ANALYSIS REPORT

DETERMINATION OF OXYSTEROLS AND CHOLESTEROL IN PLASMA FROM A STUDY INVESTIGATING THE EFFECTS OF ZURICH MRTP (DEL/C3/FR1/RDO) ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS OF APO LIPOPROTEIN E-DEFICIENT MICE USING LC-HR-MS AND LC-CAD STUDY NUMBER 150156

Program Name	Systems Toxicology (Sys Tox)
Project Name	Animal Clinic
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1 Introduction

Oxysterols are oxygenated derivatives of cholesterol. Most of the known oxysterols are formed from cholesterol by enzymatic or free radical oxidation involving reactive oxygen and nitrogen species (see Figure 1). Oxysterols play an important role in many biological processes, including cholesterol turnover, apoptosis, necrosis, inflammation, immunosuppression and cardiovascular disease (CVD).

Inhaled oxidants are major causes of environmental lung injury, and the oxidative stress caused by air pollution and tobacco smoke can contribute to the pathogenesis of chronic obstructive pulmonary disease (COPD), asthma (MacNee, 2001) and CVD (Salonen et al., 1997). The first point of contact between lung tissue and inhaled oxidants is the epithelial lining fluid, which contains pulmonary surfactant and is almost entirely composed of lipids (95%). Oxysterols are oxidized lipids which can exert a variety of biological activities including cytotoxicity, regulation of cholesterol homeostasis, suppression of the immune response and interaction with the hedgehog-signaling pathway (Brown and Jessup, 2009; Dwyer et al., 2007; Schroepfer, 2000).

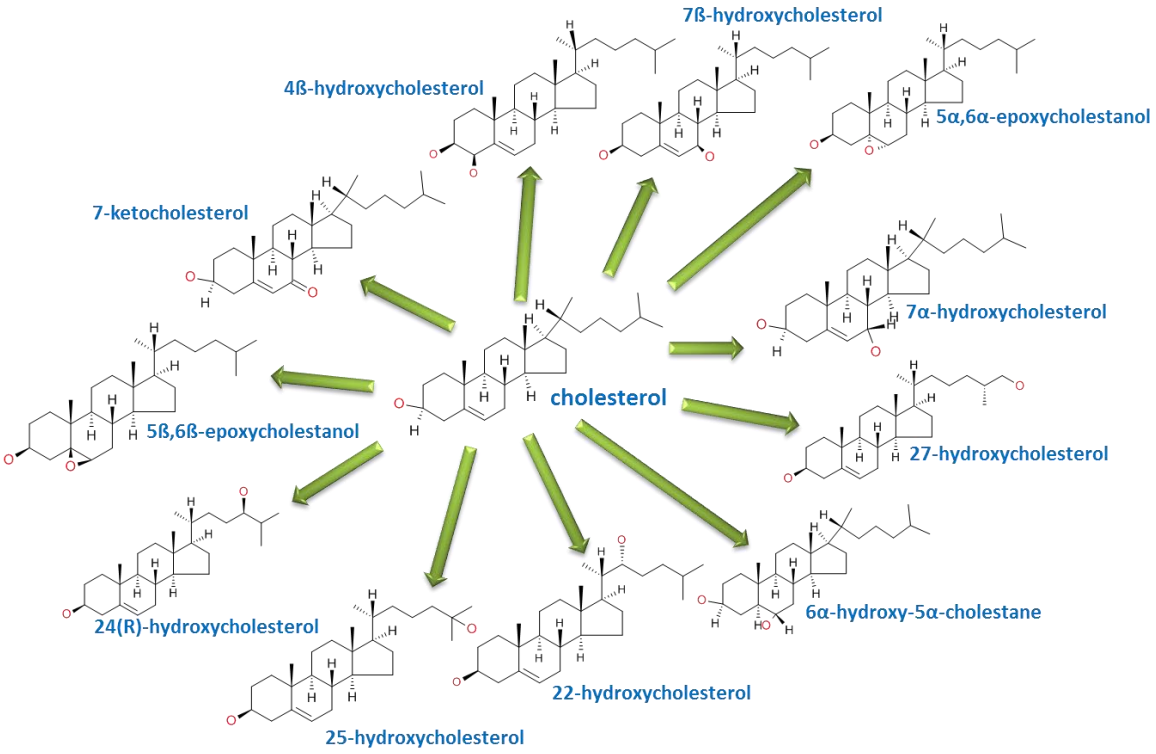


Figure 1. Important Oxysterols and Cholesterol as precursor

2 Objective

The purpose of this study was to determine the concentrations of 11 oxysterols[1] (5α,6α-epoxycholestanol, 5ß,6ß-epoxycholestanol, 6α-hydroxy-5α-cholestanol, 7-ketocholesterol, 4β-hydroxycholesterol 7α-hydroxycholesterol, 7ß-hydroxycholesterol, 22-hydroxycholesterol, 24(R)-hydroxy-cholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol) in plasma samples derived from ApoE-/- mice by liquid chromatography with high resolution mass spectrometry (LC-HR-MS). Total cholesterol was also determined in the same samples using liquid chromatography with charged aerosol detection (LC-CAD).

The samples were collected as part of Study 15015 entitled “In Vivo Evaluation of the Effects of Zurich MRTP (DEL/C3/FR1/RDO) on the Respiratory and Cardiovascular Systems of Apo Lipoprotein E-Deficient Mice”. Plasma samples were stabilized with butylhydroxytoluene (BHT) directly after collection in order to prevent any autoxidation of cholesterol during shipment and storage.

3 Materials

3.1 Test Item

The test items were defined in the protocol of the in-vivo study (Study 15015, study plan).

- MRTP Zurich FPD 4.2 sensorial medium

Description	Short Name	Batch Number
Del/C3/FR1/Rdo	ZRH	Batch 1: B-05771

- 3R4F reference cigarette

ApoE-/- mice were randomly allocated to the following groups:

i) Sham – exposed to filtered air

ii) 3R4F – aerosol from reference cigarettes (600 µg/L TPM target dose)

iii) ZRH – aerosol from an electrically heated stick from the Zurich development platform (concentration was matched to nicotine levels in the 3R4F exposure conditions)

iv) Cessation – 2 months 3R4F, followed by filtered air

v) Switching to ZRH – 2 months 3R4F, followed by aerosol from ZRH

[1] Deviation from the analysis plan. Please refer to **9.1.1** for more details.

Specific study test items for study 150156 were coded and blinded BHT stabilized plasma samples from groups i – v were delivered to Philip Morris International, Neuchatel, Switzerland, on dry ice and stored at -80°C prior to analysis.

3.2 Reference Items and Internal Standards[2]

Table 1. Reference Items and Internal Standards

Identity	Specification	Storage	Supplier	Product Nr.	Lot-Nr.
4ß-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700036P-5mg	4βOHCH-11
4ß-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700042P-5mg	4βOHCH(D7)-14
6α-hydroxy-5α-cholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700030P-5mg	6aOHCHL-11
6α-hydroxy-5α-cholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700045P-5mg	6aOH5aCHL(D7)-12
7α-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700034P-5mg	7aOHCH-12
7α-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700043P-5mg	7aOHCH(D7)-11
7ß-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700035P-5mg	7βOHCH-12
7ß-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700044P-5mg	7βOHCH(D7)-11
5α,6α-epoxycholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700032P-5mg	5a,6a-EPCHOL-10
5α,6α-epoxycholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700047P-5mg	5a,6a-EPCHOL(D7)-11
5ß,6ß-epoxycholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700033P-5mg	5β,6β-EPCHOL-11
5ß,6ß-epoxycholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700047P-5mg	5β,6β-EPCHOL(D7)-13
7-ketocholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700015P-5mg	7-KETOCH-14
7-ketocholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700046P-5mg	7-KETOCH(D7)-11
22-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700058P-5mg	22(R)OHCH-12
22-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700052P-5mg	22(R)OHCHD7-10
24(R)-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700071P-5mg	24(R)OHCH-10
24(R/S)-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700018P-5mg	24OHCH(D7)-10
25-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700019P-5mg	25OHCH-13
25-hydroxycholesterol (d6)	Purity >99% powder	-20°C	Avanti Polar Lipids	700053P-5mg	25OHCHD6-10
27-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700021P-5mg	27OHCH-13
27-hydroxycholesterol (d6)	Purity >99% powder	-20°C	Avanti Polar Lipids	700059P-5mg	27OHCHD6-10
Cholesterol	Purity >99% powder	-20°C	Sigma Aldrich	C8667	080M5304V
β-sitosterol	Purity >85% powder	-20°C	Sigma Aldrich	43623	BCBM2889V

[2] Deviation from the analysis plan. Please refer to 9.1.2 for more details.

3.3 Control Matrix

Due to unavailability of analyte free control matrix, calibration standards were prepared in solvent and treated like samples throughout the complete sample preparation. For the same reason, quality control samples were also prepared in solvent and treated like samples throughout the complete sample preparation.

3.4 Test System

3.4.1 Procedure and Instrumentation – Oxysterols

Table 2. Procedure and Instrumentation for the determination of Oxysterols

Sample Preparation	Liquid-liquid extraction with chemical derivatization
Chromatography Type	HPLC
Instrument Platform	Thermo Fisher Scientific QExactive® High Resolution Accurate Mass Spectrometer
Regression Type	Linear ($1/x^2$)
Quantitation Method	Peak Area Ratio
Assay Volume	6 µL

3.4.2 Procedure and Instrumentation – Cholesterol[3]

Table 3. Procedure and Instrumentation for the determination of cholesterol

Sample Preparation	Liquid-liquid extraction
Chromatography Type	HPLC
Instrument Platform	Charged Aerosol Detection (Dionex Corona Veo ultra RS™)
Regression Type	Quadratic (no weighting)
Quantitation Method	Peak Area Ratio
Assay Volume	5 µL

[3] Deviation from the analysis plan. Please refer to Section 9.1.3 for more details.

3.4.3 Computer Application Software[4]

Table 4. Computer Application Software

LC-HR-MS Software	Thermo Fisher Scientific Xcalibur® version 2.2 SP1.48
Quantification Software (Oxysterols)	Thermo LC Quan® for Month 3 only Thermo Fisher Scientific Trace Finder™ 3.1
Quantification Software (Cholesterol)	Dionex Chromeleon™ version 7.2
Sample Management	Labvantage®
Office Applications	Microsoft Word® 2013, Microsoft Excel® 2013

3.5 Study Samples

3.5.1 Description of Study Samples

All study samples were received at PMI Neuchatel frozen on dry ice and were in good condition. Samples from Month 3 (QC check samples, 25 in total) and Month 8 (50 in total) were analysed and reported in Section 5.6.

Table 5. Study Samples

Identity	Number of Samples	Oxysterol Replicates	Cholesterol Replicates
Month 3 QC Check[5]	25	1	NA
Month 8	50	3	1

Only study samples from Month 8 were analysed for the determination of total cholesterol. The determination for oxysterols was performed on samples from both Month 3 and Month 8 collection timepoints.

