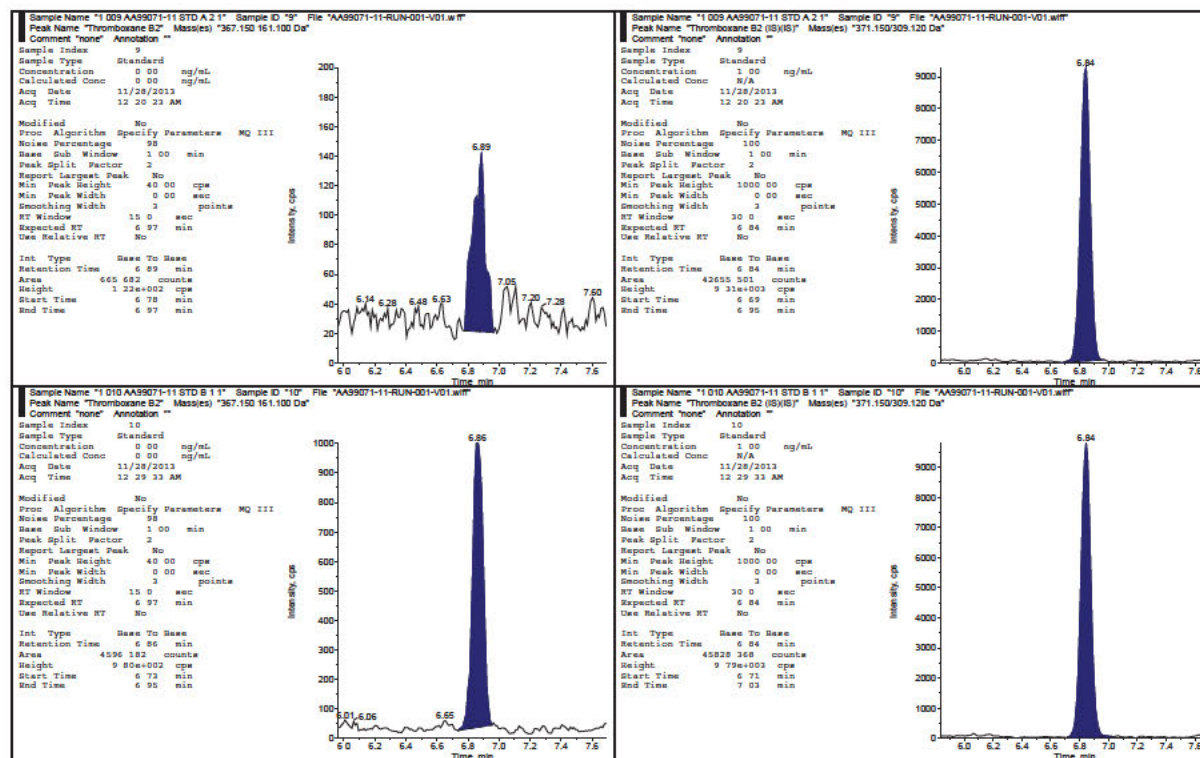


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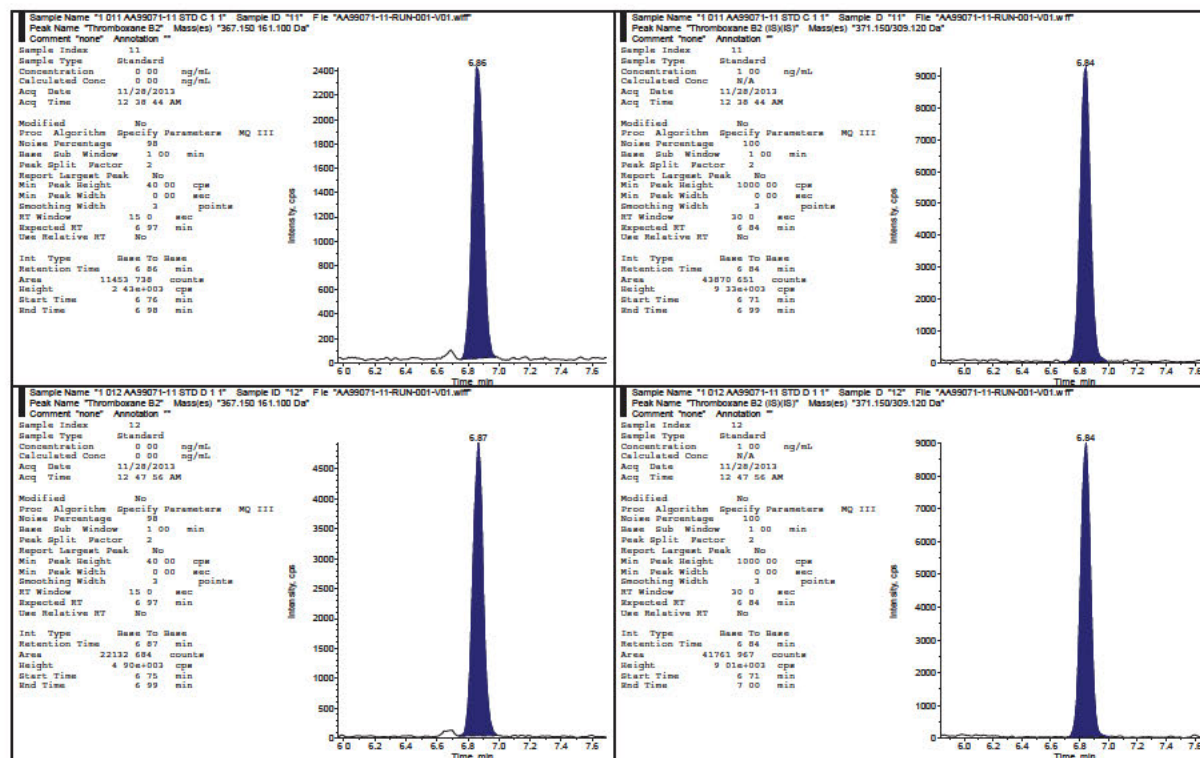


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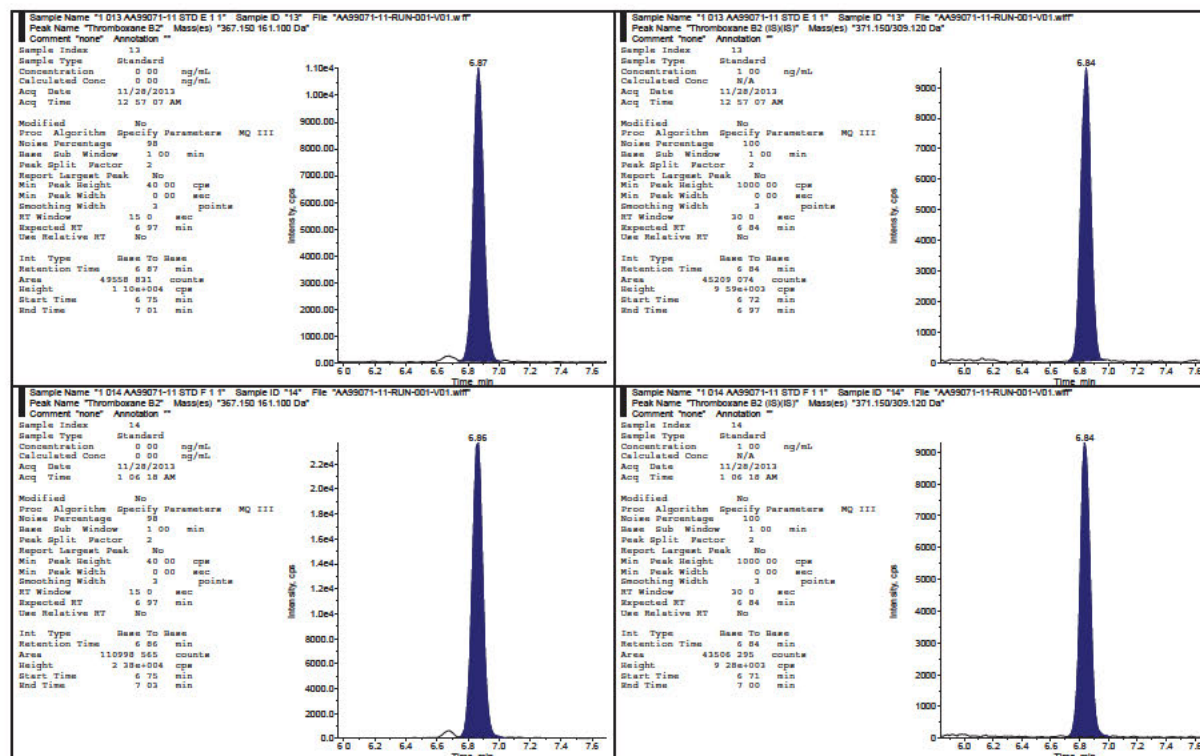


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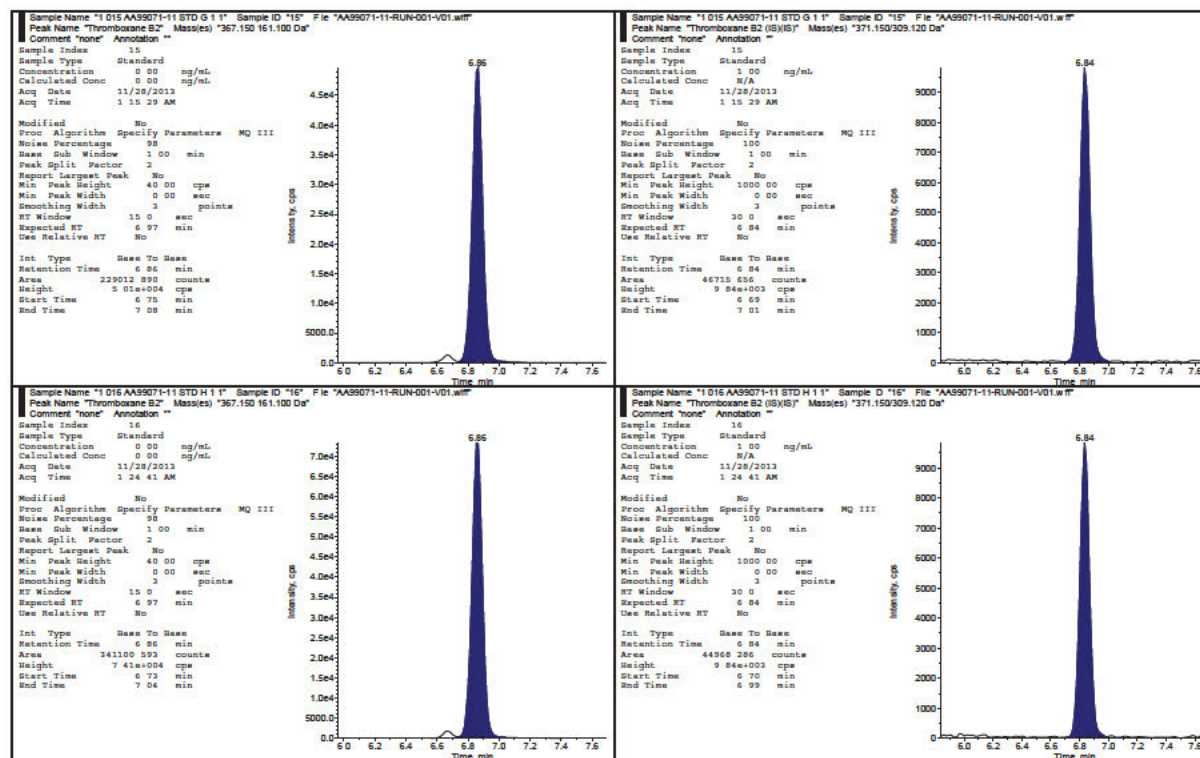


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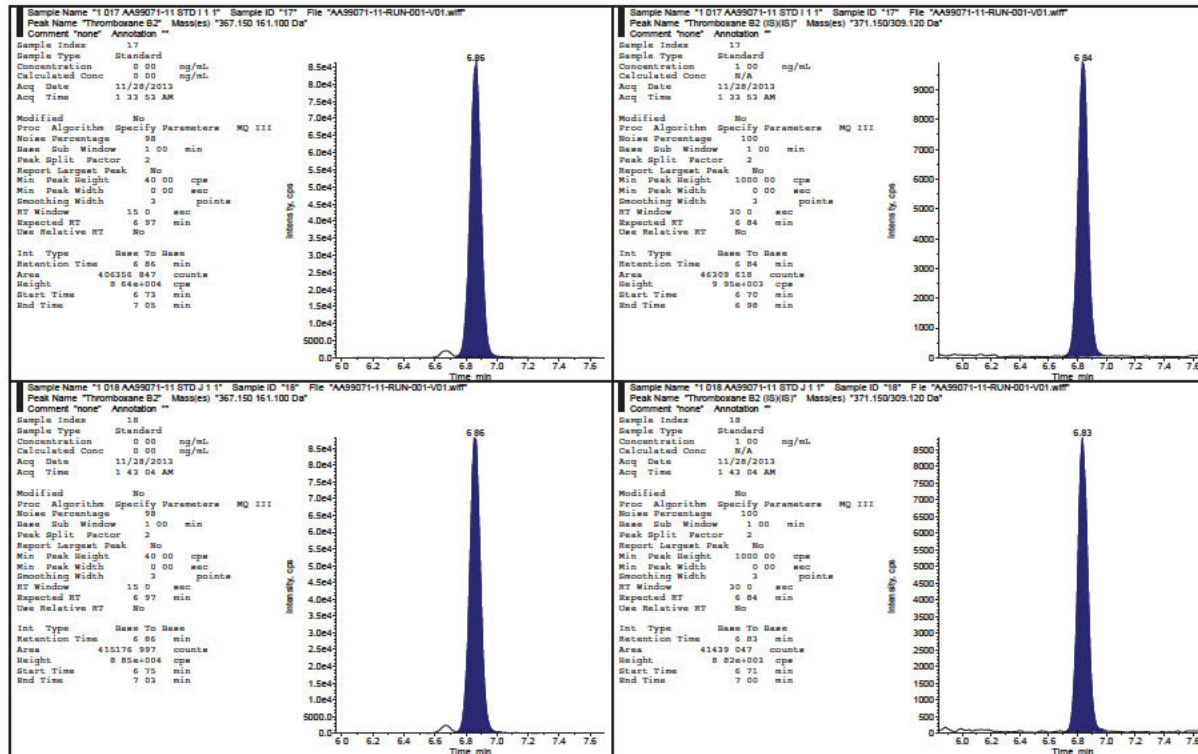