3.5.2 Sample Management

After arrival at PMI Neuchatel the study samples were lodged into the LIMS (laboratory information management system) of PMI R&D BSR. The Study Director was informed regarding sample arrival and any discrepancy between expected and actual number of received samples, sample labels and the shipment documentation.

[4] Deviation from the analysis plan. Please refer to Section 9.1.4 for more details.

[5] Month 3 QC Check samples were analyzed in order to verify the effectiveness of the stabilization procedure. Therefore a statistical representative number of replicates was not analyzed. The method for total cholesterol was not available at the time of sample arrival.

3.5.3 Sample Identification

Study samples were identified based on the unique identification (CAN) number and time point identification given on the sample label. All study samples could be identified, therefore all received samples could be considered for analysis. The coded samples were blinded, therefore no group affiliation could be applied during analysis and results have been reported based upon the CAN identifier.

3.5.4 Sample Storage

From receipt until analysis the study samples were stored frozen at nominal -80°C (-65°C to -90°C). After submission of the final report the study samples will be further stored under the same conditions for up to 1 month. Then, upon agreement with the Study Director, the study samples will be either transferred to a location defined by the Study Director, stored frozen for a longer period of time or destroyed.

4 Sample Analysis

4.1 Analytical Method

The determination of oxysterols in mouse plasma samples was carried out using LC-HR-MS using independent calibration ranges for each oxysterol respectively, in accordance with PMI_RD_WKI_01241, 'Determination of oxysterols in plasma using LC-HR-MS'.

Calibration ranges were as follows:

Table 6. Lower Limit of Quantification and Upper Limit of Quantification for Oxysterols

Analyte	LLOQ [ng/mL]	ULOQ [ng/mL]
4ß-hydroxycholesterol	6	820
5α,6α-epoxycholestanol	7	956
5ß,6ß-epoxycholestanol	16	1031
6α-hydroxy-5α-cholestanol	8	1053
7-ketocholesterol	10	1100
7α-hydroxycholesterol	8	1035
7ß-hydroxycholesterol	8	989
22(R)-hydroxycholesterol	6	727
24(R)-hydroxycholesterol	4	1008
25-hydroxycholesterol	3	1446
27-hydroxycholesterol	7	890

The determination of total cholesterol in mouse plasma samples was carried out using LC-CAD in accordance with PMI_RD_WKI_01240, 'Determination of cholesterol in plasma using LC-CAD'.

Calibration range was as follows:

Table 7. Lower Limit of Quantification and Upper Limit of Quantification for Cholesterol

Analyte	LLOQ [mg/mL]	ULOQ [mg/mL]
Cholesterol	0.5	5

4.2 Description of an Analytical Run

An analytical run, as a minimum contained:

- a sufficient number of blank samples to reflect at least 2% of the study samples (with at least one blank sample positioned after a known high concentration sample),
- two standard zero samples (blank sample with internal standard (IS) added),
- one set of at least 6 non-zero calibration standards at the beginning of a sample run,
- one set of at least 6 non-zero calibration standards at the end of a sample run,
- a minimum number of three QCs (one replicate at three concentration levels) to reflect at least 5% of the number of study samples,
- if study samples were diluted: three dilution QC (DQC) replicates diluted by the same factor as used for the dilution of the study samples were included.

4.3 Acceptance Criteria

4.3.1 Analytical Run Acceptance Criteria[6]

An analytical run was acceptable if all of the following criteria were met:

- at least 75% of the non-zero calibration standards were within ± 15% (± 20% for the lower limit of quantification (LLOQ) calibration standard) of their nominal concentration,
- at least two-thirds of the QC samples were within ± 15% of their nominal concentration,
- at least 50% of the standard zero samples were free of interference at the retention time of the analyte(s) of interest,

[6] Deviation from the analysis plan. Please refer to Section 9.1.6 for more details.

- at least 50% of the blank samples were 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 was 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 was defined as a response greater than 5% of the mean IS response of the LLOQ calibration standard(s).

4.3.2 Rejection of Calibration Standards

After initial evaluation, non-zero calibration standards that were out of their acceptance criteria were rejected and were not used to define the final calibration function. If not otherwise documented and justified in the raw data the rejection was performed starting with the calibration standard that has the most deviating response factor versus the mean response factor of all non-zero calibration standards. If applicable, other subsequent calibration standards were rejected following the same principle.

If the LLOQ calibration standard was rejected or the above given interference criteria were not met, the LLOQ was raised to the next lowest calibration standard provided that:

- At least 50% of the blank and standard zero samples fulfilled the above described interference criteria with respect to the designated raised LLOQ level, and
- The back-calculated concentration of designated LLOQ calibration standard was within ± 20% of the nominal concentration.

Rejected calibration standards were coded as ‘rejected’ in the raw data, and the rejected data was flagged in the reporting section.

4.4 Reassays

4.4.1 Reassay of Samples from Rejected Analytical Runs

Study samples from analytical runs that did not fulfil the acceptance criteria were reassayed in another analytical run, provided that sufficient sample volume was available. The analytical data from study samples, calibration standards and QC samples obtained from rejected analytical runs and the reasons for rejection were documented in the study file.

4.4.2 Reassays for Analytical Reasons

Study samples were reassayed in triplicate (for oxysterols, for cholesterol as a single repeat analysis) if no result could be obtained or the original result had to be rejected for analytical reasons, and provided that sufficient sample volume was available. The samples were coded as defined below and the rejected analytical data were documented in the study file. The same coding and documentation procedure applied to calibration standards and QC samples failing to meet acceptance criteria.

In case a reassay value was generated for a study sample for which a valid result has already been determined, the reassay value was not considered and was coded 'reassayed in error'. If such a reassay was performed in the same analytical run, then the result of the sample assayed first in the run was reported and the other sample result was disregarded.

5 Administrative Aspects

5.1 Study Timelines

Table 8. Study Timelines

Proposed experimental starting date:	25th October 2013
Proposed experimental completion date:	15th August 2014
Proposed date for draft study report:	19th October 2014

5.2 Names and Addresses

Table 9. Administrative Study Addresses

Sponsor	Peitsch, Manuel VP Biological Systems Research PHILIP MORRIS INTERNATIONAL CH-2000 Neuchâtel, Switzerland
Test Facility	PHILIP MORRIS INTERNATIONAL Complex Matrix Analysis CH-2000 Neuchâtel, Switzerland
Study Director	Phillips, Blaine Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall Singapore Science Park II, Singapore 117406 Tel.: +65-6631-4510 Fax: +65 64130948 Email: Blaine.Phillips@pmi.com
Deputy Study Director	Alberto Oviedo Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall Singapore Science Park II, Singapore 117406 Tel.: +65-6631-4591 Fax: +65 64130948 Email: Alberto.Oviedo@pmi.com
Principal Investigator for Oxysterols/Cholesterol	Daniel Arndt PHILIP MORRIS INTERNATIONAL CH-2000 Neuchâtel, Switzerland Tel: +41(0)58 242 2134 Fax: +41(0)58 242 28 11 e-mail: Daniel.Arndt@pmi.com

5.3 Quality Assurance

This study was not conducted in accordance with Good Laboratory Practice (GLP).

Since this study was not performed in accordance with GLP, there was no requirement for the involvement of Quality Assurance activities. However, Quality Assurance may review the study report and any amendments, and inspect the raw data and the final report and any amendments according to PMI procedures. Any findings will be reported to the Study Director. A Quality Assurance statement will not be issued.

5.4 Study Plan Amendments

No Study Plan Amendments were issued.

5.5 Deviations

The validations of the assays “Determination of Oxysterols in Plasma using LC-HR-MS” with the corresponding WKI (PMI_RD_WKI_01241) and the “Determination of Cholesterol in Plasma using LC-CAD” with the corresponding WKI (PMI_RD_WKI_01240) were ongoing during this explorative study. Assay validations were finalized after completion of the experimental part of this explorative study. All unintended deviations are summarized in section 9.1.

5.6 Reporting

5.6.1 Study Sample Results

Study sample results from Month 3 QC Check samples and from Month 8 samples were provided directly after completion of each experimental part for the respective time point to the Study Director and the Work Package Leader & SME for Inflammation. Results were reported in numerical format (i.e. xxxx.xx) for oxysterols (ng/mL) and cholesterol (mg/mL) in the form of an .XLS file.

Table 10. Sample details

Report ID	n of samples	n of replicates Cholesterol	n of replicates Oxysterols	Date of report
Month 3 QC Check samples	25	0	1	17th January 2014
Month 8 samples	50	1	3	31st July 2014

If the concentration for a study sample could not be determined, the sample result was reported using one of the codes as described in the following table:

Table 11. Sample Coding

Reason (Code)	Description
Below the Limit of Quantification (BLQ)	Identifies a study sample having a concentration that is below the LLOQ
Dilution Concentration Unreliable (DCU)	Identifies a study sample that was coded DCU and insufficient sample volume remained for reassay
Insufficient Sample for Analysis (ISA)	Identifies a study sample that had insufficient sample volume for initial analysis
Insufficient Sample Volume for Reanalysis (IVR)	Identifies a study sample that had insufficient sample volume for reanalysis
Not Received	Identifies a study sample that was not received
Not Available (N/AV)	Identifies a study sample or a value that was coded N/AV. No value for this sample is available due to e.g. the assay was not performed on this sample.
Not Reportable (NR)	Identifies a study sample for which (due to any other reason not covered above) it was not possible to determine a result
Above the Accepted Range (AAR)	Identifies a study sample whose calculated concentration is greater than the upper limit of quantitation (ULOQ). These study samples were diluted before being reassayed
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 DQC statistics

Table continued on next page

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
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 was documented by the analyst
Unacceptable Chromatography (UCR)	Identifies a study sample, calibration standard, or QC sample judged to have demonstrated unacceptable chromatography
Unacceptable Internal Standard Response (UISR)	Identifies a study sample, non-zero-calibration standard, or QC sample for which - There is no IS response observed when expected - The IS response is < 50% or >180% of the mean IS response of the non-zero standards and QC samples (including DQC samples) meeting their acceptance criteria in the respective analytical run Identifies a standard-zero sample if the IS response is < 50% of the mean IS response as defined above and, at the same time, is lower than the lowest IS response observed in the lowest acceptable calibration standard(s).

The same sample coding mentioned in Table 11 is used for coding the results in this final study report and is documented in the study file.

5.6.1.1 Month 3 sample Results

The results are presented for each oxysterol as a single value. The determination of cholesterol was not performed for Month 3 samples.