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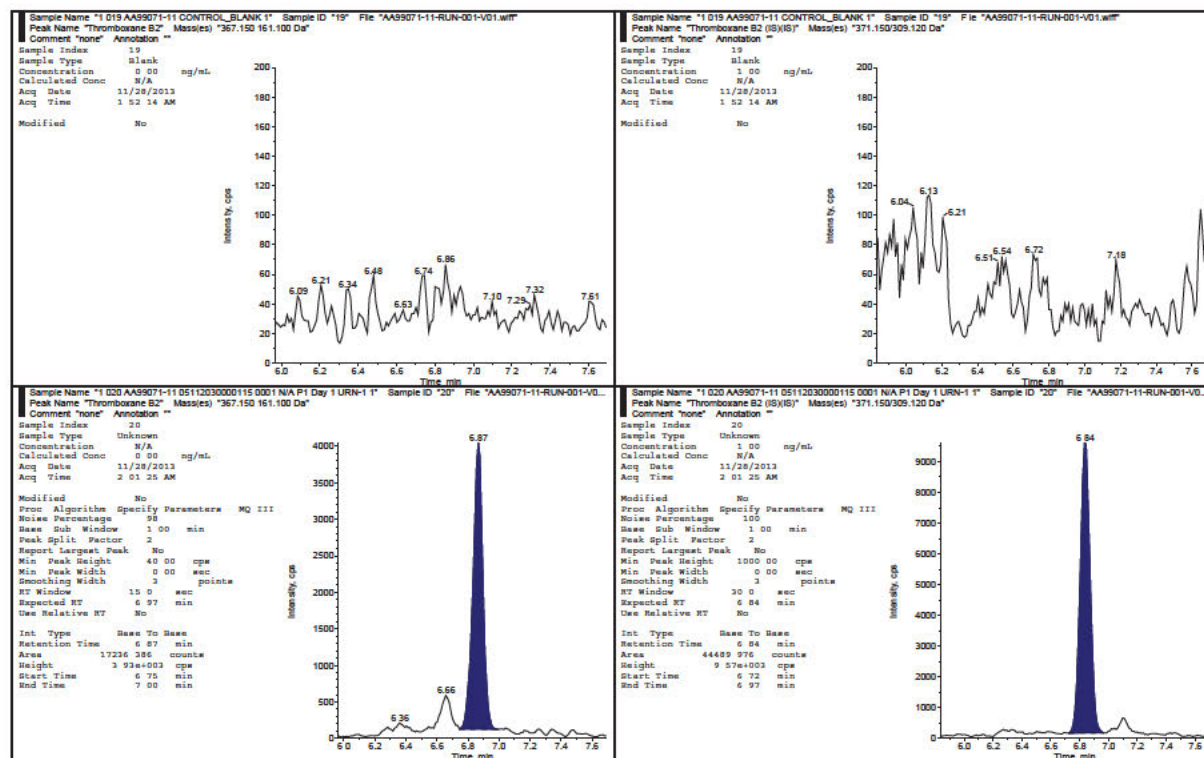


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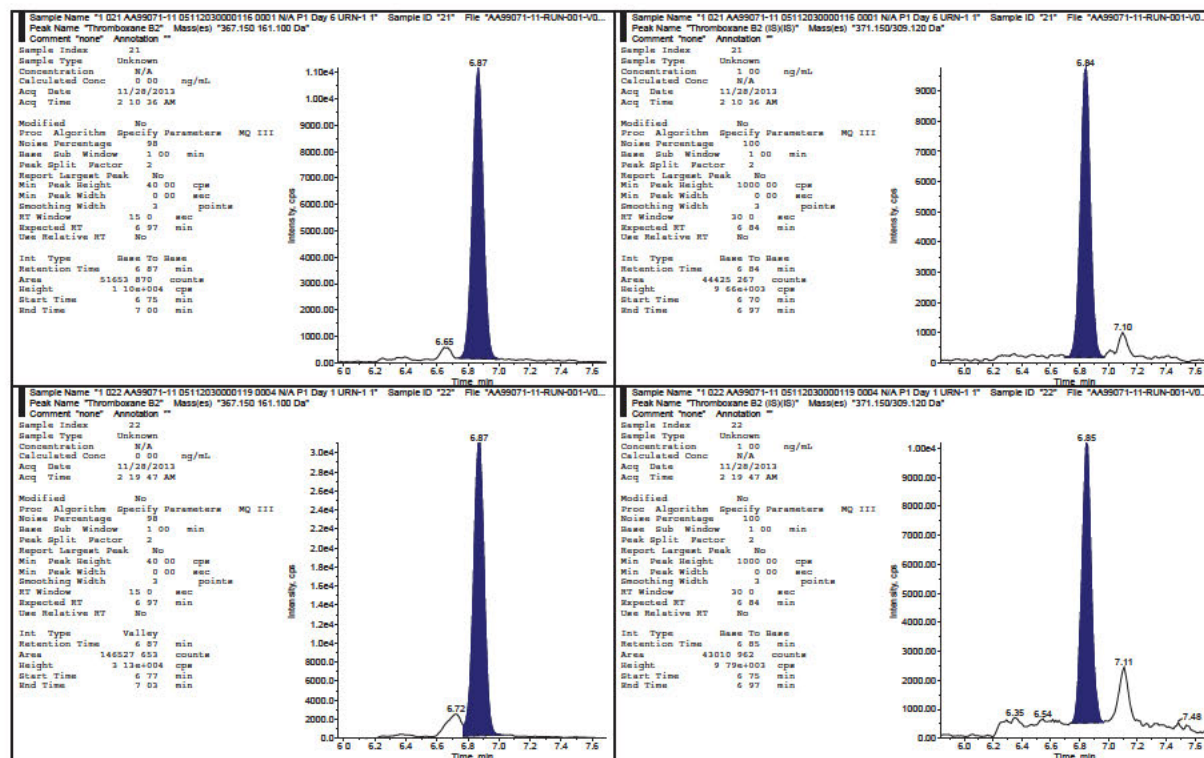


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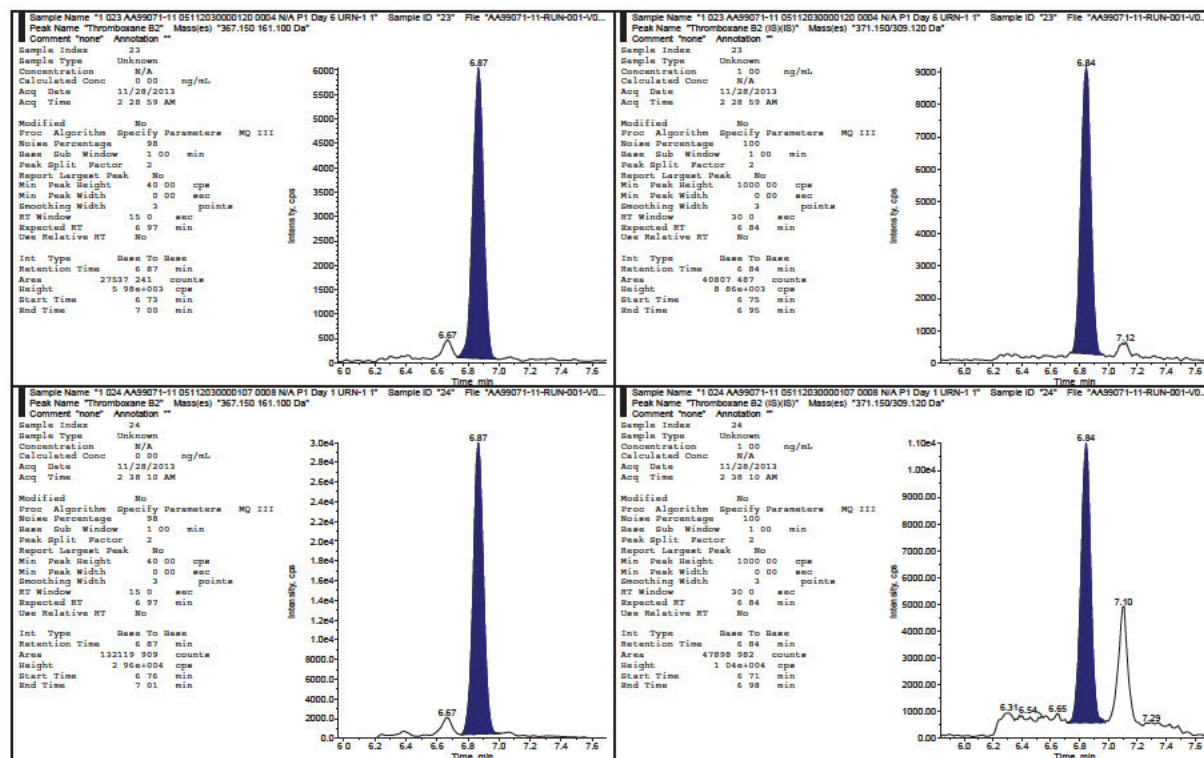


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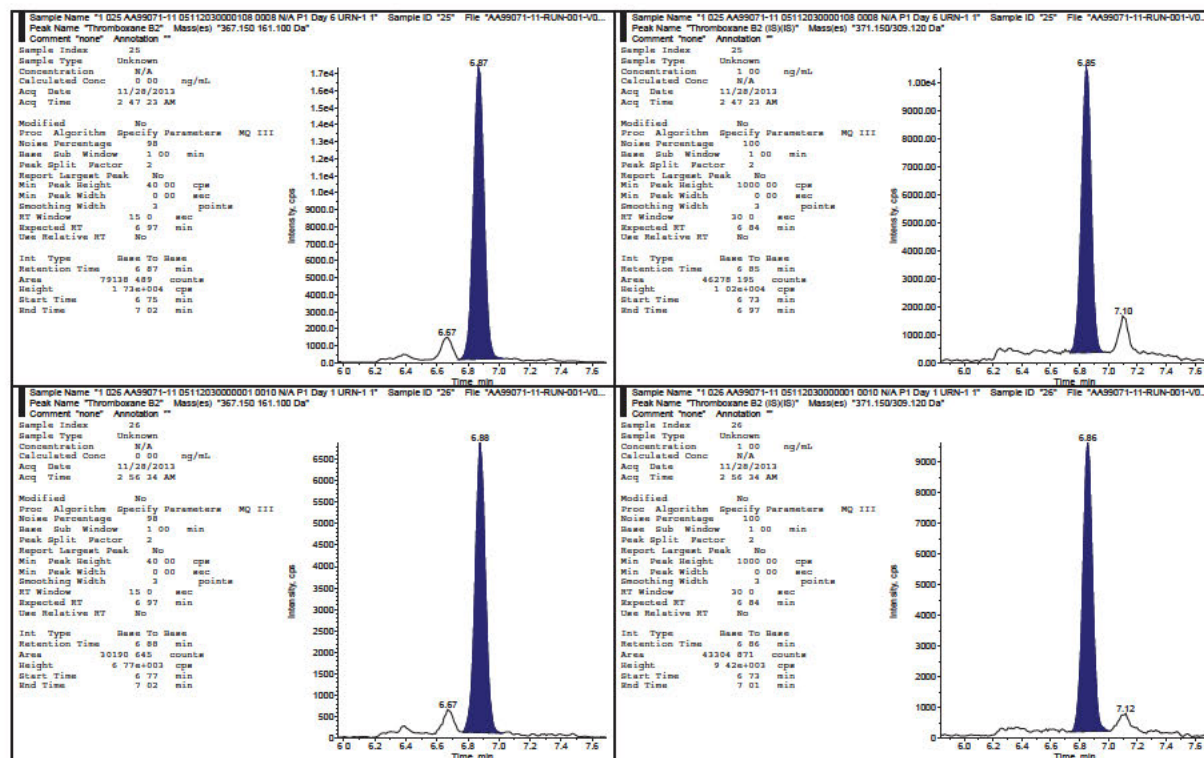


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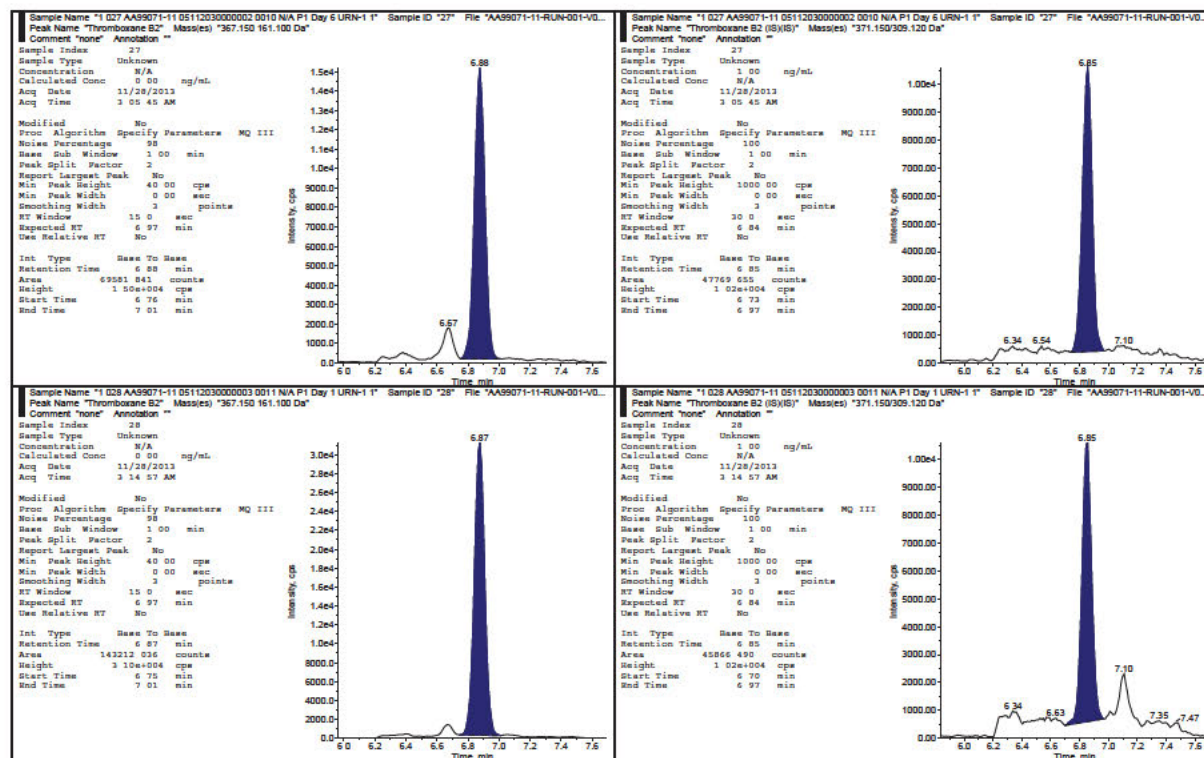


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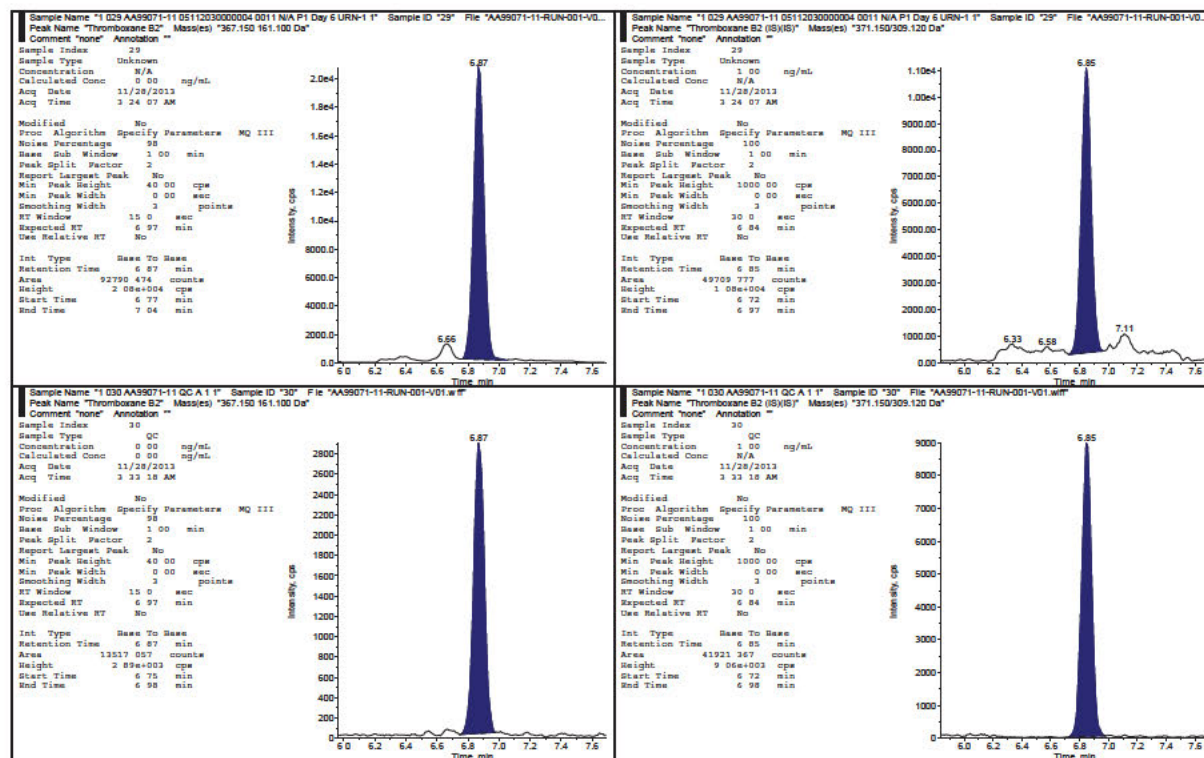