Table 12. Oxysterols results for Month 3 samples

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. (mg/mL)	22-Hydroxycholesterol Conc. (ng/mL)	24-Hydroxycholesterol Conc. (ng/mL)	25-Hydroxycholesterol Conc. (ng/mL)	27-Hydroxycholesterol Conc. (ng/mL)	4β-Hydroxycholesterol Conc. (ng/mL)	5α6α-Epoxycholestanol Conc. (ng/mL)	5β6β-Epoxycholestanol Conc. (ng/mL)	7α-Hydroxycholesterol Conc. (ng/mL)	7β-Hydroxycholesterol Conc. (ng/mL)	7-Ketocholesterol Conc. (ng/mL)	6αOHSHydroxyCholestanol Conc. (ng/mL)
S-000019881	Plasma (Oxysterol)	920003	6/12/2013 0:00	15015	N/AV*	7.44	84.03	35.56	86.85	335.72	23.13	61.84	181.57	44.92	44.26	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019897	Plasma (Oxysterol)	920064	6/12/2013 0:00	15015	N/AV*	7.77	117.90	46.46	132.40	386.85	38.63	112.31	204.81	58.42	55.12	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019882	Plasma (Oxysterol)	920150	6/12/2013 0:00	15015	N/AV*	8.69	97.89	29.08	96.76	438.96	34.47	106.28	227.22	82.89	58.47	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019899	Plasma (Oxysterol)	920179	6/12/2013 0:00	15015	N/AV*	7.28	80.94	27.07	81.21	368.37	24.53	75.52	249.74	55.96	54.29	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019901	Plasma (Oxysterol)	920221	6/12/2013 0:00	15015	N/AV*	8.32	88.03	31.45	96.97	387.27	21.52	59.02	179.50	54.44	39.98	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019883	Plasma (Oxysterol)	920238	6/12/2013 0:00	15015	N/AV*	8.74	81.45	39.24	88.58	357.70	26.99	77.89	220.21	54.17	45.97	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**

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Table 12. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. (mg/mL)	22-Hydroxycholesterol Conc. (ng/mL)	24-Hydroxycholesterol Conc. (ng/mL)	25-Hydroxycholesterol Conc. (ng/mL)	27-Hydroxycholesterol Conc. (ng/mL)	4β-Hydroxycholesterol Conc. (ng/mL)	5α6α-Epoxycholestanol Conc. (ng/mL)	5β6β-Epoxycholestanol Conc. (ng/mL)	7α-Hydroxycholesterol Conc. (ng/mL)	7β-Hydroxycholesterol Conc. (ng/mL)	7-Ketocholesterol Conc. (ng/mL)	6αOHSHydroxyCholestanol Conc. (ng/mL)
S-000019878	Plasma (Oxysterol)	920335	6/12/2013 0:00	15015	N/AV*	BLQ**	58.20	27.83	76.56	291.14	23.71	80.34	150.45	51.42	39.48	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019891	Plasma (Oxysterol)	920352	6/12/2013 0:00	15015	N/AV*	9.83	96.02	33.74	103.10	328.71	25.74	77.43	339.52	68.94	50.49	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019879	Plasma (Oxysterol)	920417	6/12/2013 0:00	15015	N/AV*	12.29	107.68	52.30	138.57	404.22	27.61	77.77	232.63	52.09	45.53	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019888	Plasma (Oxysterol)	920422	6/12/2013 0:00	15015	N/AV*	11.25	115.61	37.31	123.40	517.00	31.33	83.75	172.16	51.53	53.36	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019890	Plasma (Oxysterol)	920429	6/12/2013 0:00	15015	N/AV*	11.31	118.68	55.96	144.23	400.81	44.33	111.02	169.16	67.53	74.47	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019889	Plasma (Oxysterol)	920440	6/12/2013 0:00	15015	N/AV*	10.42	163.79	57.44	150.03	460.79	32.83	96.95	177.13	84.35	70.78	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**

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Table 12. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. (mg/mL)	22-Hydroxycholesterol Conc. (ng/mL)	24-Hydroxycholesterol Conc. (ng/mL)	25-Hydroxycholesterol Conc. (ng/mL)	27-Hydroxycholesterol Conc. (ng/mL)	4β-Hydroxycholesterol Conc. (ng/mL)	5α6α-Epoxycholestanol Conc. (ng/mL)	5β6β-Epoxycholestanol Conc. (ng/mL)	7α-Hydroxycholesterol Conc. (ng/mL)	7β-Hydroxycholesterol Conc. (ng/mL)	7-Ketocholesterol Conc. (ng/mL)	6αOHSHydroxyCholestanol Conc. (ng/mL)
S-000019884	Plasma (Oxysterol)	920479	6/12/2013 0:00	15015	N/AV*	8.04	90.31	50.48	104.23	265.76	33.15	92.54	170.65	45.26	55.01	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019893	Plasma (Oxysterol)	920500	6/12/2013 0:00	15015	N/AV*	9.72	106.38	36.93	104.78	340.63	27.71	86.51	258.18	63.82	63.82	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019900	Plasma (Oxysterol)	920521	6/12/2013 0:00	15015	N/AV*	9.20	123.27	38.52	117.48	480.01	40.65	135.71	245.33	68.54	72.94	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019898	Plasma (Oxysterol)	920544	6/12/2013 0:00	15015	N/AV*	8.90	105.94	39.43	109.90	421.75	25.23	75.25	155.13	56.08	49.27	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019886	Plasma (Oxysterol)	920738	6/12/2013 0:00	15015	N/AV*	8.58	90.72	35.53	84.07	341.49	44.18	140.40	187.12	64.72	55.44	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019892	Plasma (Oxysterol)	920801	6/12/2013 0:00	15015	N/AV*	12.10	122.97	50.86	135.40	449.89	34.22	98.41	289.53	103.51	72.43	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**

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Table 12. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. (mg/mL)	22-Hydroxycholesterol Conc. (ng/mL)	24-Hydroxycholesterol Conc. (ng/mL)	25-Hydroxycholesterol Conc. (ng/mL)	27-Hydroxycholesterol Conc. (ng/mL)	4β-Hydroxycholesterol Conc. (ng/mL)	5α6α-Epoxycholestanol Conc. (ng/mL)	5β6β-Epoxycholestanol Conc. (ng/mL)	7α-Hydroxycholesterol Conc. (ng/mL)	7β-Hydroxycholesterol Conc. (ng/mL)	7-Ketocholesterol Conc. (ng/mL)	6αOHSHydroxyCholestanol Conc. (ng/mL)
S-000019896	Plasma (Oxysterol)	920810	6/12/2013 0:00	15015	N/AV*	11.45	114.87	62.96	124.34	396.14	37.55	102.99	177.20	57.37	62.60	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019894	Plasma (Oxysterol)	920814	6/12/2013 0:00	15015	N/AV*	12.23	116.83	63.19	136.22	415.47	42.58	105.24	193.57	64.55	73.20	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019887	Plasma (Oxysterol)	920821	6/12/2013 0:00	15015	N/AV*	9.80	76.75	34.14	79.17	427.12	22.61	63.54	200.76	53.21	46.03	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019895	Plasma (Oxysterol)	920933	6/12/2013 0:00	15015	N/AV*	7.77	104.26	39.49	104.53	356.83	38.74	113.51	241.01	61.16	65.45	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019902	Plasma (Oxysterol)	920969	6/12/2013 0:00	15015	N/AV*	8.97	76.85	28.25	87.68	398.92	15.19	44.97	145.16	32.53	40.40	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
S-000019880	Plasma (Oxysterol)	920986	6/12/2013 0:00	15015	N/AV*	9.91	114.32	41.02	107.45	387.49	38.12	115.57	158.46	52.45	57.93	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**

Table continued on next page

Table 12. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. (mg/mL)	22-Hydroxycholesterol Conc. (ng/mL)	24-Hydroxycholesterol Conc. (ng/mL)	25-Hydroxycholesterol Conc. (ng/mL)	27-Hydroxycholesterol Conc. (ng/mL)	4β-Hydroxycholesterol Conc. (ng/mL)	5α6α-Epoxycholestanol Conc. (ng/mL)	5β6β-Epoxycholestanol Conc. (ng/mL)	7α-Hydroxycholesterol Conc. (ng/mL)	7β-Hydroxycholesterol Conc. (ng/mL)	7-Ketocholesterol Conc. (ng/mL)	6αOHSHydroxyCholestanol Conc. (ng/mL)
S-000019885	Plasma (Oxysterol)	920992	6/12/2013 0:00	15015	N/AV*	6.85	72.21	32.69	88.22	325.08	26.34	73.26	272.82	49.72	48.70	BLQ*
					Median	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					Average	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					SD	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**
					RSD%	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**	N/AV**

N/AV - Not Available

- N/AV* - determination of total cholesterol was not performed on Month 3 samples. As this time the determination of total cholesterol was under development.
- N/AV** - No descriptive statistics could be performed due to single analysis for the determination of oxysterols.

5.6.1.2 Month 3 Chromatograms Oxysterols

This section provides random selected example chromatograms of oxysterol analysis representing at least 5% of total sample chromatograms

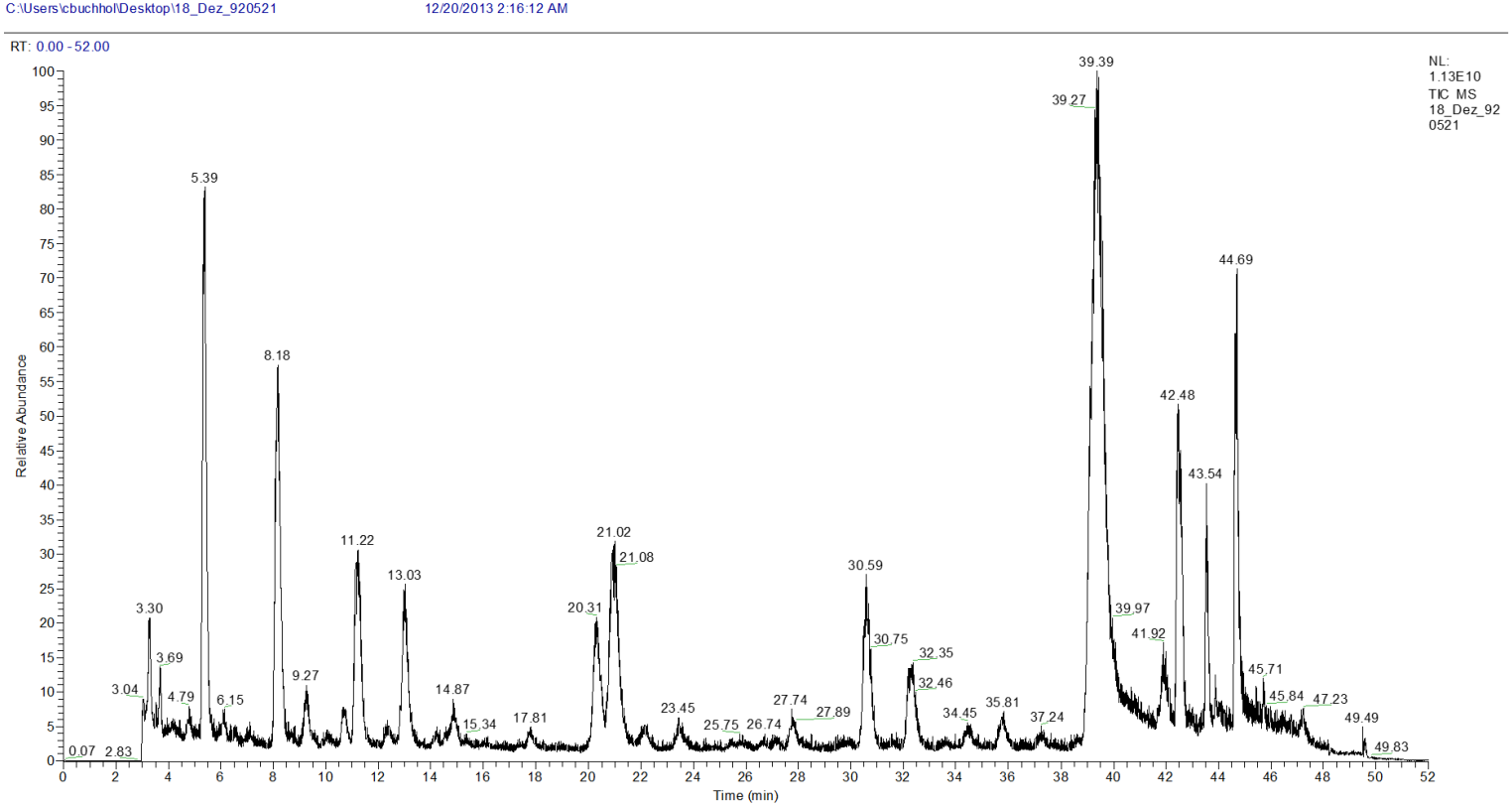


Figure 2. Oxysterols total ion chromatogram (TIC) example month 3 for sample 920521

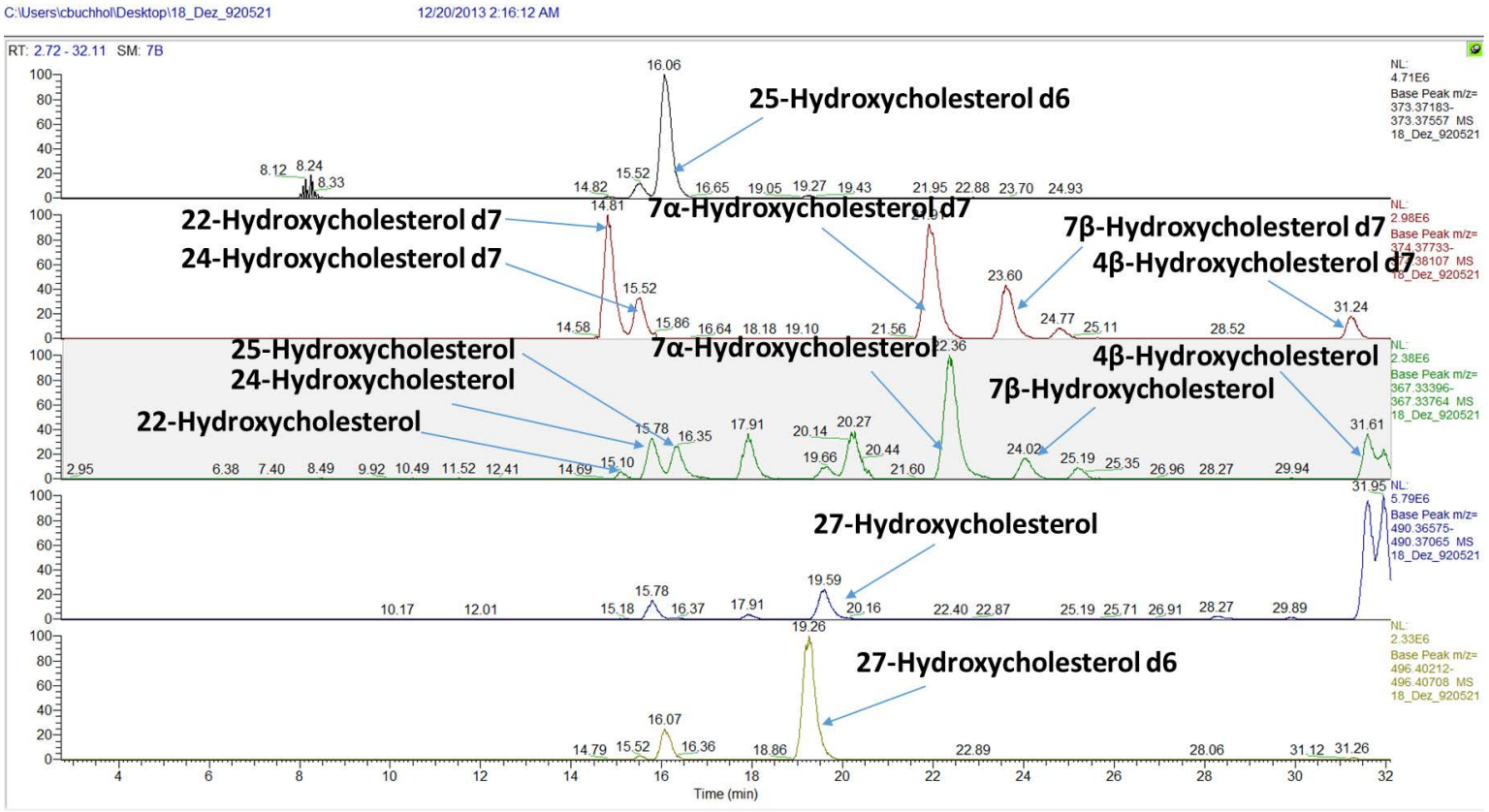


Figure 3. Oxysterols extracted ion chromatogram (XIC) example month 3 for sample 920521

Figure continues on next page

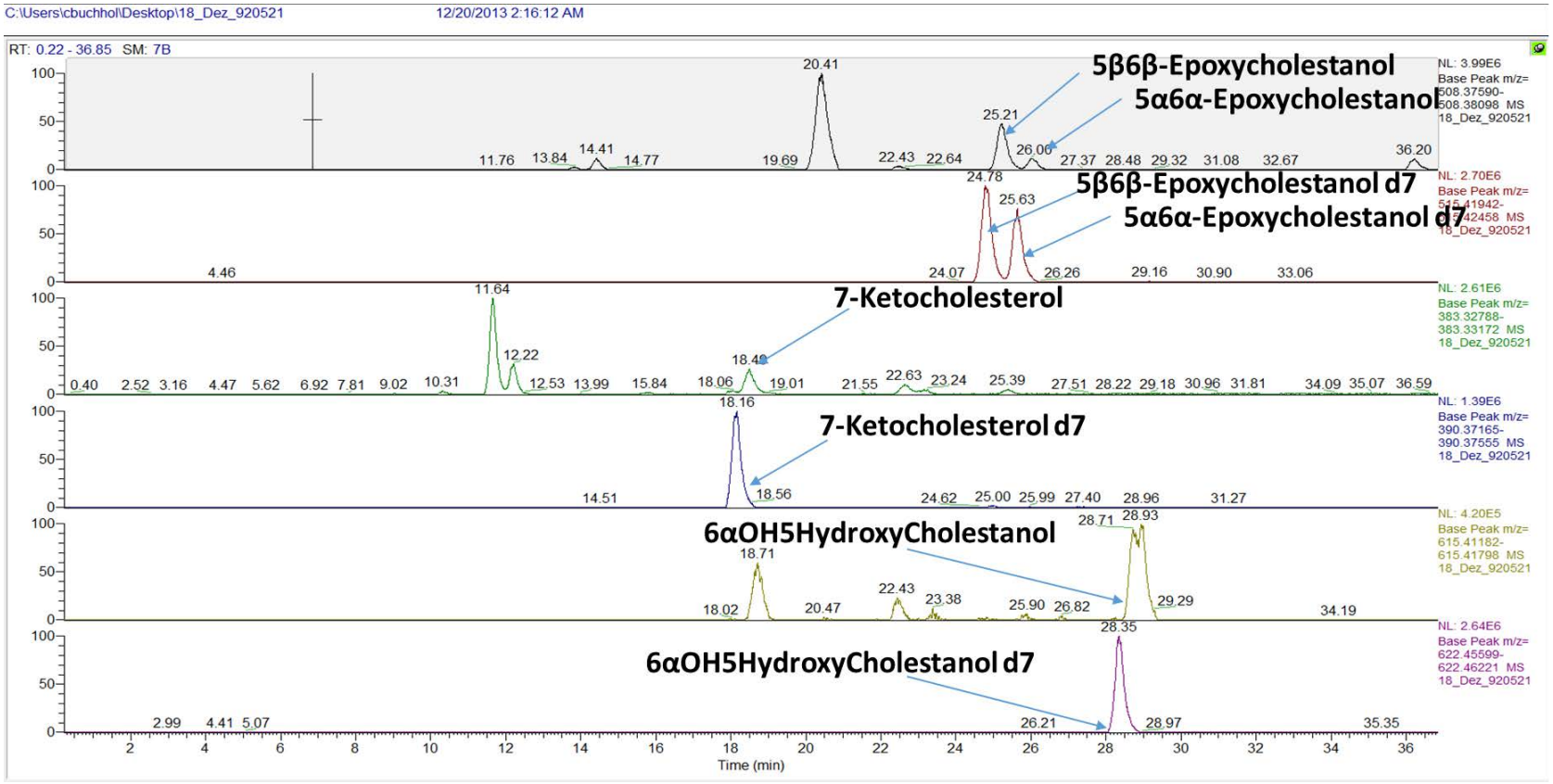


Figure 3. (Continued)