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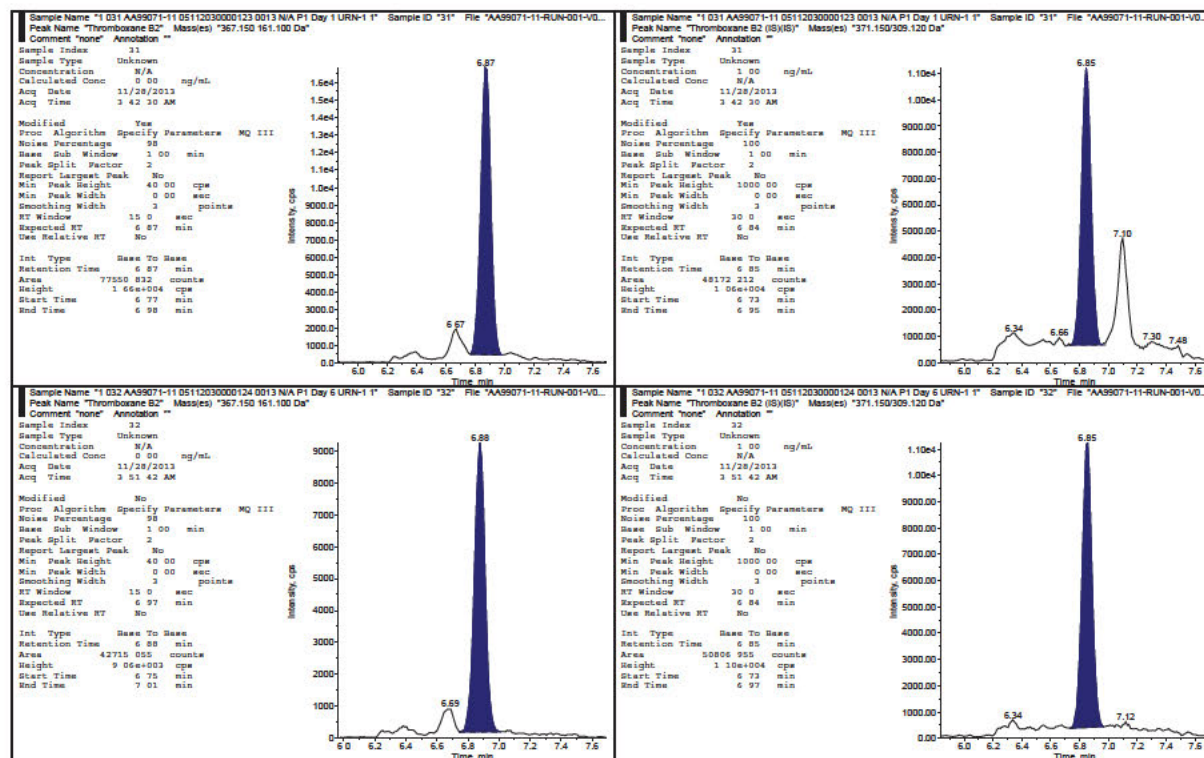


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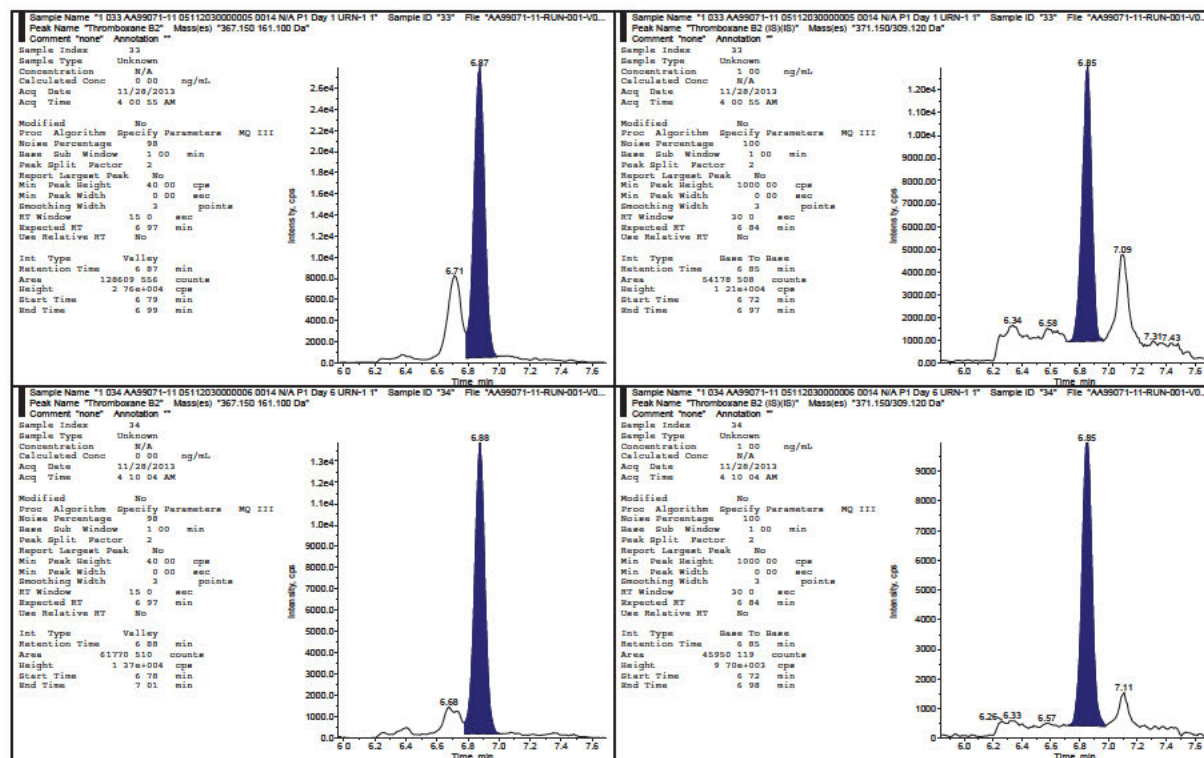


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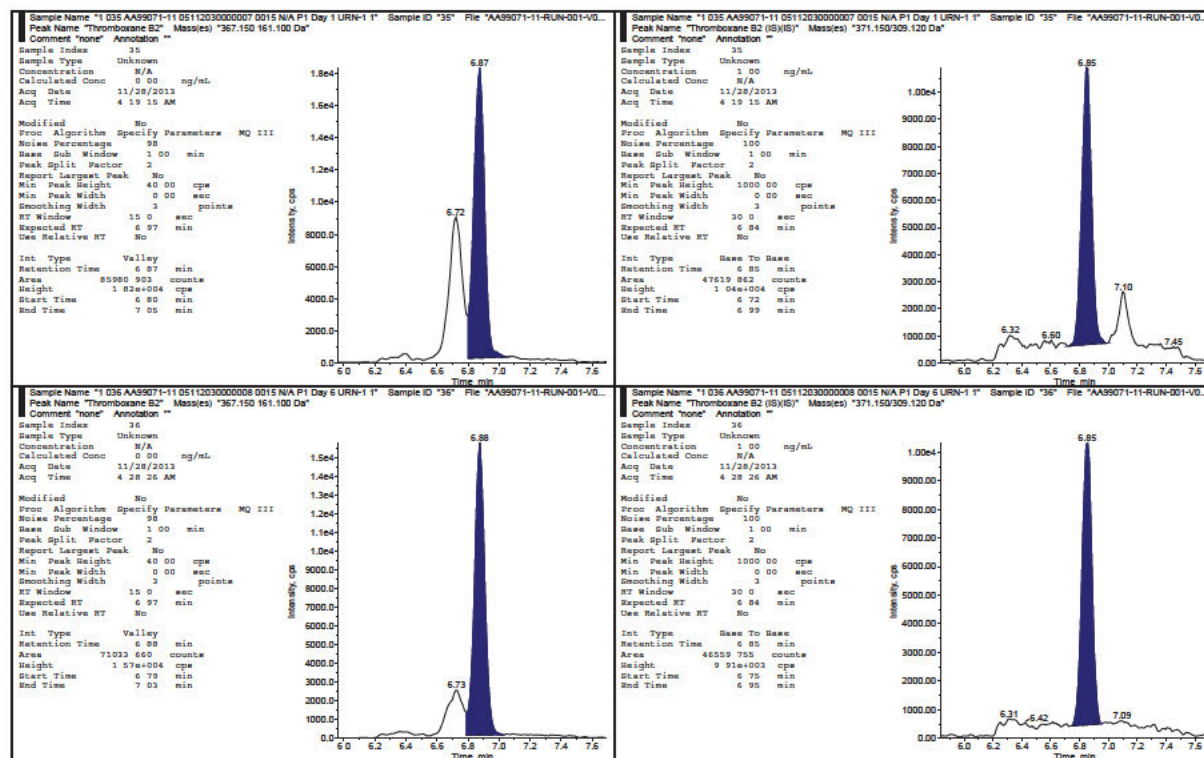


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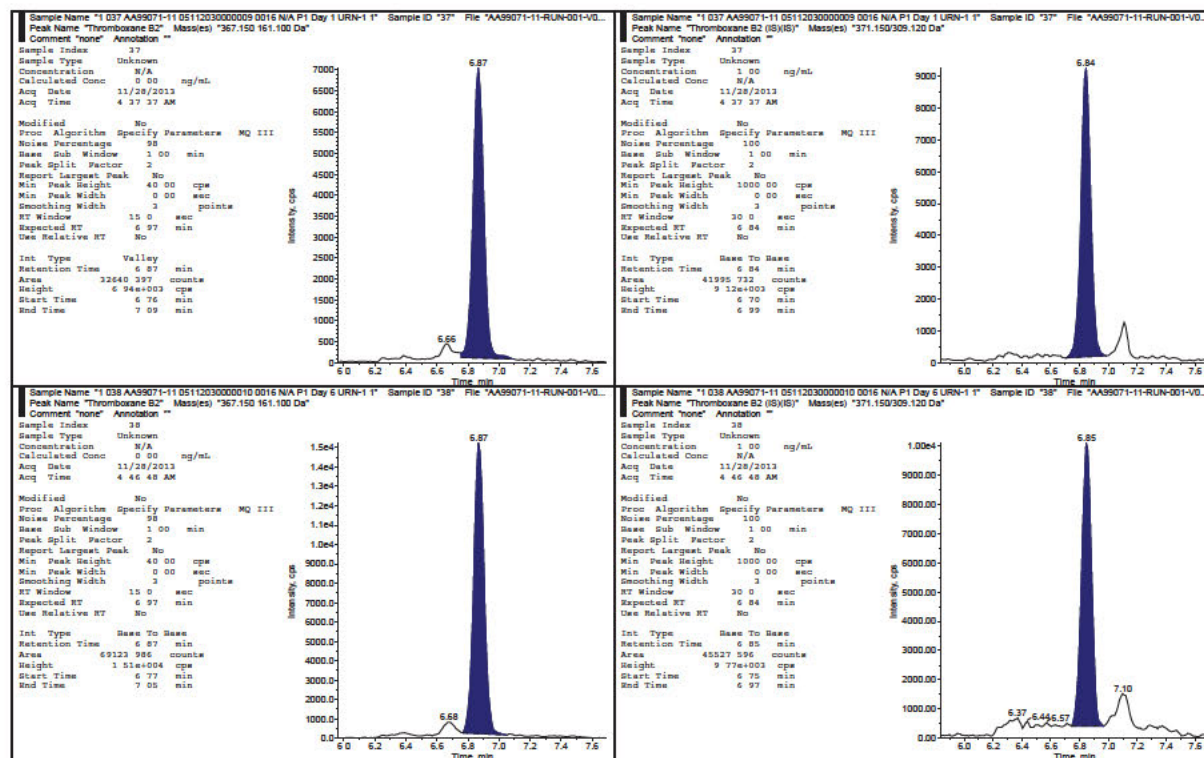


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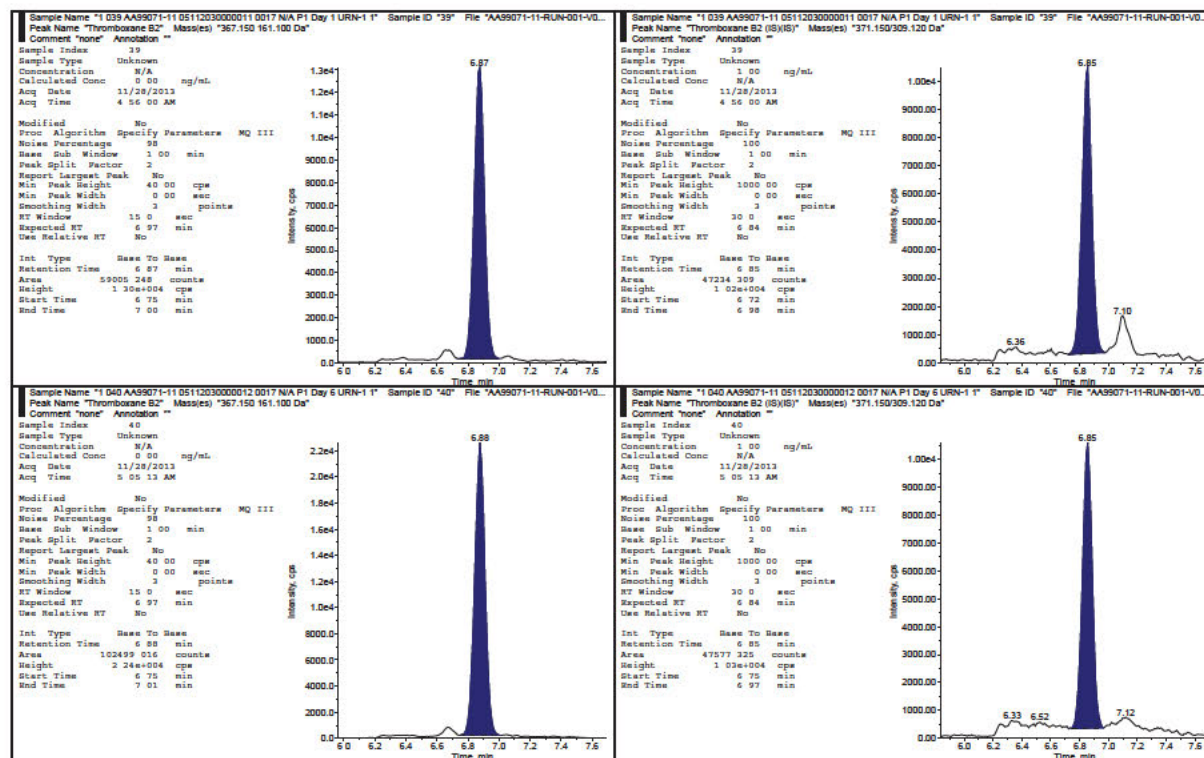