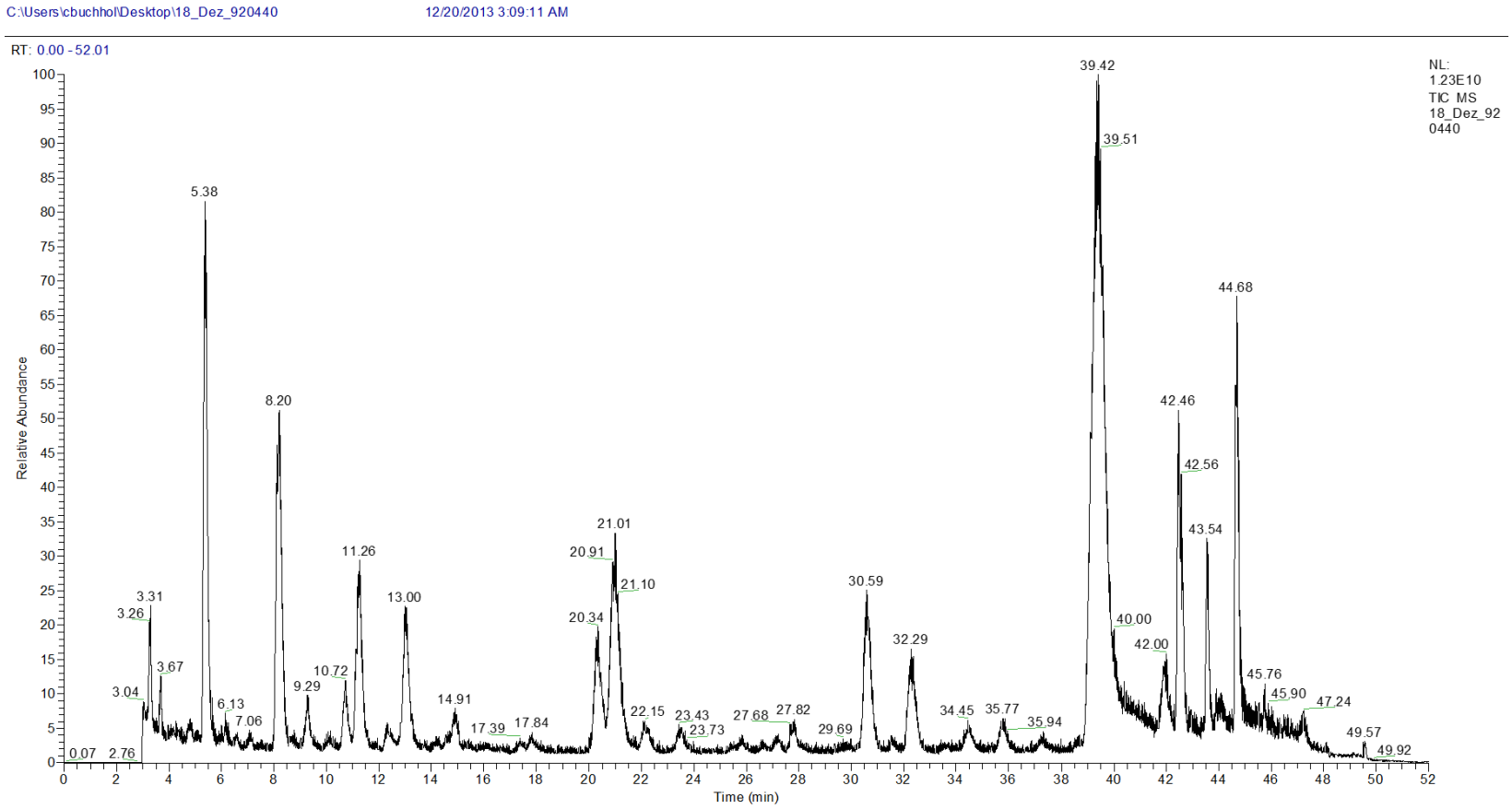


Figure 4. Oxysterols total ion chromatogram (TIC) example month 3 for sample 920440

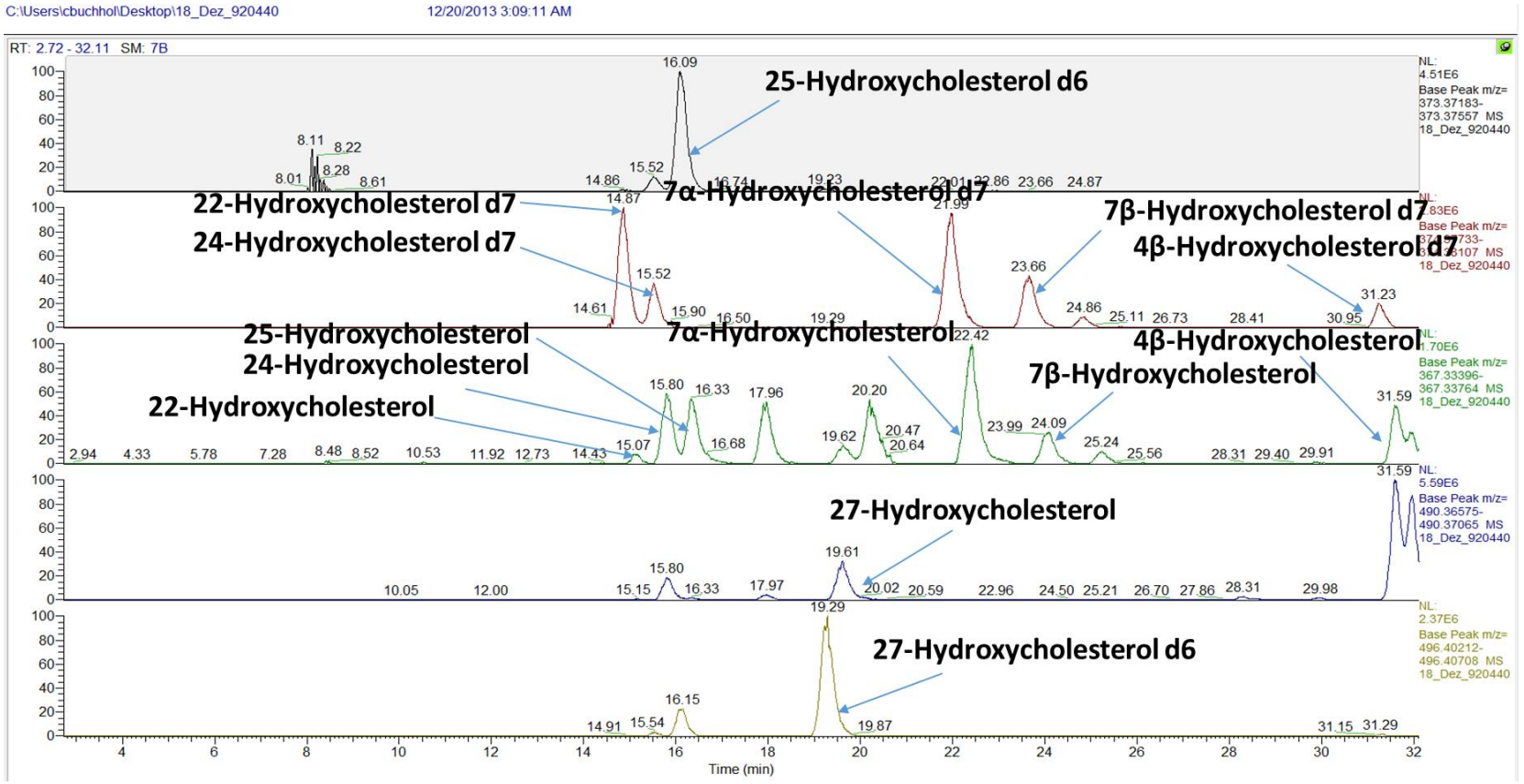


Figure 5. Oxysterols extracted ion chromatogram (XIC) example month 3 for sample 920440

Figure continues on next page

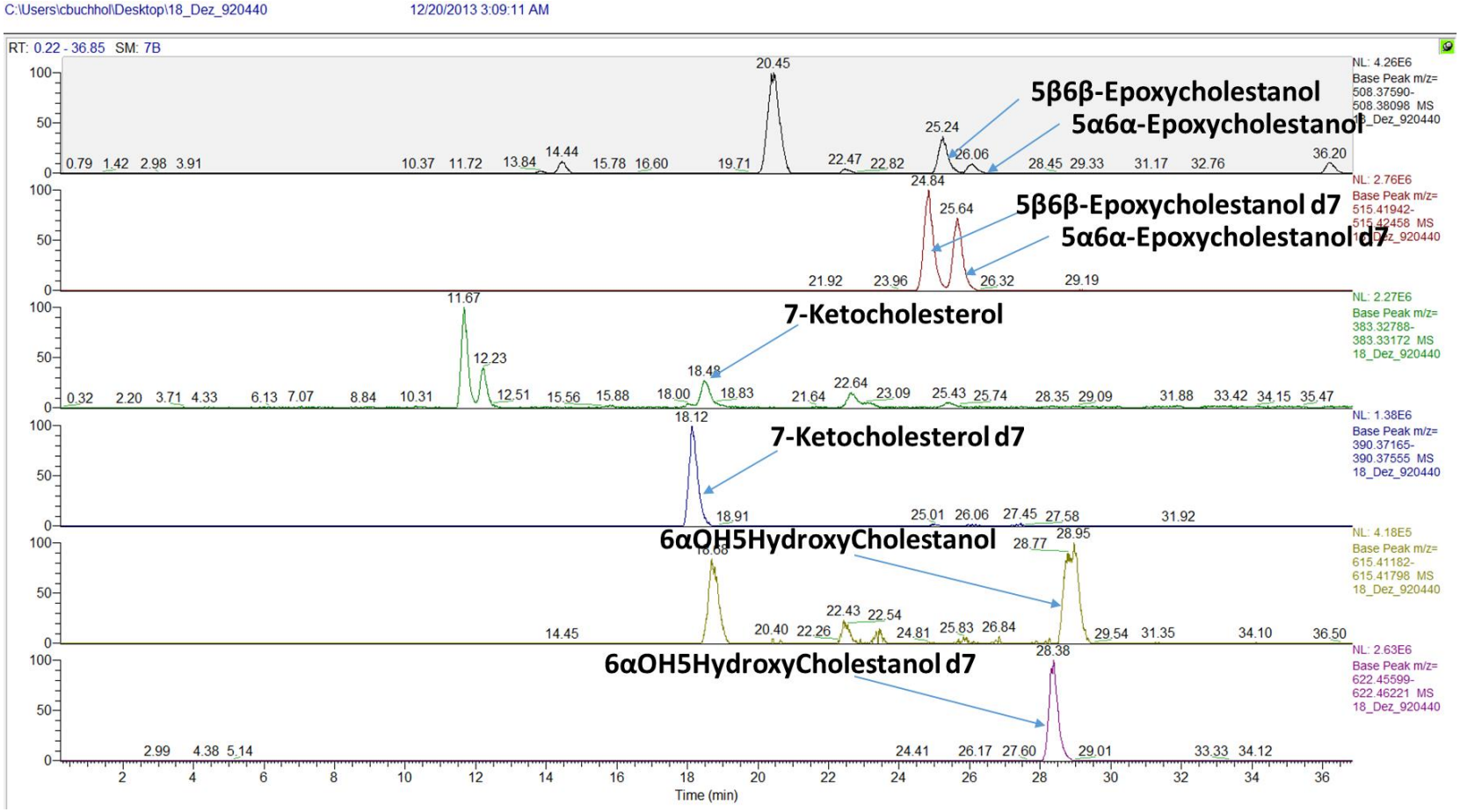


Figure 5. (continued)

5.6.1.3 Month 8 Results

The results are presented for each oxysterol as the median of the three analytical replicates incl. individual values for each replicate. Single values are reported for total cholesterol.