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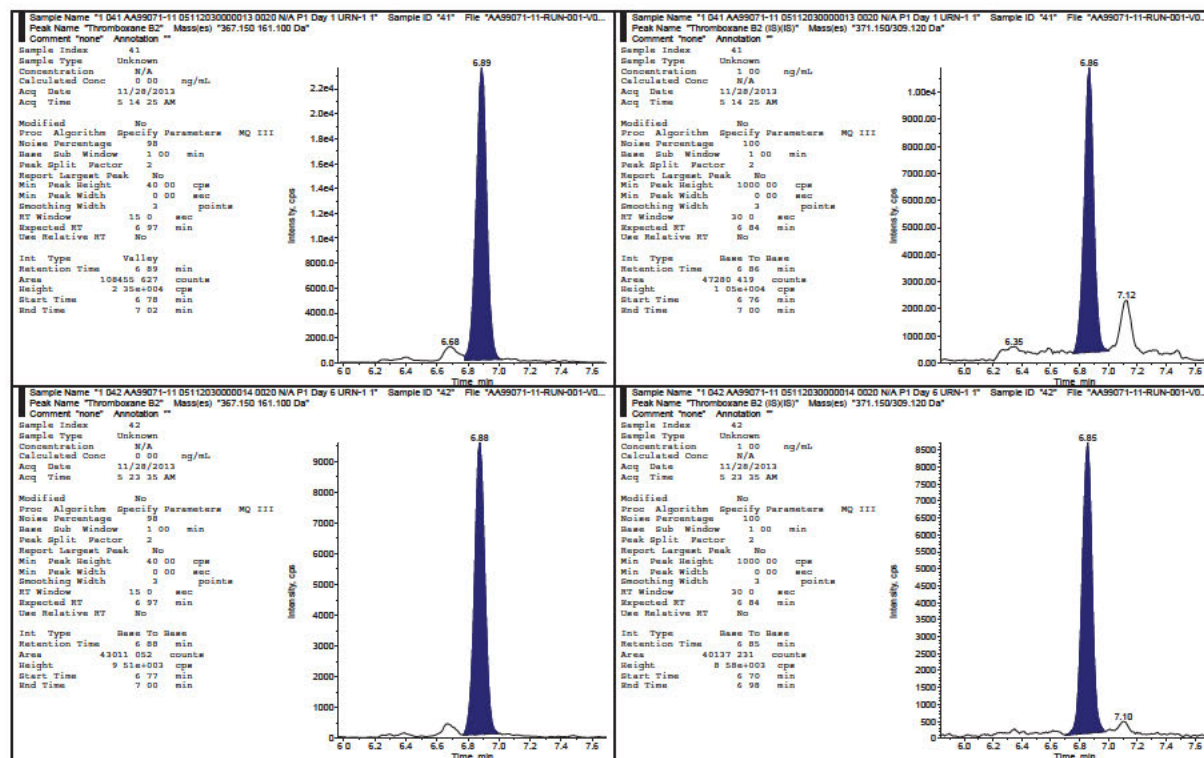


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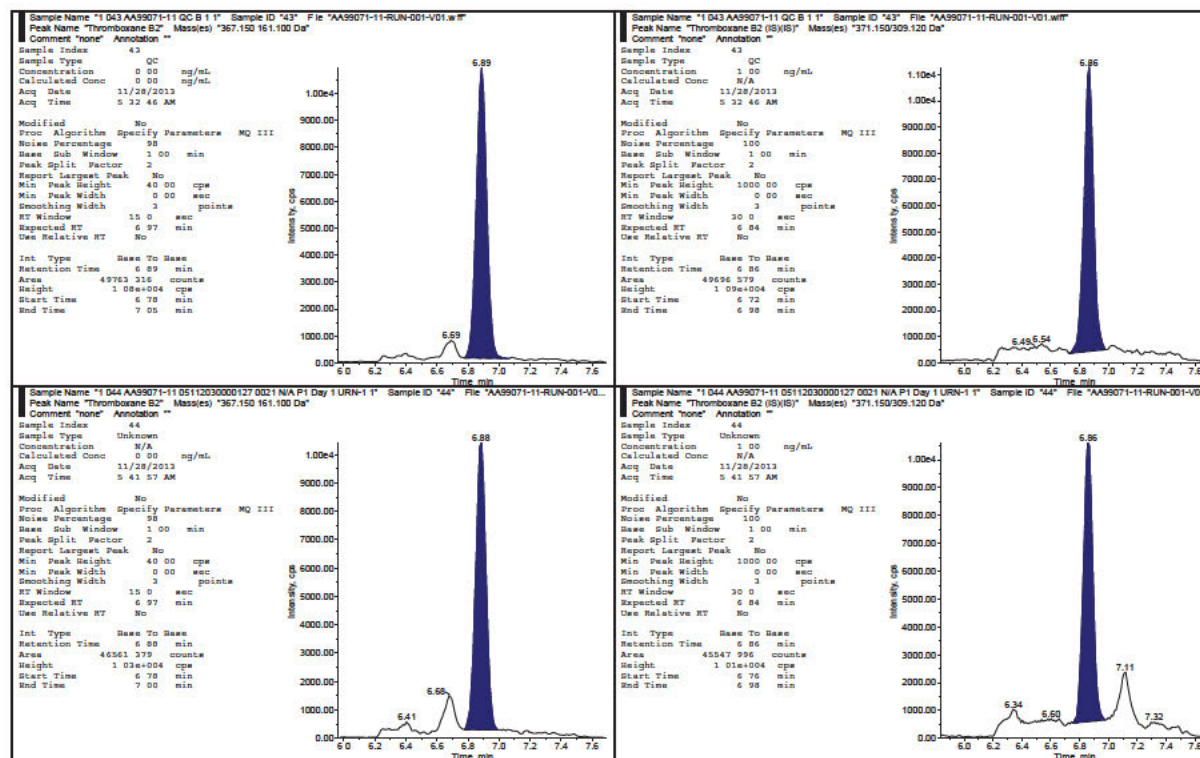


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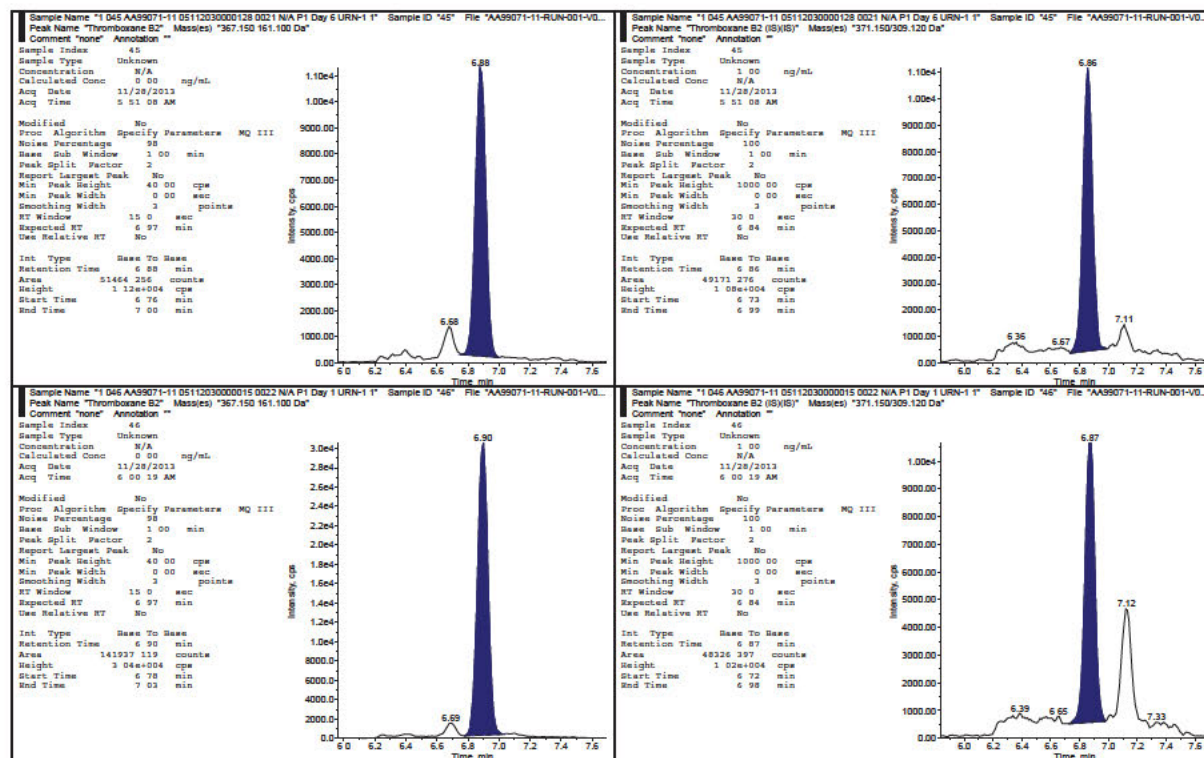


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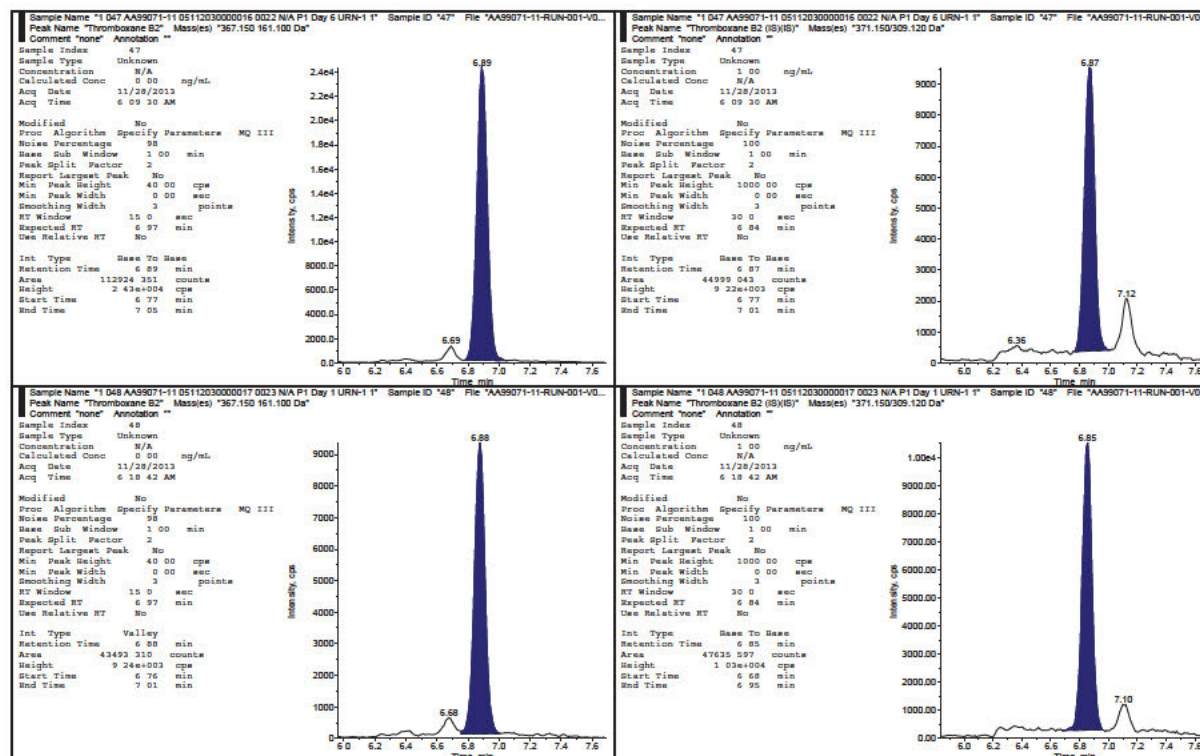


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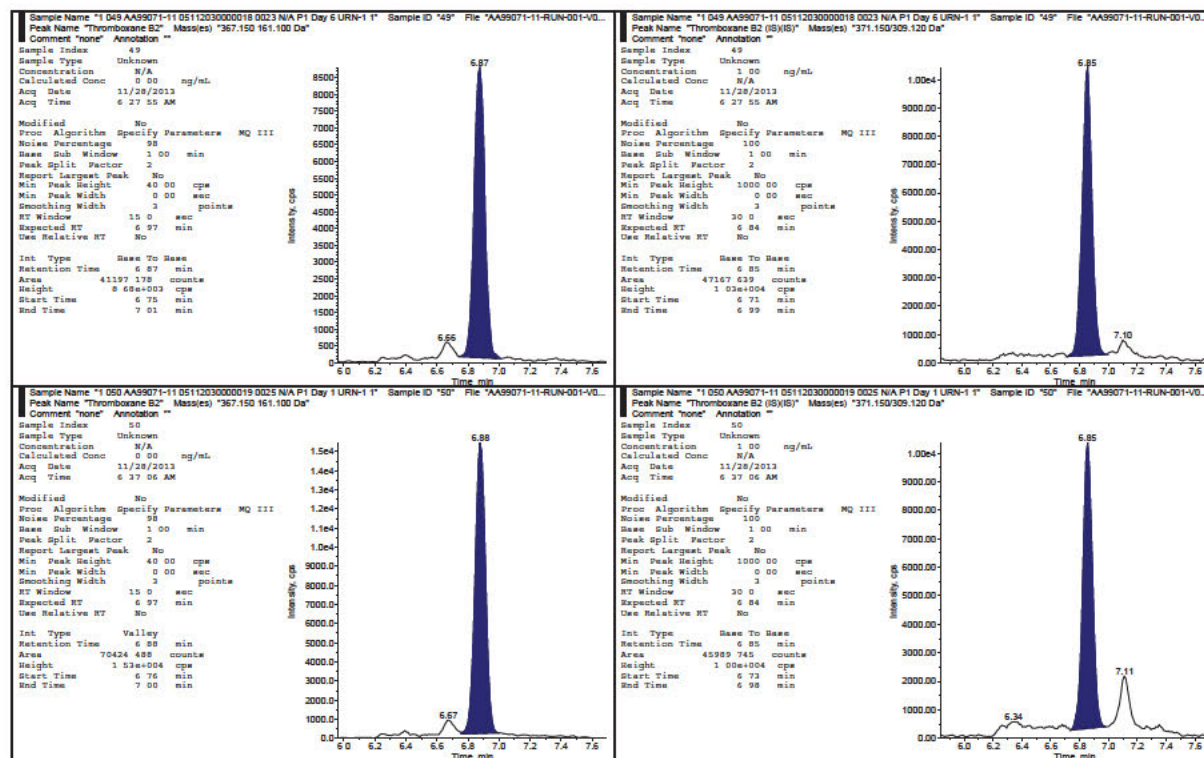


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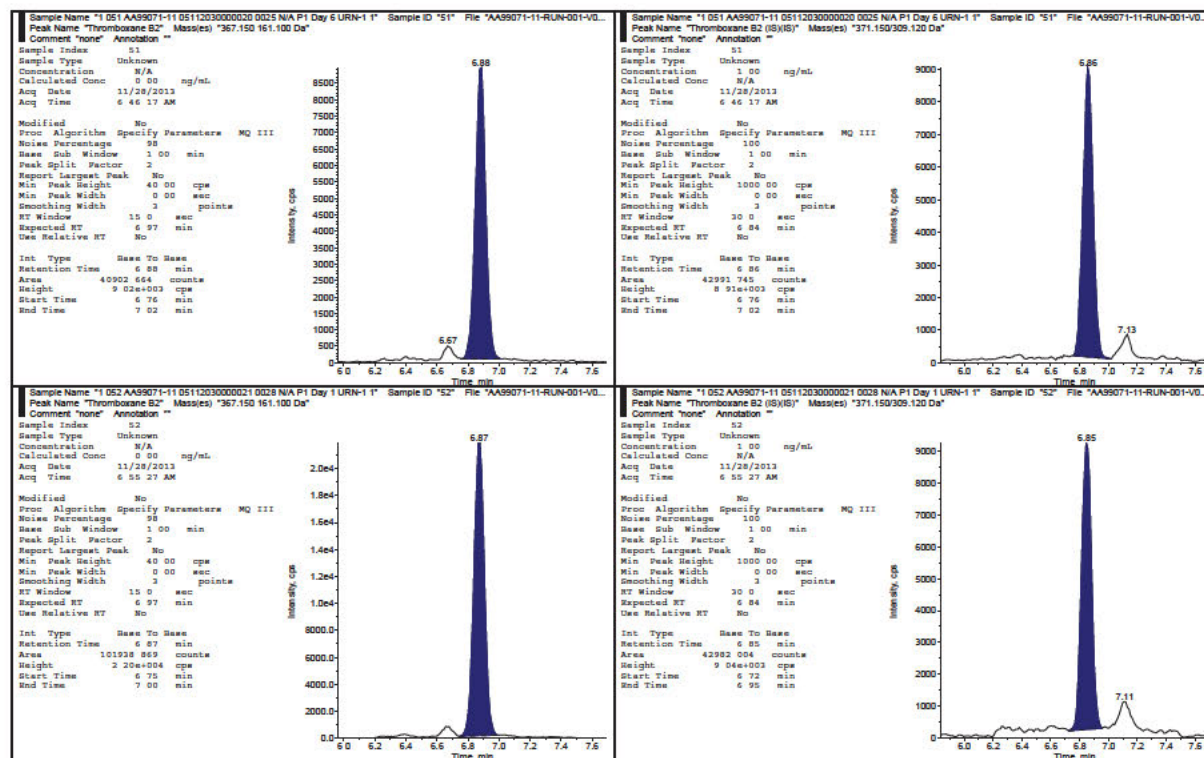