Table 13. Oxysterols and Cholesterol results for Month 8 samples

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028577	Plasma (Oxysterol)	920218	5/2/14 12:00 AM	15015		7.19	91.09	33.41	110.57	225.10	44.67	112.16	176.64	41.91	34.11	BLQ
					3.63	8.47	105.62	38.55	135.45	257.10	33.05	102.63	205.28	50.12	33.80	BLQ
						7.40	104.45	37.19	121.55	205.24	22.77	70.28	190.84	47.18	28.54	BLQ
					Median	**7.40**	**104.45**	**37.19**	**121.55**	**225.10**	**33.05**	**102.63**	**190.84**	**47.18**	**33.80**	**N/AV******
					Average	**7.68**	**100.39**	**36.38**	**122.52**	**229.15**	**33.50**	**95.02**	**190.92**	**46.40**	**32.15**	**N/AV******
					SD	0.69	8.07	2.67	12.46	26.17	10.96	21.95	14.32	4.16	3.13	N/AV****
					RSD%	**8.92**	**8.04**	**7.33**	**10.17**	**11.42**	**32.72**	**23.10**	**7.50**	**8.97**	**9.74**	**N/AV******
S-000028578	Plasma (Oxysterol)	920617	5/2/14 12:00 AM	15015		13.39	98.44	41.66	154.87	310.57	33.96	97.74	357.68	66.14	38.20	BLQ
					5.13	15.66	110.56	47.34	180.04	336.88	40.07	108.26	388.64	73.06	39.90	BLQ
						15.13	104.44	45.73	164.00	320.08	44.38	139.97	362.64	69.45	41.44	BLQ
					Median	**15.13**	**104.44**	**45.73**	**164.00**	**320.08**	**40.07**	**108.26**	**362.64**	**69.45**	**39.90**	**N/AV******
					Average	**14.73**	**104.48**	**44.91**	**166.30**	**322.51**	**39.47**	**115.32**	**369.65**	**69.55**	**39.85**	**N/AV******
					SD	1.19	6.06	2.93	12.74	13.33	5.23	21.98	16.63	3.46	1.62	N/AV****
					RSD%	**8.06**	**5.80**	**6.52**	**7.66**	**4.13**	**13.26**	**19.06**	**4.50**	**4.98**	**4.06**	**N/AV******
S-000028581	Plasma (Oxysterol)	921062	5/2/14 12:00 AM	15015		14.20	113.05	45.64	157.47	227.17	31.63	93.18	224.84	70.53	45.66	BLQ
					4.69	13.66	118.72	45.80	160.64	215.10	39.84	133.65	223.18	68.47	49.39	BLQ
						14.27	110.57	45.97	148.56	223.76	45.44	138.51	219.94	66.79	40.01	BLQ
					Median	**14.20**	**113.05**	**45.80**	**157.47**	**223.76**	**39.84**	**133.65**	**223.18**	**68.47**	**45.66**	**N/AV******
					Average	**14.04**	**114.11**	**45.80**	**155.56**	**222.01**	**38.97**	**121.78**	**222.65**	**68.60**	**45.02**	**N/AV******
					SD	0.33	4.18	0.16	6.27	6.23	6.94	24.89	2.49	1.88	4.72	N/AV****
					RSD%	**2.38**	**3.66**	**0.35**	**4.03**	**2.80**	**17.82**	**20.43**	**1.12**	**2.74**	**10.49**	**N/AV******
S-000028583	Plasma (Oxysterol)	920627	5/2/14 12:00 AM	15015		13.17	101.10	44.52	129.92	97.68	52.38	136.52	200.73	57.62	55.22	BLQ
					4.63	13.63	103.33	43.72	135.92	100.04	60.34	177.40	206.93	62.08	56.15	BLQ
						12.87	100.89	42.68	142.18	123.65	48.19	114.40	198.13	57.67	50.62	BLQ
					Median	**13.17**	**101.10**	**43.72**	**135.92**	**100.04**	**52.38**	**136.52**	**200.73**	**57.67**	**55.22**	**N/AV******
					Average	**13.22**	**101.77**	**43.64**	**136.01**	**107.12**	**53.64**	**142.77**	**201.93**	**59.12**	**54.00**	**N/AV******
					SD	0.39	1.36	0.92	6.13	14.36	6.17	31.96	4.52	2.56	2.96	N/AV****
					RSD%	**2.92**	**1.33**	**2.12**	**4.51**	**13.41**	**11.51**	**22.39**	**2.24**	**4.33**	**5.48**	**N/AV******
S-000028593	Plasma (Oxysterol)	920601	5/2/14 12:00 AM	15015		11.95	90.86	35.46	144.51	240.87	33.33	109.09	158.58	52.99	34.38	BLQ
					3.95	11.91	93.60	34.94	138.53	250.26	33.91	103.81	164.48	54.15	36.08	BLQ
						11.19	89.61	33.82	133.81	238.15	28.04	77.83	148.29	48.94	32.16	BLQ
					Median	**11.91**	**90.86**	**34.94**	**138.53**	**240.87**	**33.33**	**103.81**	**158.58**	**52.99**	**34.38**	**N/AV******
					Average	**11.68**	**91.36**	**34.74**	**138.95**	**243.09**	**31.76**	**96.91**	**157.12**	**52.03**	**34.21**	**N/AV******
					SD	0.43	2.04	0.84	5.36	6.35	3.24	16.73	8.19	2.73	1.97	N/AV****
					RSD%	**3.68**	**2.23**	**2.41**	**3.86**	**2.61**	**10.20**	**17.26**	**5.21**	**5.26**	**5.76**	**N/AV******
S-000028580	Plasma (Oxysterol)	920339	5/2/14 12:00 AM	15015		11.91	91.09	38.61	129.30	226.25	45.13	168.28	181.58	54.88	43.01	BLQ
					4.10	13.01	97.50	37.71	147.05	228.19	40.14	113.36	184.27	51.76	42.46	BLQ
						12.62	96.66	37.89	139.77	217.06	35.67	122.34	175.19	50.30	37.80	BLQ
					Median	**12.62**	**96.66**	**37.89**	**139.77**	**226.25**	**40.14**	**122.34**	**181.58**	**51.76**	**42.46**	**N/AV******
					Average	**12.51**	**95.08**	**38.07**	**138.71**	**223.83**	**40.31**	**134.66**	**180.35**	**52.31**	**41.09**	**N/AV******
					SD	0.56	3.48	0.47	8.92	5.95	4.73	29.46	4.67	2.34	2.86	N/AV****
					RSD%	**4.47**	**3.66**	**1.24**	**6.43**	**2.66**	**11.74**	**21.87**	**2.59**	**4.47**	**6.97**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOH5HydroxyCholestanol Conc. [ng/mL]
S-000028579	Plasma (Oxysterol)	920762	5/2/14 12:00 AM	15015		9.08	98.28	38.38	149.80	94.44	36.76	124.04	208.49	56.98	38.25	BLQ
					5.09	9.77	102.58	39.20	171.78	100.85	35.78	94.37	222.07	60.22	37.57	BLQ
						9.22	101.74	37.32	157.87	101.72	37.77	145.18	214.72	60.69	41.10	BLQ
					Median	**9.22**	**101.74**	**38.38**	**157.87**	**100.85**	**36.76**	**124.04**	**214.72**	**60.22**	**38.25**	**N/AV******
					Average	**9.35**	**100.87**	**38.30**	**159.82**	**99.00**	**36.77**	**121.20**	**215.09**	**59.29**	**38.98**	**N/AV******
					SD	0.37	2.28	0.94	11.12	3.98	0.99	25.53	6.80	2.02	1.87	N/AV****
					RSD%	**3.90**	**2.26**	**2.45**	**6.96**	**4.02**	**2.70**	**21.06**	**3.16**	**3.41**	**4.80**	**N/AV******
S-000028582	Plasma (Oxysterol)	920266	5/2/14 12:00 AM	15015		15.41	113.10	46.50	162.48	232.71	38.58	104.70	288.96	83.99	41.82	BLQ
					4.81	15.32	113.82	44.83	151.52	196.58	44.18	161.97	274.88	78.75	46.57	BLQ
						16.19	116.11	46.83	159.56	192.63	45.61	146.22	291.72	83.37	51.70	BLQ
					Median	**15.41**	**113.82**	**46.50**	**159.56**	**196.58**	**44.18**	**146.22**	**288.96**	**83.37**	**46.57**	**N/AV******
					Average	**15.64**	**114.34**	**46.05**	**157.85**	**207.30**	**42.79**	**137.63**	**285.19**	**82.03**	**46.70**	**N/AV******
					SD	0.48	1.57	1.07	5.68	22.09	3.71	29.58	9.03	2.86	4.95	N/AV****
					RSD%	**3.06**	**1.38**	**2.33**	**3.60**	**10.65**	**8.68**	**21.49**	**3.17**	**3.49**	**10.59**	**N/AV******
S-000028587	Plasma (Oxysterol)	920197	5/2/14 12:00 AM	15015		18.95	114.82	70.02	182.01	262.19	43.29	94.45	228.67	54.70	41.25	BLQ
					6.95	17.33	116.83	71.39	176.19	259.05	44.09	97.48	226.55	53.59	40.99	BLQ
						18.58	112.21	71.09	170.91	237.76	42.09	97.28	221.95	53.13	38.69	BLQ
					Median	**18.58**	**114.82**	**71.09**	**176.19**	**259.05**	**43.29**	**97.28**	**226.55**	**53.59**	**40.99**	**N/AV******
					Average	**18.29**	**114.62**	**70.83**	**176.37**	**253.00**	**43.16**	**96.40**	**225.72**	**53.81**	**40.31**	**N/AV******
					SD	0.85	2.32	0.72	5.55	13.29	1.01	1.70	3.44	0.81	1.41	N/AV****
					RSD%	**4.65**	**2.02**	**1.01**	**3.15**	**5.25**	**2.34**	**1.76**	**1.52**	**1.50**	**3.49**	**N/AV******
S-000028596	Plasma (Oxysterol)	921019	5/2/14 12:00 AM	15015		15.85	90.66	38.89	155.69	184.67	37.08	97.43	450.27	67.97	32.10	BLQ
					4.24	17.11	99.05	44.01	181.27	210.86	42.51	122.17	508.02	77.38	37.96	BLQ
						17.59	87.53	38.95	161.79	183.31	37.21	107.41	448.38	65.73	34.20	BLQ
					Median	**17.11**	**90.66**	**38.95**	**161.79**	**184.67**	**37.21**	**107.41**	**450.27**	**67.97**	**34.20**	**N/AV******
					Average	**16.85**	**92.41**	**40.61**	**166.25**	**192.94**	**38.93**	**109.00**	**468.89**	**70.36**	**34.75**	**N/AV******
					SD	0.90	5.96	2.94	13.36	15.53	3.10	12.45	33.90	6.18	2.97	N/AV****
					RSD%	**5.33**	**6.44**	**7.24**	**8.03**	**8.05**	**7.95**	**11.42**	**7.23**	**8.79**	**8.54**	**N/AV******
S-000028584	Plasma (Oxysterol)	920970	5/2/14 12:00 AM	15015		10.05	74.01	32.93	116.03	272.93	31.96	93.89	167.93	49.12	31.19	BLQ
					4.05	9.95	71.29	33.92	120.16	279.80	29.92	88.67	162.10	48.33	30.84	BLQ
						8.60	64.79	31.51	102.54	273.05	28.98	84.70	149.44	45.38	37.75	BLQ
					Median	**9.95**	**71.29**	**32.93**	**116.03**	**273.05**	**29.92**	**88.67**	**162.10**	**48.33**	**31.19**	**N/AV******
					Average	**9.53**	**70.03**	**32.79**	**112.91**	**275.26**	**30.29**	**89.09**	**159.82**	**47.61**	**33.26**	**N/AV******
					SD	0.81	4.74	1.21	9.21	3.93	1.52	4.61	9.45	1.97	3.89	N/AV****
					RSD%	**8.51**	**6.76**	**3.70**	**8.16**	**1.43**	**5.02**	**5.17**	**5.91**	**4.13**	**11.70**	**N/AV******
S-000028586	Plasma (Oxysterol)	920896	5/2/14 12:00 AM	15015		7.17	64.13	24.22	94.77	153.09	21.58	60.74	85.59	26.56	25.06	BLQ
					2.59	7.26	57.84	22.25	77.32	150.35	20.68	63.09	78.41	22.54	24.58	BLQ
						6.68	59.68	23.17	75.80	161.41	19.71	57.18	77.91	20.71	23.92	BLQ
					Median	**7.17**	**59.68**	**23.17**	**77.32**	**153.09**	**20.68**	**60.74**	**78.41**	**22.54**	**24.58**	**N/AV******
					Average	**7.04**	**60.55**	**23.21**	**82.63**	**154.95**	**20.66**	**60.34**	**80.64**	**23.27**	**24.52**	**N/AV******
					SD	0.31	3.23	0.98	10.54	5.76	0.93	2.97	4.30	3.00	0.57	N/AV****
					RSD%	**4.45**	**5.34**	**4.24**	**12.75**	**3.72**	**4.52**	**4.93**	**5.33**	**12.87**	**2.33**	**N/AV******