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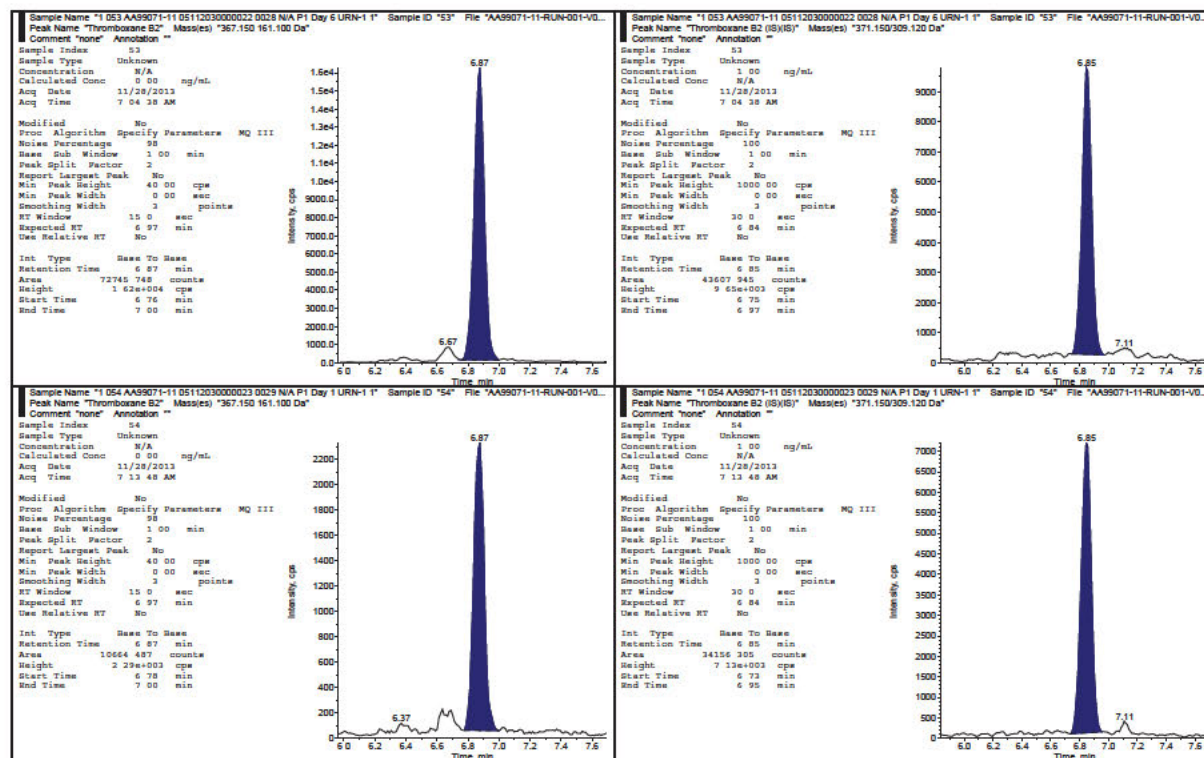


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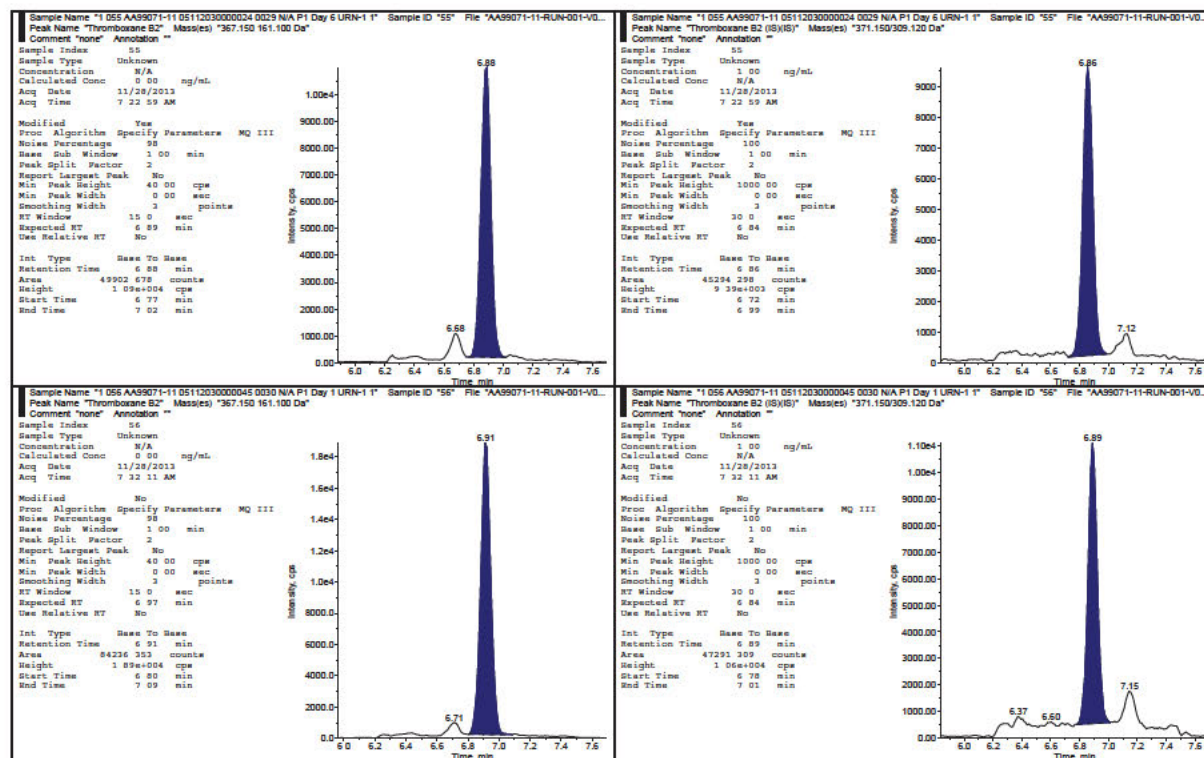


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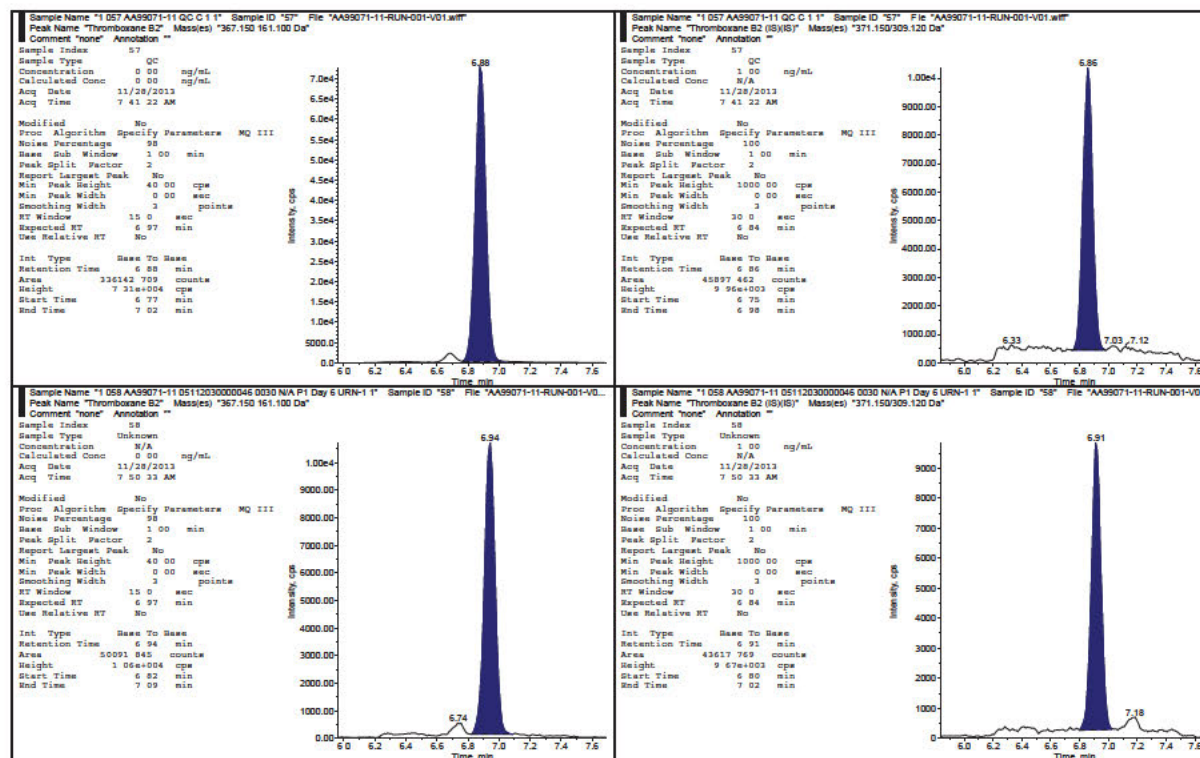


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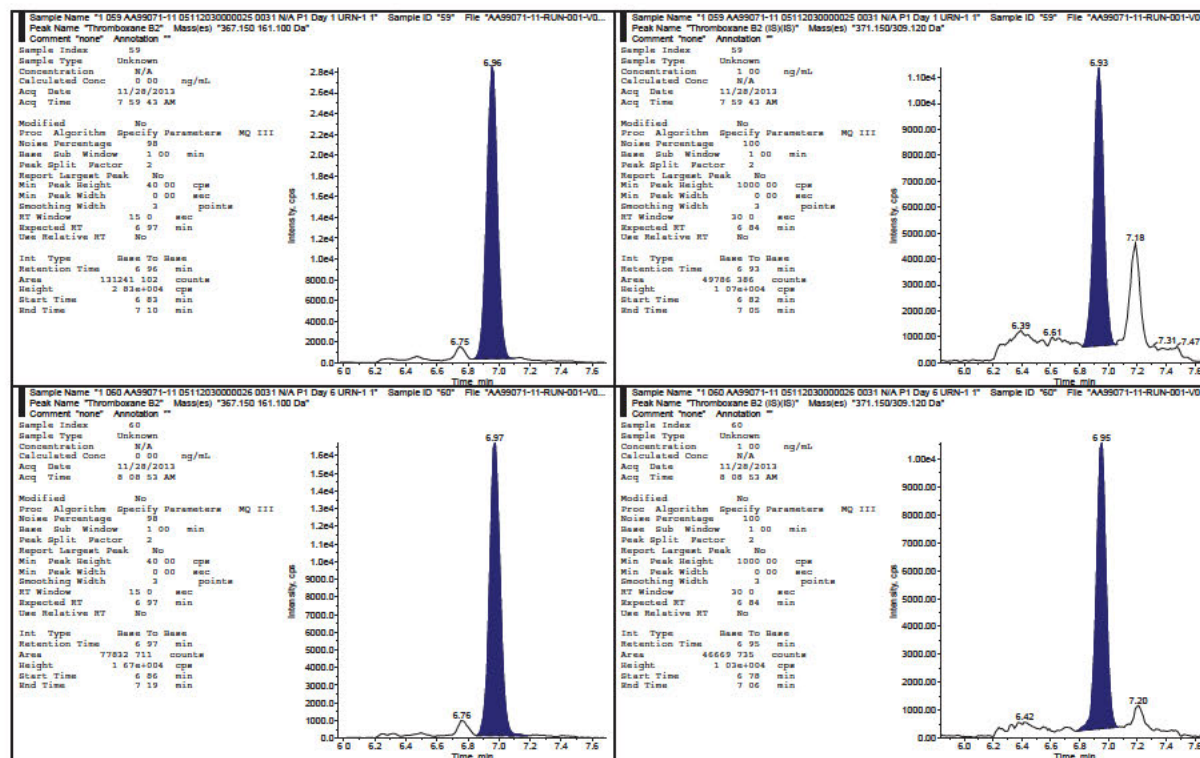


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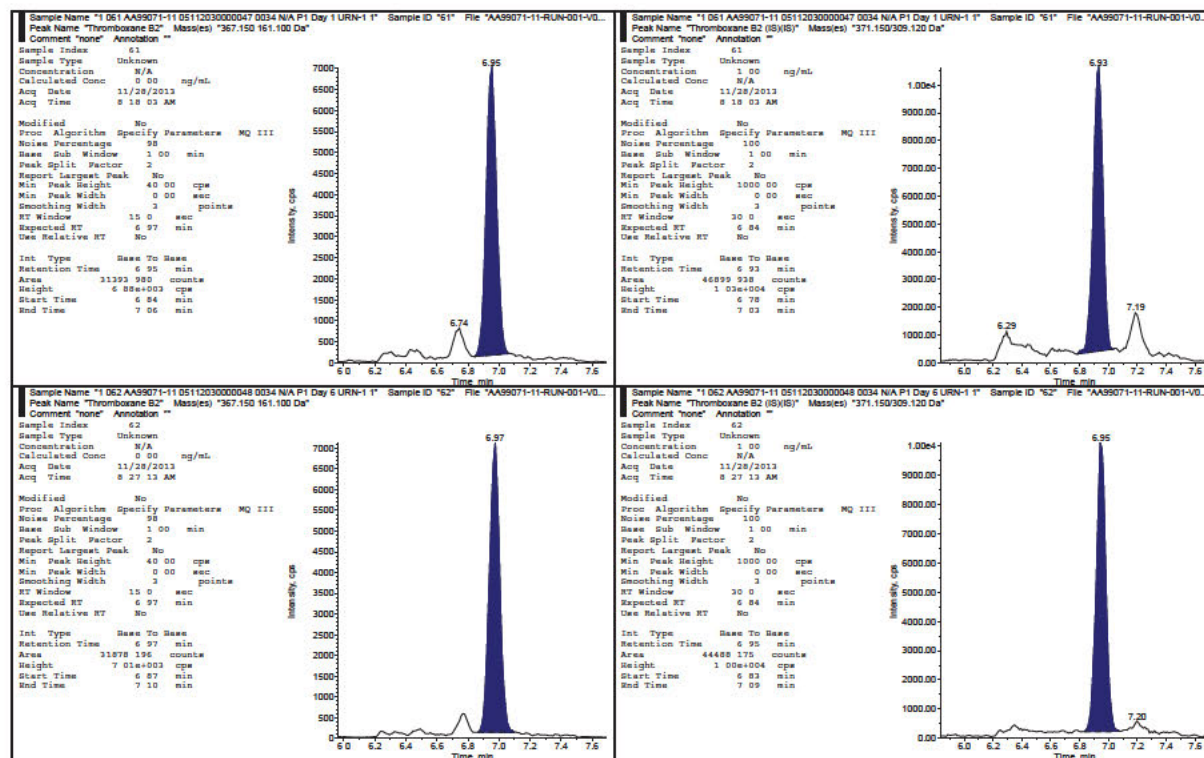


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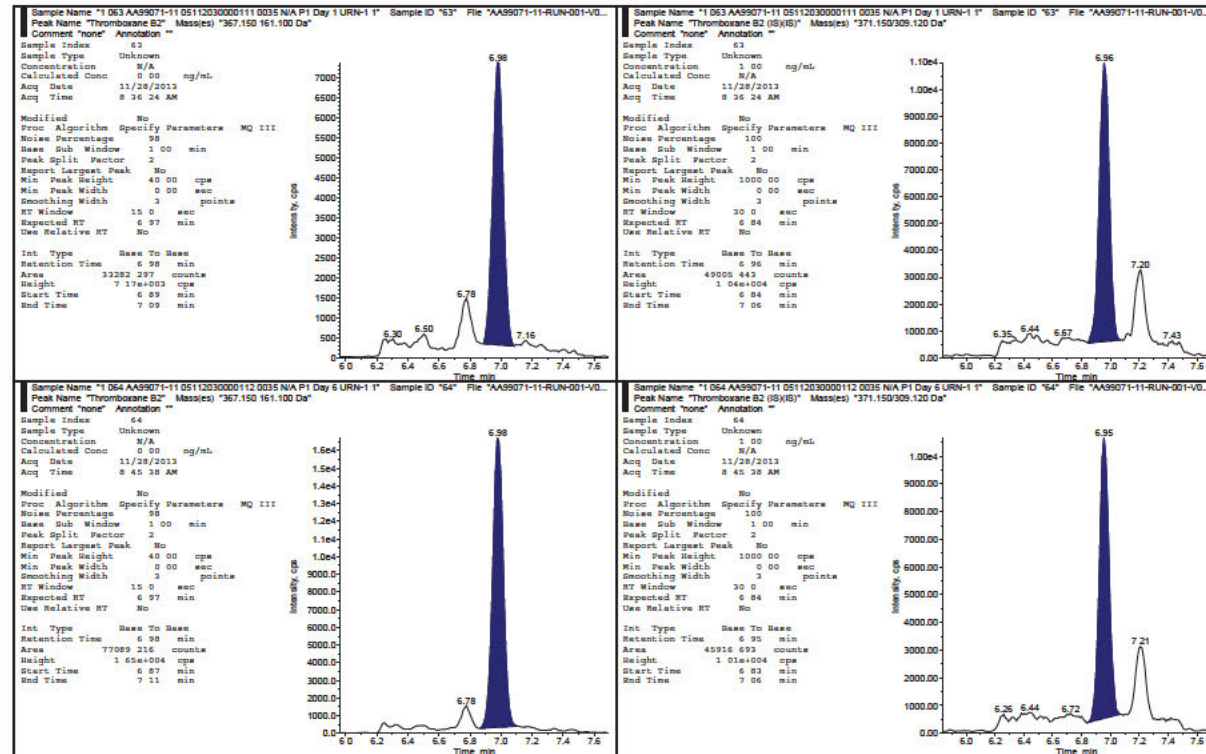


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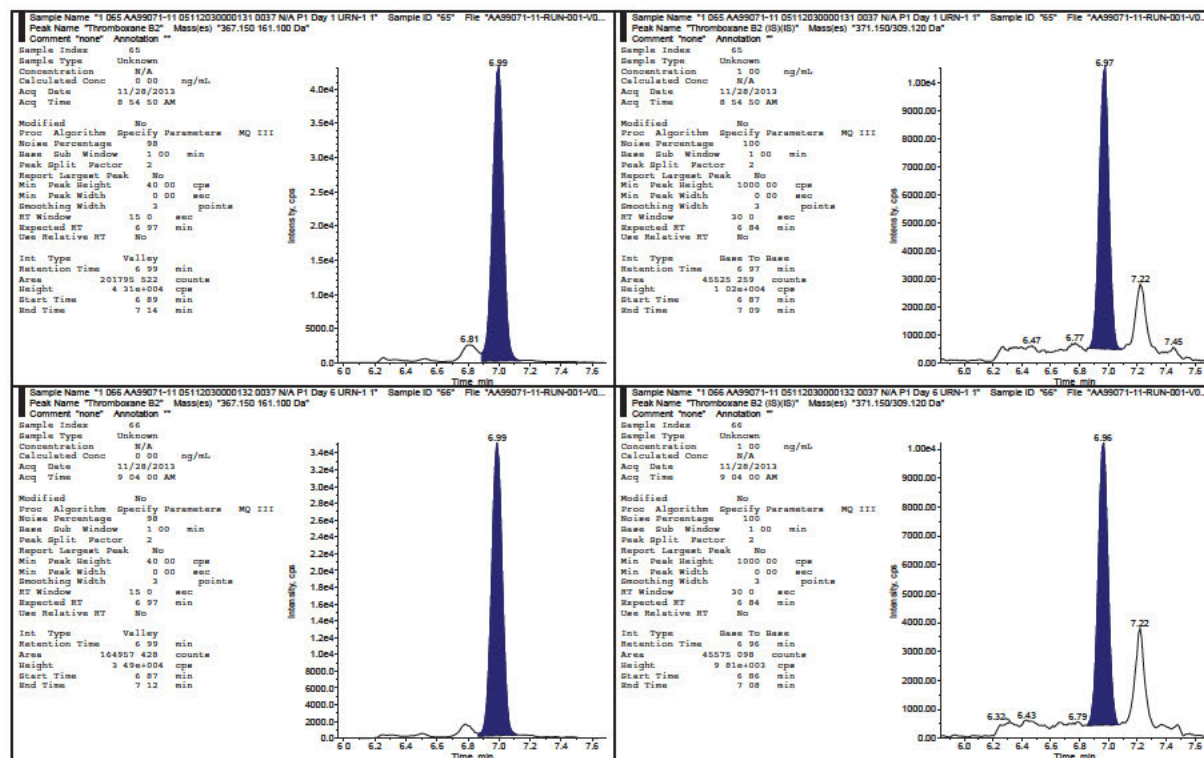


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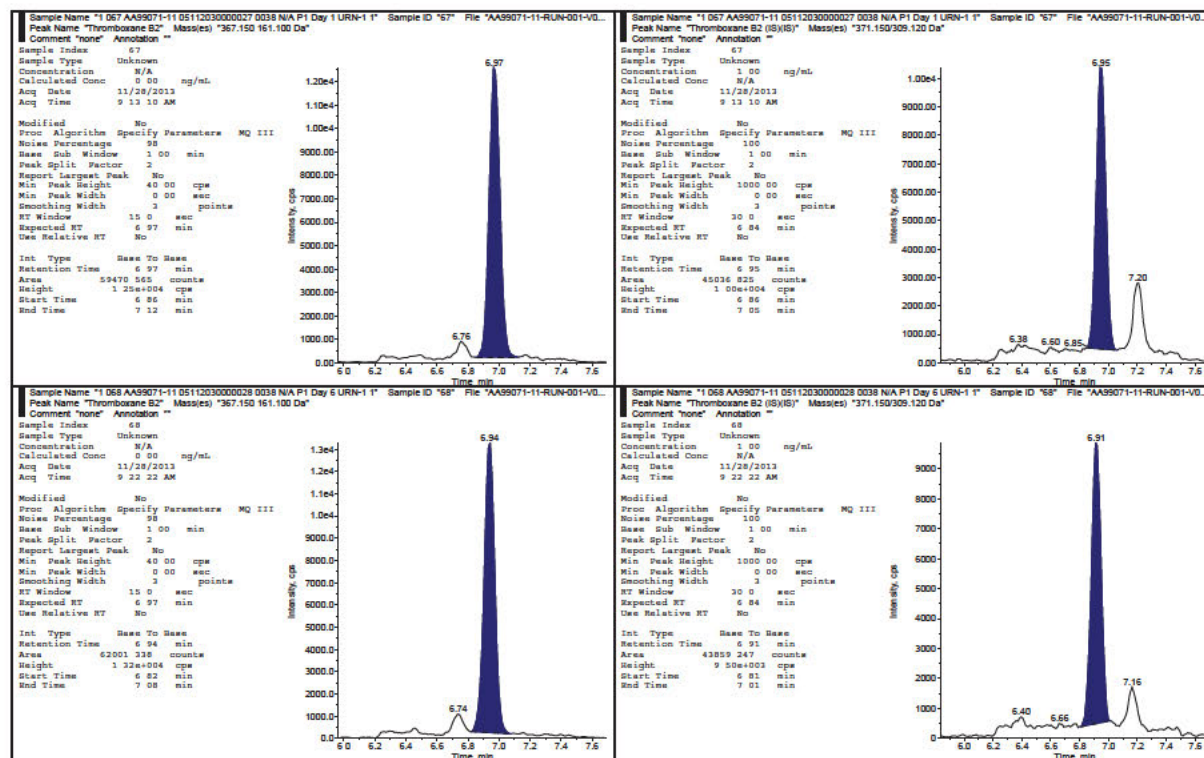


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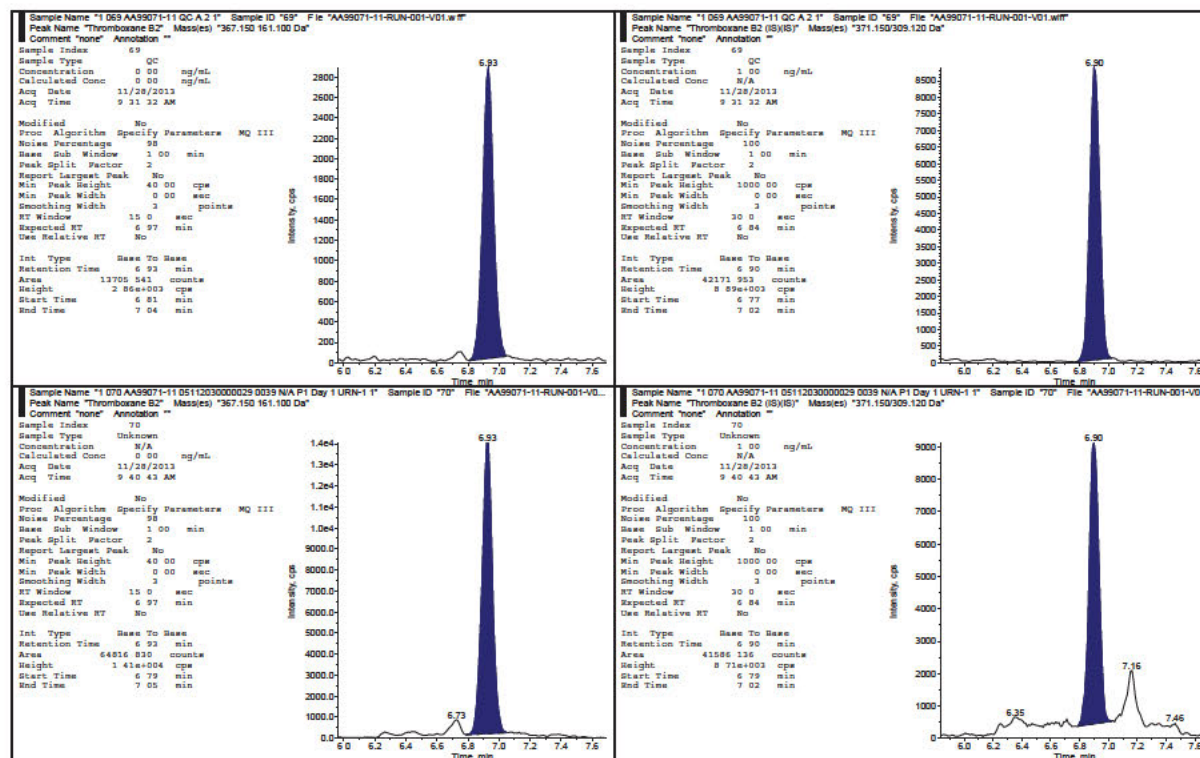


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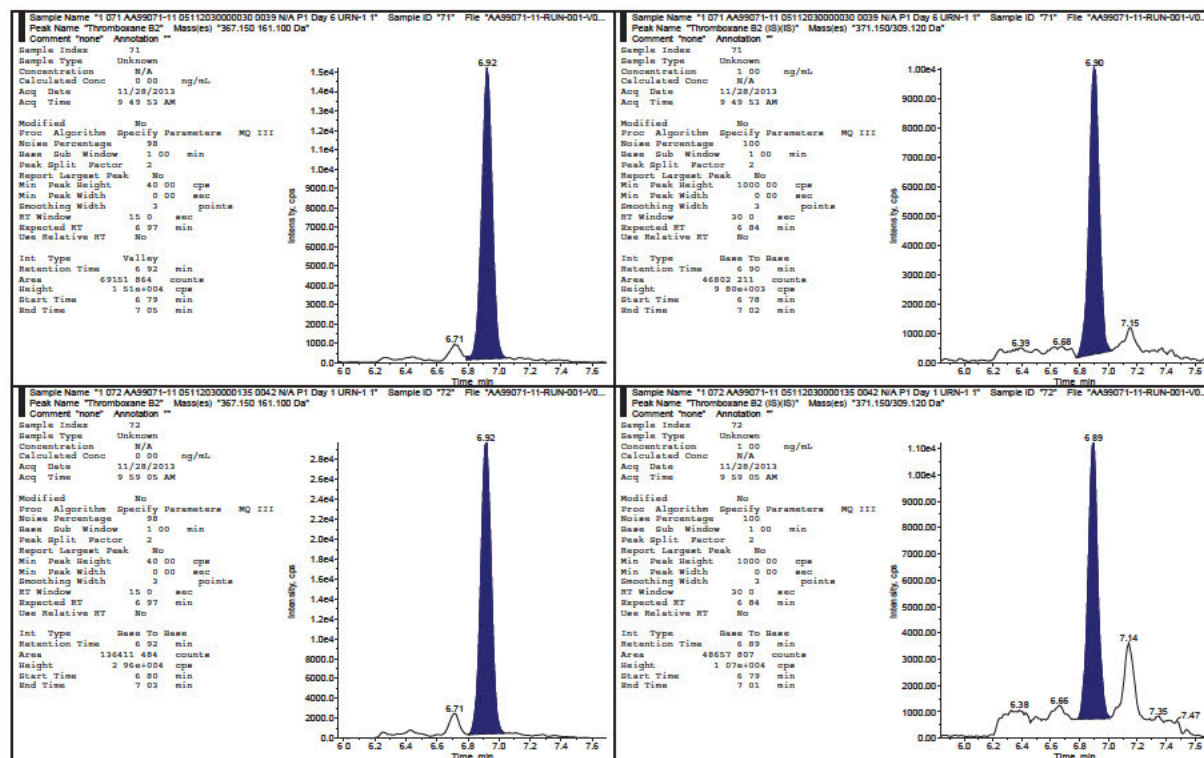