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Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028585	Plasma (Oxysterol)	920793	5/2/14 12:00 AM	15015	4.12	9.52	76.27	31.12	123.70	153.48	30.37	86.97	138.06	43.78	41.36	BLQ
						10.30	81.85	33.00	132.91	172.88	26.91	76.20	136.85	45.83	40.80	BLQ
						11.28	79.31	32.82	126.83	176.80	28.64	86.50	137.71	46.88	40.71	BLQ
					Median	**10.30**	**79.31**	**32.82**	**126.83**	**172.88**	**28.64**	**86.50**	**137.71**	**45.83**	**40.80**	**N/AV******
					Average	**10.37**	**79.14**	**32.31**	**127.81**	**167.72**	**28.64**	**83.22**	**137.54**	**45.49**	**40.96**	**N/AV******
					SD	0.88	2.80	1.04	4.68	12.49	1.73	6.09	0.62	1.58	0.35	N/AV****
					RSD%	**8.53**	**3.53**	**3.22**	**3.66**	**7.45**	**6.05**	**7.31**	**0.45**	**3.46**	**0.85**	**N/AV******
S-000028589	Plasma (Oxysterol)	920722	5/2/14 12:00 AM	15015	6.81	16.64	119.70	64.71	169.06	199.04	43.62	101.49	182.72	54.24	51.88	BLQ
						16.47	112.47	58.25	156.59	194.46	41.13	95.85	164.00	52.03	44.82	BLQ
						16.68	117.63	60.76	170.39	174.62	40.22	90.35	176.25	55.38	52.56	BLQ
					Median	**16.64**	**117.63**	**60.76**	**169.06**	**194.46**	**41.13**	**95.85**	**176.25**	**54.24**	**51.88**	**N/AV******
					Average	**16.59**	**116.60**	**61.24**	**165.35**	**189.37**	**41.66**	**95.90**	**174.33**	**53.88**	**49.75**	**N/AV******
					SD	0.11	3.72	3.25	7.61	12.98	1.76	5.57	9.51	1.70	4.29	N/AV****
					RSD%	**0.68**	**3.19**	**5.31**	**4.60**	**6.86**	**4.22**	**5.81**	**5.45**	**3.16**	**8.62**	**N/AV******
S-000028598	Plasma (Oxysterol)	920700	5/2/14 12:00 AM	15015	5.18	12.59	102.27	43.19	153.48	191.05	33.29	91.59	303.49	79.66	42.79	BLQ
						14.29	117.20	46.03	170.67	223.87	38.75	108.80	332.28	81.01	52.10	BLQ
						14.35	108.16	44.72	165.58	206.31	32.41	96.09	305.22	76.88	45.57	BLQ
					Median	**14.29**	**108.16**	**44.72**	**165.58**	**206.31**	**33.29**	**96.09**	**305.22**	**79.66**	**45.57**	**N/AV******
					Average	**13.74**	**109.21**	**44.65**	**163.24**	**207.08**	**34.82**	**98.83**	**313.66**	**79.18**	**46.82**	**N/AV******
					SD	1.00	7.52	1.42	8.83	16.42	3.43	8.93	16.14	2.11	4.78	N/AV****
					RSD%	**7.27**	**6.89**	**3.18**	**5.41**	**7.93**	**9.86**	**9.03**	**5.15**	**2.66**	**10.21**	**N/AV******
S-000028588	Plasma (Oxysterol)	920147	5/2/14 12:00 AM	15015	4.35	11.77	85.36	36.63	133.76	197.42	32.35	81.89	156.05	55.90	51.46	BLQ
						13.03	86.23	37.77	141.30	202.62	33.53	81.68	153.27	52.65	43.59	BLQ
						11.48	83.17	36.11	133.29	191.32	30.33	77.62	152.63	48.72	45.01	BLQ
					Median	**11.77**	**85.36**	**36.63**	**133.76**	**197.42**	**32.35**	**81.68**	**153.27**	**52.65**	**45.01**	**N/AV******
					Average	**12.09**	**84.92**	**36.84**	**136.12**	**197.12**	**32.07**	**80.40**	**153.98**	**52.42**	**46.69**	**N/AV******
					SD	0.82	1.58	0.85	4.49	5.66	1.62	2.41	1.82	3.60	4.19	N/AV****
					RSD%	**6.81**	**1.86**	**2.31**	**3.30**	**2.87**	**5.04**	**3.00**	**1.18**	**6.86**	**8.98**	**N/AV******
S-000028590	Plasma (Oxysterol)	920865	5/2/14 12:00 AM	15015	5.15	15.29	111.07	46.01	155.11	257.47	41.67	113.42	266.23	74.47	59.52	BLQ
						15.02	112.46	44.75	152.14	282.82	37.17	99.78	269.70	77.16	57.44	BLQ
						15.72	112.35	42.94	148.28	245.74	40.16	103.57	269.50	75.13	64.16	BLQ
					Median	**15.29**	**112.35**	**44.75**	**152.14**	**257.47**	**40.16**	**103.57**	**269.50**	**75.13**	**59.52**	**N/AV******
					Average	**15.35**	**111.96**	**44.57**	**151.84**	**262.01**	**39.67**	**105.59**	**268.48**	**75.59**	**60.37**	**N/AV******
					SD	0.35	0.77	1.54	3.42	18.95	2.29	7.04	1.95	1.40	3.45	N/AV****
					RSD%	**2.29**	**0.69**	**3.46**	**2.26**	**7.23**	**5.77**	**6.67**	**0.73**	**1.85**	**5.71**	**N/AV******
S-000028592	Plasma (Oxysterol)	920011	5/2/14 12:00 AM	15015	7.39	19.40	120.12	46.47	183.90	306.11	34.66	96.43	298.67	79.13	49.72	BLQ
						19.26	115.28	46.22	175.65	313.70	45.96	117.98	289.61	78.85	49.74	BLQ
						19.44	114.05	42.98	170.46	327.39	29.03	84.47	279.41	73.85	39.48	BLQ
					Median	**19.40**	**115.28**	**46.22**	**175.65**	**313.70**	**34.66**	**96.43**	**289.61**	**78.85**	**49.72**	**N/AV******
					Average	**19.37**	**116.48**	**45.22**	**176.67**	**315.73**	**36.55**	**99.63**	**289.23**	**77.27**	**46.31**	**N/AV******
					SD	0.10	3.21	1.95	6.78	10.79	8.62	16.99	9.64	2.97	5.92	N/AV****
					RSD%	**0.50**	**2.76**	**4.31**	**3.84**	**3.42**	**23.59**	**17.05**	**3.33**	**3.84**	**12.78**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028591	Plasma (Oxysterol)	920379	5/2/14 12:00 AM	15015		13.01	71.65	41.99	121.38	139.91	37.75	74.45	184.99	38.43	37.75	BLQ
					3.75	10.20	74.05	41.10	116.49	130.47	42.60	75.68	188.17	37.31	39.99	BLQ
						11.20	71.35	40.93	123.92	135.79	40.51	78.25	181.94	36.07	42.97	BLQ
					Median	**11.20**	**71.65**	**41.10**	**121.38**	**135.79**	**40.51**	**75.68**	**184.99**	**37.31**	**39.99**	**N/AV******
					Average	**11.47**	**72.35**	**41.34**	**120.60**	**135.39**	**40.29**	**76.12**	**185.03**	**37.27**	**40.23**	**N/AV******
					SD	1.43	1.48	0.57	3.78	4.73	2.43	1.94	3.12	1.18	2.62	N/AV****
					RSD%	**12.43**	**2.05**	**1.37**	**3.13**	**3.50**	**6.04**	**2.55**	**1.68**	**3.17**	**6.51**	**N/AV******
S-000028607	Plasma (Oxysterol)	920856	5/2/14 12:00 AM	15015		12.03	80.45	33.65	133.11	179.65	26.23	61.60	205.94	54.59	34.12	BLQ
					4.30	13.01	81.78	34.46	138.42	185.08	29.02	74.09	202.36	56.85	31.34	BLQ
						11.64	81.43	30.82	127.61	179.41	25.93	69.20	195.45	54.51	29.24	BLQ
					Median	**12.03**	**81.43**	**33.65**	**133.11**	**179.65**	**26.23**	**69.20**	**202.36**	**54.59**	**31.34**	**N/AV******
					Average	**12.23**	**81.22**	**32.98**	**133.05**	**181.38**	**27.06**	**68.30**	**201.25**	**55.31**	**31.56**	**N/AV******
					SD	0.70	0.69	1.91	5.40	3.21	1.71	6.29	5.33	1.33	2.45	N/AV****
					RSD%	**5.74**	**0.85**	**5.79**	**4.06**	**1.77**	**6.31**	**9.21**	**2.65**	**2.40**	**7.75**	**N/AV******
S-000028597	Plasma (Oxysterol)	920816	5/2/14 12:00 AM	15015		12.45	93.97	39.43	148.03	270.96	50.29	204.44	355.51	68.18	53.95	BLQ
					4.87	13.73	85.65	39.43	159.24	240.10	38.35	126.60	326.19	68.96	40.86	BLQ
						13.30	92.23	39.95	165.15	251.89	39.30	117.97	353.88	71.06	43.79	BLQ
					Median	**13.30**	**92.23**	**39.43**	**159.24**	**251.89**	**39.30**	**126.60**	**353.88**	**68.96**	**43.79**	**N/AV******
					Average	**13.16**	**90.61**	**39.60**	**157.47**	**254.32**	**42.64**	**149.67**	**345.19**	**69.40**	**46.20**	**N/AV******
					SD	0.65	4.39	0.30	8.70	15.57	6.64	47.63	16.48	1.49	6.87	N/AV****
					RSD%	**4.97**	**4.84**	**0.75**	**5.52**	**6.12**	**15.56**	**31.82**	**4.77**	**2.15**	**14.87**	**N/AV******
S-000028601	Plasma (Oxysterol)	920830	5/2/14 12:00 AM	15015		14.41	121.86	46.03	180.09	301.38	43.86	186.02	254.33	72.49	42.94	BLQ
					4.73	14.96	108.76	42.63	166.41	270.09	33.21	102.00	233.50	63.64	36.00	BLQ
						13.65	114.43	42.80	176.95	287.01	36.67	117.30	242.78	66.64	41.74	BLQ