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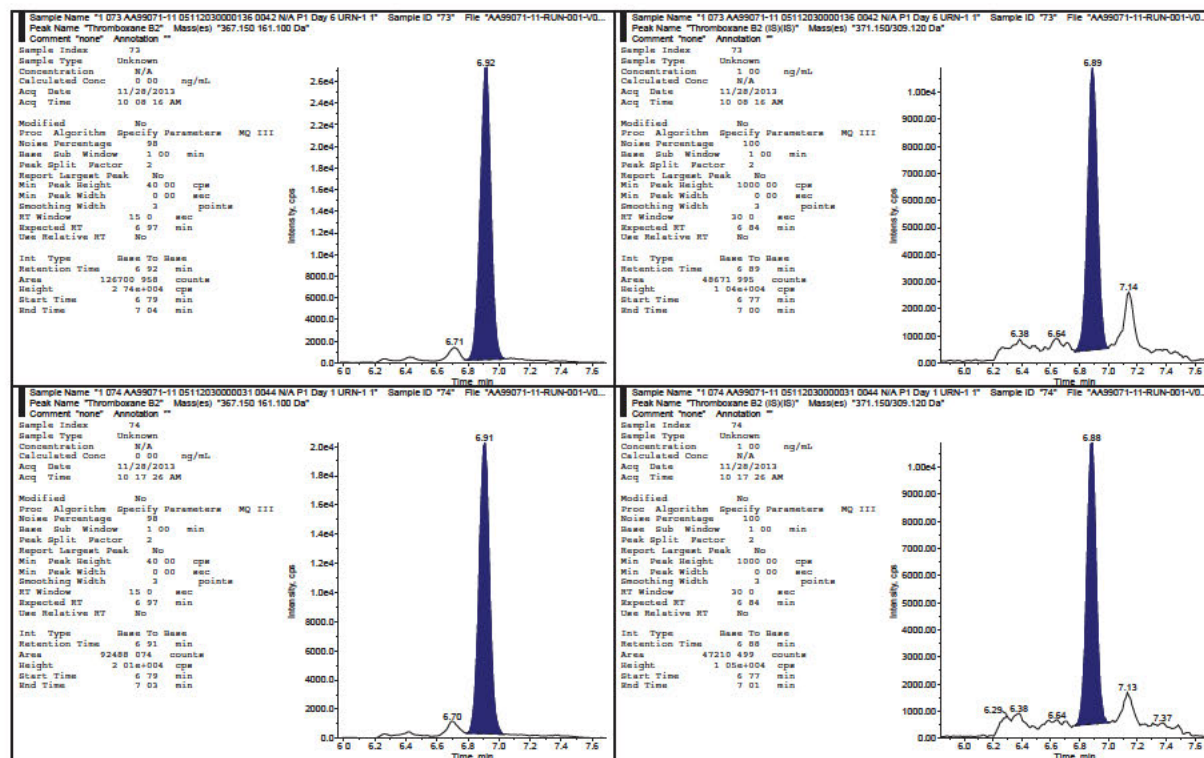


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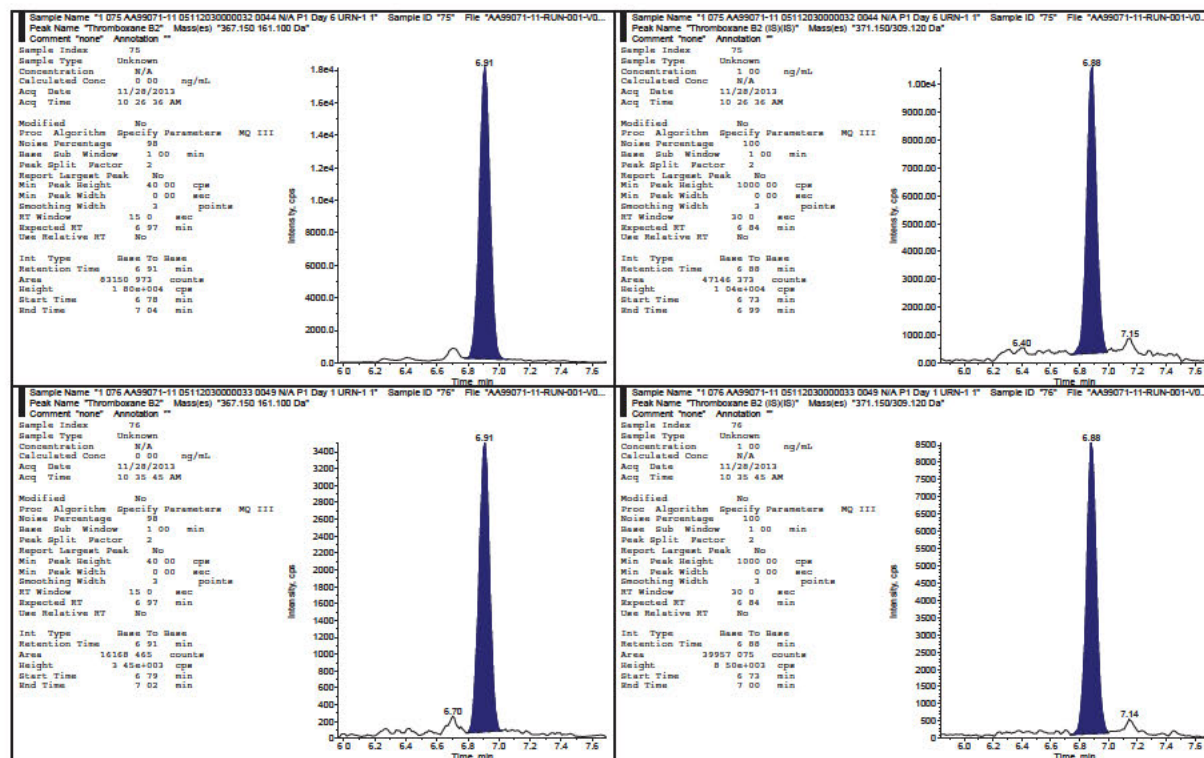


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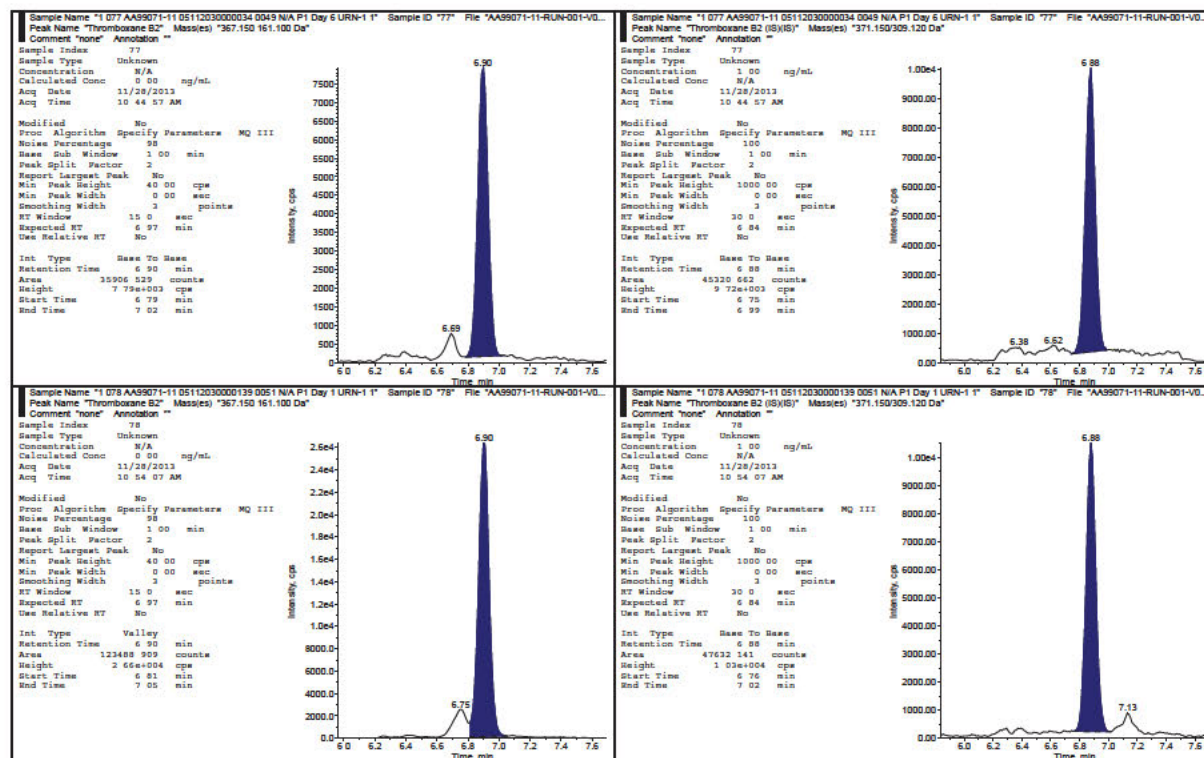


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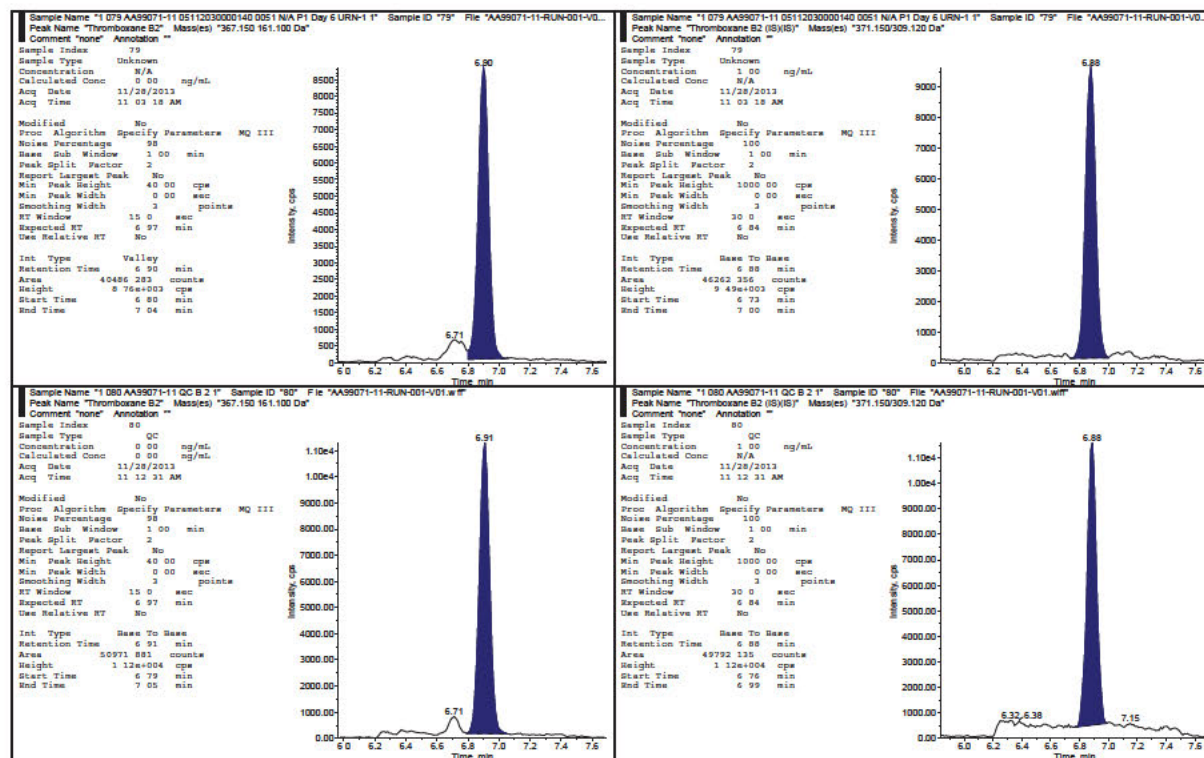


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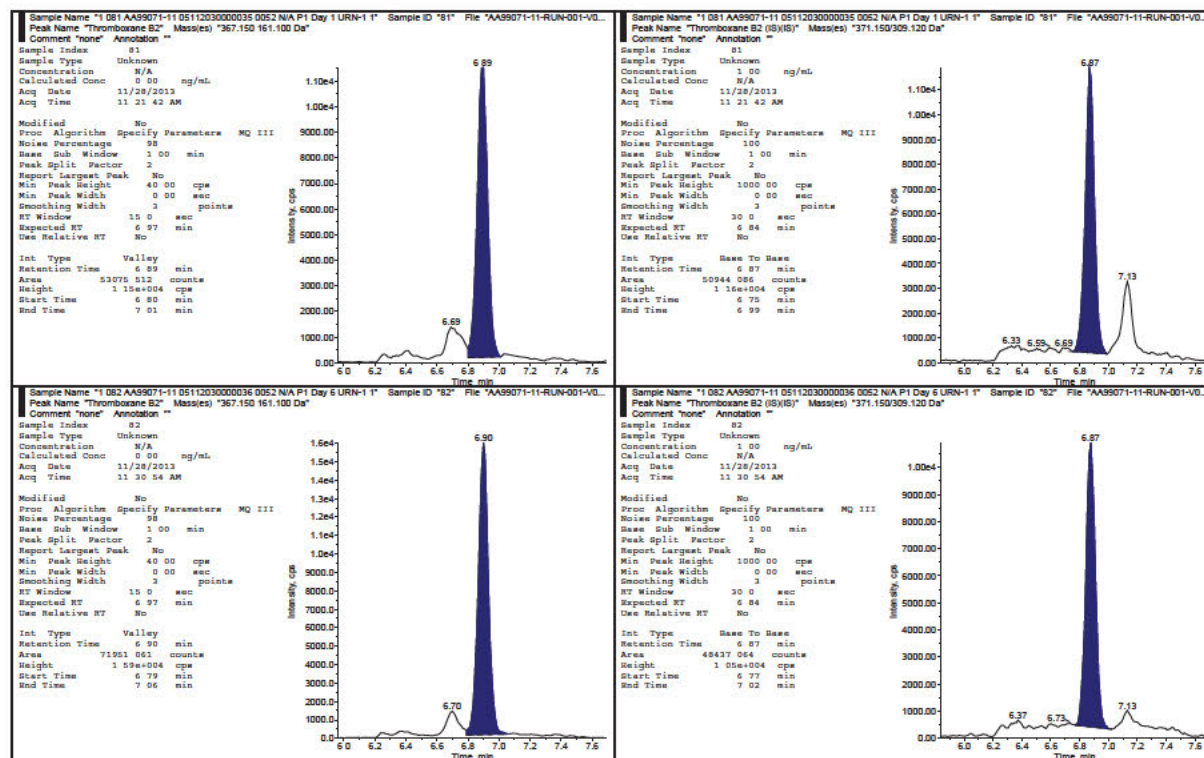


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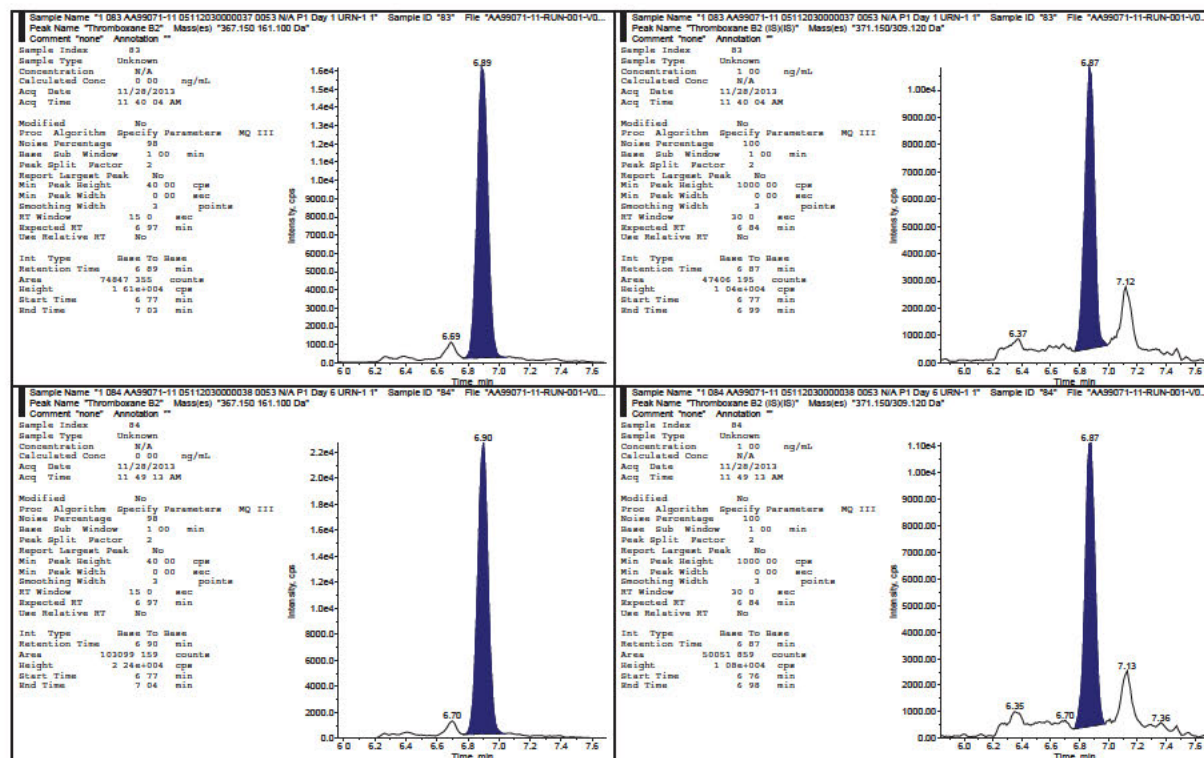


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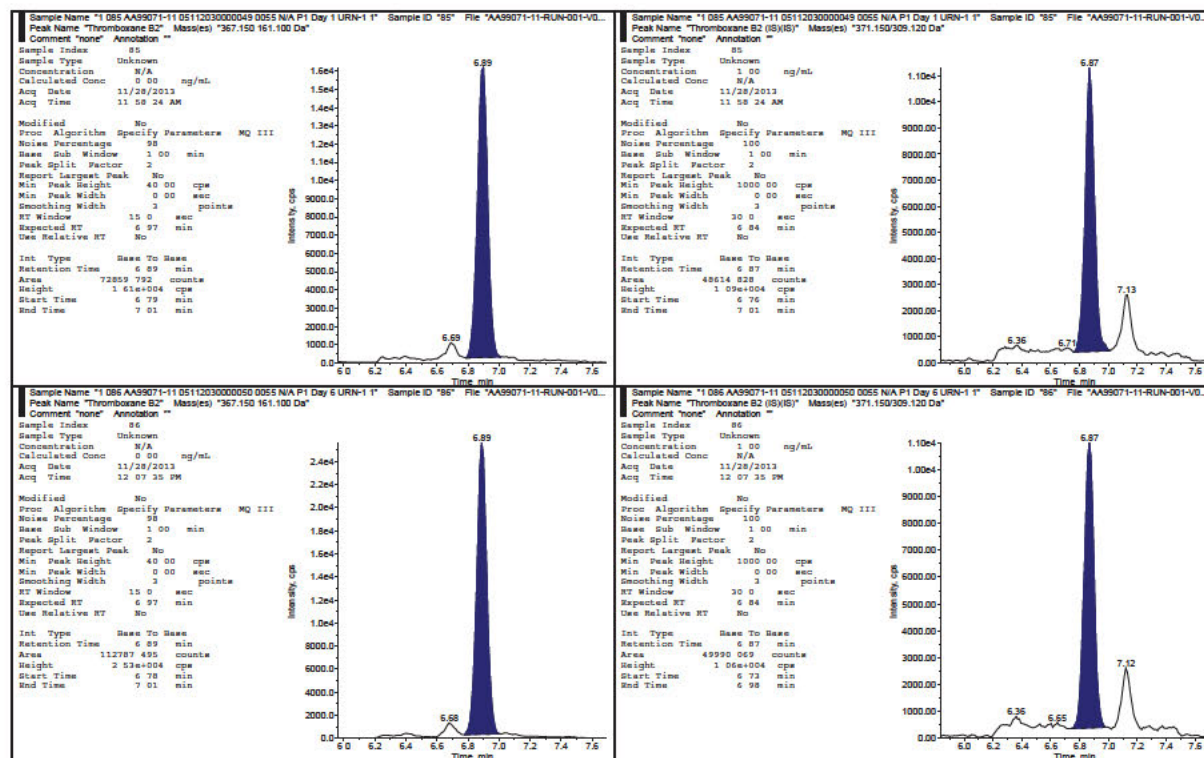


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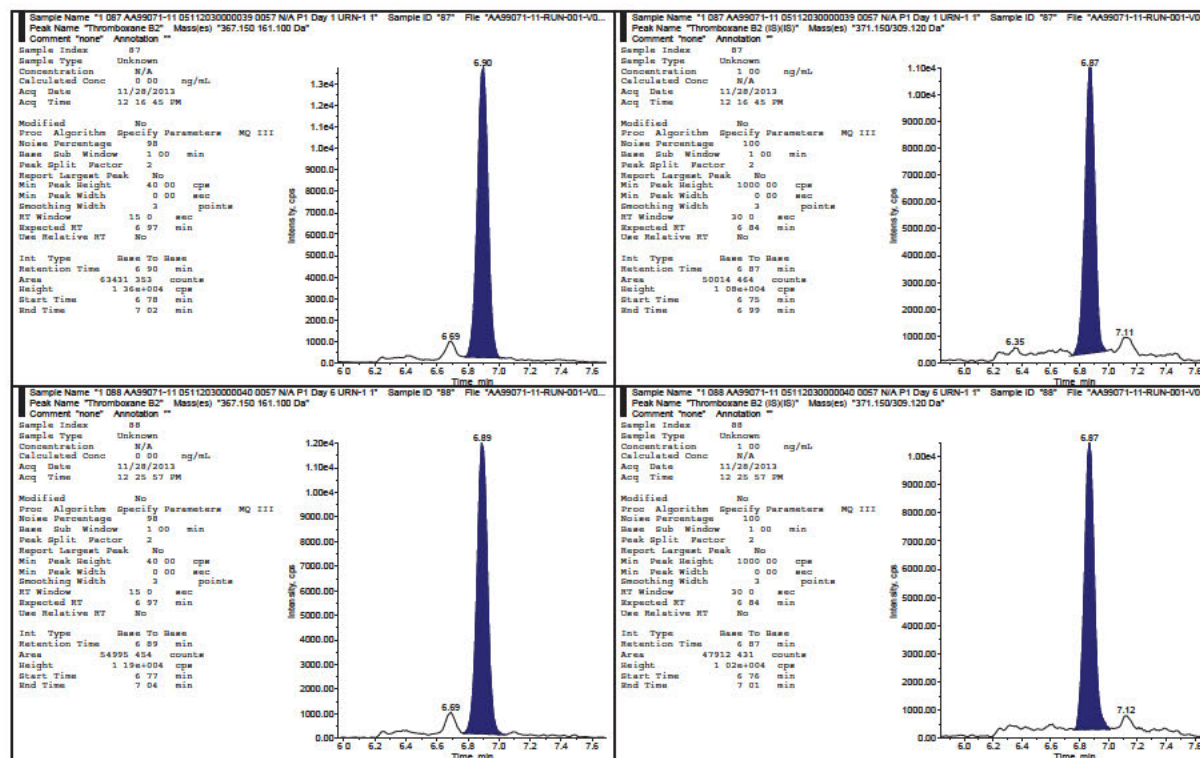


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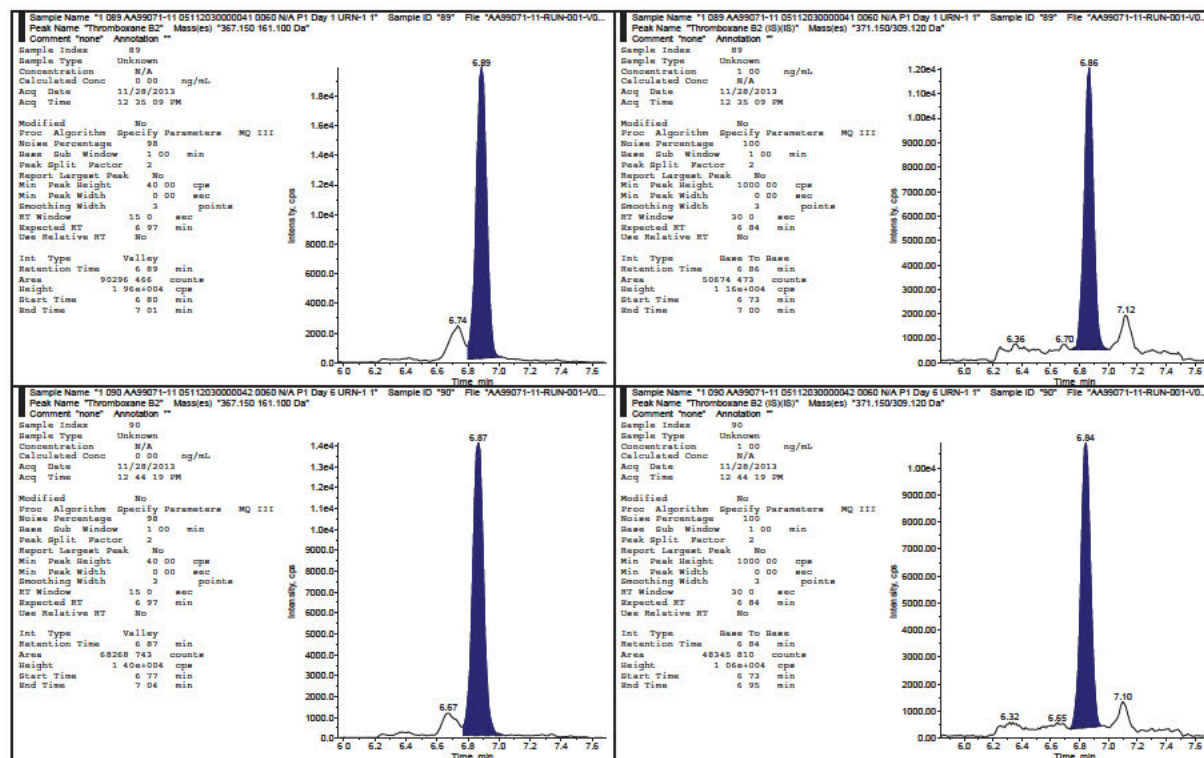


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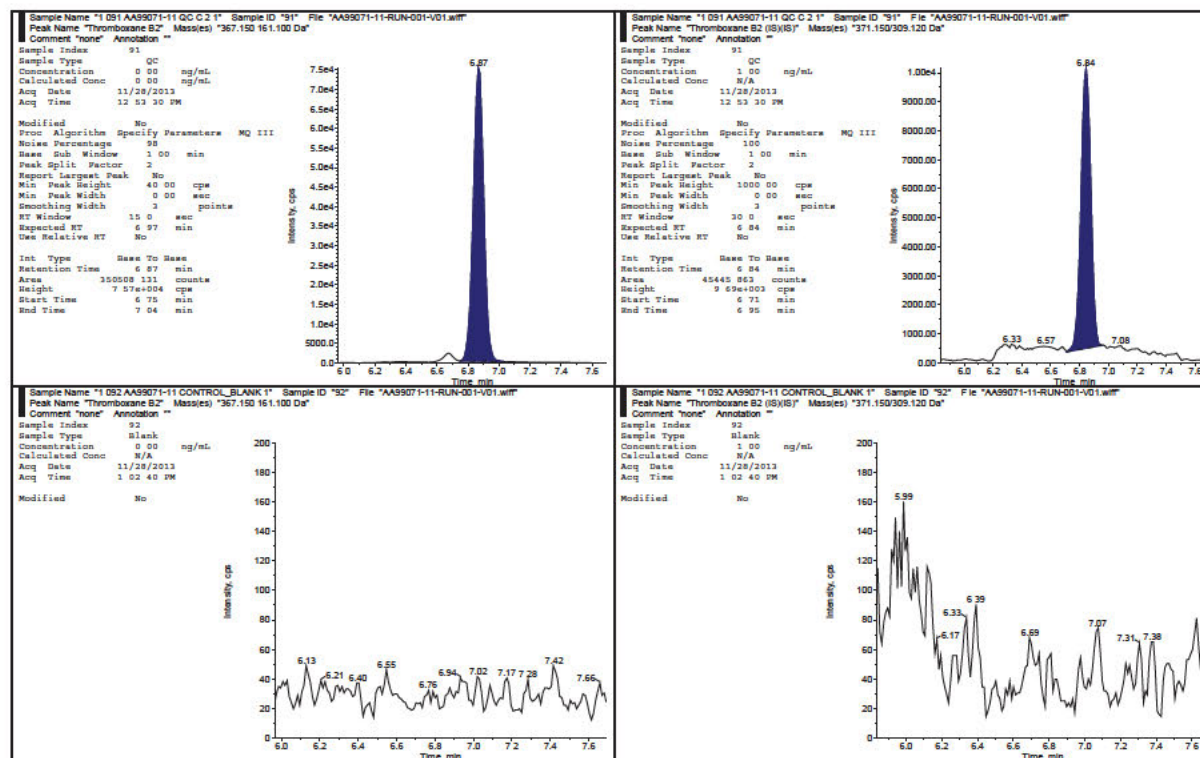


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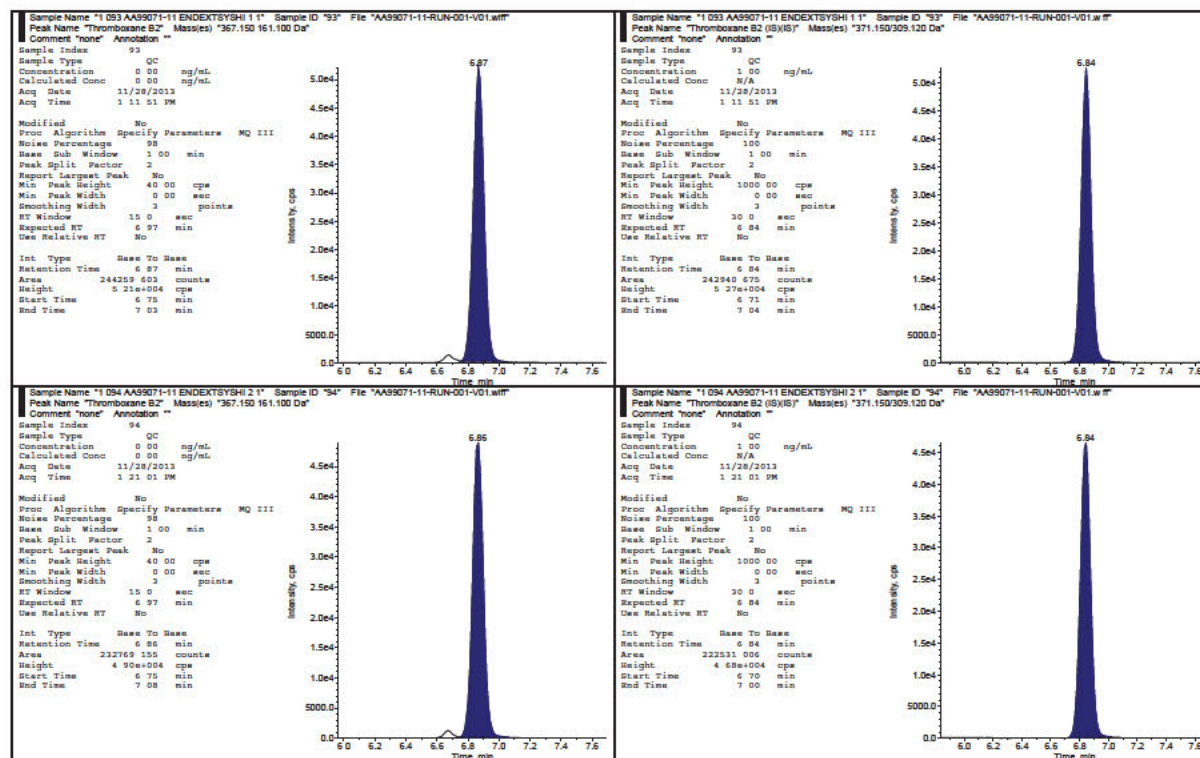


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