					Median	**14.41**	**114.43**	**42.80**	**176.95**	**287.01**	**36.67**	**117.30**	**242.78**	**66.64**	**41.74**	**N/AV******
					Average	**14.34**	**115.02**	**43.82**	**174.48**	**286.16**	**37.92**	**135.11**	**243.54**	**67.59**	**40.23**	**N/AV******
					SD	0.66	6.57	1.92	7.17	15.66	5.43	44.75	10.44	4.50	3.71	N/AV****
					RSD%	**4.58**	**5.71**	**4.38**	**4.11**	**5.47**	**14.33**	**33.12**	**4.29**	**6.66**	**9.21**	**N/AV******
S-000028599	Plasma (Oxysterol)	920330	5/2/14 12:00 AM	15015		13.02	99.23	38.01	139.85	206.74	37.04	97.90	298.72	64.61	36.89	BLQ
					3.66	5.41**ISP	42.70**ISP	15.65**ISP	62.32**ISP	80.38**ISP	13.64**ISP	46.87**ISP	118.92**ISP	24.66**ISP	14.23**ISP	BLQ
						10.71	95.97	33.72	125.91	199.16	33.43	101.04	283.83	57.11	37.29	BLQ
					Median	**11.86**	**97.60**	**35.87**	**132.88**	**202.95**	**35.23**	**99.47**	**291.27**	**60.86**	**37.09**	**N/AV******
					Average	**11.86**	**97.60**	**35.87**	**132.88**	**202.95**	**35.23**	**99.47**	**291.27**	**60.86**	**37.09**	**N/AV******
					SD	1.63	2.31	3.04	9.86	5.37	2.55	2.22	10.53	5.30	0.28	N/AV****
					RSD%	**13.75**	**2.36**	**8.47**	**7.42**	**2.64**	**7.24**	**2.23**	**3.61**	**8.72**	**0.76**	**N/AV******
S-000028594	Plasma (Oxysterol)	920556	5/2/14 12:00 AM	15015		20.60	155.94	89.64	223.60	271.61	57.12	148.32	317.90	92.99	60.24	BLQ
					7.36	22.86	157.94	88.82	215.44	303.43	65.55	196.80	327.40	96.59	78.70	BLQ
						23.40	164.16	90.59	223.84	282.54	55.37	147.94	328.43	96.68	71.32	BLQ
					Median	**22.86**	**157.94**	**89.64**	**223.60**	**282.54**	**57.12**	**148.32**	**327.40**	**96.59**	**71.32**	**N/AV******
					Average	**22.29**	**159.34**	**89.68**	**220.96**	**285.86**	**59.34**	**164.35**	**324.58**	**95.42**	**70.09**	**N/AV******
					SD	1.49	4.29	0.88	4.78	16.17	5.44	28.10	5.81	2.11	9.29	N/AV****
					RSD%	**6.68**	**2.69**	**0.98**	**2.16**	**5.66**	**9.17**	**17.10**	**1.79**	**2.21**	**13.26**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028610	Plasma (Oxysterol)	920709	5/2/14 12:00 AM	15015		11.14	87.51	41.45	122.68	190.51	29.12	99.56	162.95	49.42	31.72	BLQ
					3.65	9.50	92.25	38.25	120.10	179.08	31.65	97.25	162.35	46.10	26.72	BLQ
						8.39	82.12	36.93	108.33	195.39	27.95	84.58	155.21	43.43	28.11	BLQ
					Median	**9.50**	**87.51**	**38.25**	**120.10**	**190.51**	**29.12**	**97.25**	**162.35**	**46.10**	**28.11**	**N/AV******
					Average	**9.68**	**87.29**	**38.88**	**117.04**	**188.33**	**29.57**	**93.80**	**160.17**	**46.32**	**28.85**	**N/AV******
					SD	1.38	5.07	2.33	7.65	8.37	1.89	8.06	4.31	3.00	2.58	N/AV****
					RSD%	**14.29**	**5.81**	**5.99**	**6.54**	**4.45**	**6.39**	**8.60**	**2.69**	**6.47**	**8.94**	**N/AV******
S-000028603	Plasma (Oxysterol)	920020	5/2/14 12:00 AM	15015		12.08	99.02	39.28	136.42	175.69	40.60	90.58	279.62	53.80	43.16	BLQ
					3.85	12.46	93.72	37.84	132.76	167.41	44.84	105.31	262.97	52.54	46.77	BLQ
						14.23	107.59	40.59	148.47	180.27	44.56	106.93	290.92	56.58	48.22	BLQ
					Median	**12.46**	**99.02**	**39.28**	**136.42**	**175.69**	**44.56**	**105.31**	**279.62**	**53.80**	**46.77**	**N/AV******
					Average	**12.92**	**100.11**	**39.24**	**139.22**	**174.45**	**43.33**	**100.94**	**277.84**	**54.31**	**46.05**	**N/AV******
					SD	1.15	7.00	1.38	8.22	6.52	2.37	9.00	14.06	2.07	2.60	N/AV****
					RSD%	**8.89**	**6.99**	**3.51**	**5.91**	**3.74**	**5.47**	**8.92**	**5.06**	**3.81**	**5.66**	**N/AV******
S-000028605	Plasma (Oxysterol)	920251	5/2/14 12:00 AM	15015		11.34	109.10	38.91	132.68	171.09	41.09	108.57	162.75	51.69	40.14	BLQ
					3.86	11.29	119.35	42.27	140.30	217.67	39.47	102.75	168.19	51.11	33.11	BLQ
						11.80	115.42	41.53	135.53	205.74	36.74	95.10	163.91	48.04	31.55	BLQ
					Median	**11.34**	**115.42**	**41.53**	**135.53**	**205.74**	**39.47**	**102.75**	**163.91**	**51.11**	**33.11**	**N/AV******
					Average	**11.47**	**114.62**	**40.90**	**136.17**	**198.16**	**39.10**	**102.14**	**164.95**	**50.28**	**34.93**	**N/AV******
					SD	0.28	5.17	1.77	3.85	24.20	2.20	6.75	2.87	1.96	4.58	N/AV****
					RSD%	**2.46**	**4.51**	**4.32**	**2.83**	**12.21**	**5.62**	**6.61**	**1.74**	**3.90**	**13.10**	**N/AV******
S-000028600	Plasma (Oxysterol)	920791	5/2/14 12:00 AM	15015		14.48	110.33	39.75	169.52	195.14	53.45	159.97	264.44	85.98	55.19	BLQ
					4.62	13.54	111.43	39.57	154.50	218.66	50.45	129.54	257.05	81.94	48.50	BLQ
						12.82	105.11	37.37	156.55	190.61	46.41	123.66	240.22	77.55	44.12	BLQ
					Median	**13.54**	**110.33**	**39.57**	**156.55**	**195.14**	**50.45**	**129.54**	**257.05**	**81.94**	**48.50**	**N/AV******
					Average	**13.62**	**108.96**	**38.90**	**160.19**	**201.47**	**50.10**	**137.72**	**253.90**	**81.82**	**49.27**	**N/AV******
					SD	0.83	3.38	1.32	8.14	15.06	3.54	19.49	12.41	4.21	5.57	N/AV****
					RSD%	**6.12**	**3.10**	**3.40**	**5.08**	**7.47**	**7.06**	**14.15**	**4.89**	**5.15**	**11.31**	**N/AV******
S-000028595	Plasma (Oxysterol)	920646	5/2/14 12:00 AM	15015		18.00	116.20	56.89	167.21	155.80	39.67	130.90	239.01	55.39	33.08	BLQ
					4.53	17.33	107.83	53.30	163.25	156.73	35.55	88.94	223.47	56.57	31.43	BLQ
						16.95	106.37	55.14	162.59	150.20	34.08	91.74	227.23	53.95	40.29	BLQ
					Median	**17.33**	**107.83**	**55.14**	**163.25**	**155.80**	**35.55**	**91.74**	**227.23**	**55.39**	**33.08**	**N/AV******
					Average	**17.43**	**110.13**	**55.11**	**164.35**	**154.25**	**36.43**	**103.86**	**229.90**	**55.30**	**34.93**	**N/AV******
					SD	0.53	5.30	1.80	2.50	3.53	2.89	23.46	8.11	1.31	4.71	N/AV****
					RSD%	**3.04**	**4.82**	**3.26**	**1.52**	**2.29**	**7.94**	**22.59**	**3.53**	**2.37**	**13.49**	**N/AV******
S-000028613	Plasma (Oxysterol)	920140	5/2/14 12:00 AM	15015		14.69	114.14	46.42	154.89	289.39	57.12	191.77	204.14	60.95	45.76	BLQ
					4.17	15.06	114.70	50.83	155.08	275.13	46.23	116.66	212.44	61.80	44.40	BLQ
						14.15	109.01	48.30	141.62	292.93	40.96	95.65	198.04	54.80	38.40	BLQ
					Median	**14.69**	**114.14**	**48.30**	**154.89**	**289.39**	**46.23**	**116.66**	**204.14**	**60.95**	**44.40**	**N/AV******
					Average	**14.63**	**112.62**	**48.52**	**150.53**	**285.82**	**48.10**	**134.69**	**204.87**	**59.19**	**42.85**	**N/AV******
					SD	0.45	3.14	2.22	7.72	9.43	8.24	50.53	7.23	3.82	3.92	N/AV****
					RSD%	**3.11**	**2.78**	**4.57**	**5.13**	**3.30**	**17.14**	**37.52**	**3.53**	**6.45**	**9.14**	**N/AV******

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Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028609	Plasma (Oxysterol)	920835	5/2/14 12:00 AM	15015		10.80	103.52	36.25	139.85	214.25	28.12	85.97	251.59	51.83	41.67	BLQ
					3.94	11.11	92.43	33.78	126.44	200.46	26.99	77.46	221.20	50.56	42.41	BLQ
						10.53	94.66	34.62	126.37	186.97	29.15	72.68	220.16	48.51	41.70	BLQ
					Median	**10.80**	**94.66**	**34.62**	**126.44**	**200.46**	**28.12**	**77.46**	**221.20**	**50.56**	**41.70**	**N/AV******
					Average	**10.81**	**96.87**	**34.88**	**130.89**	**200.56**	**28.09**	**78.70**	**230.98**	**50.30**	**41.92**	**N/AV******
					SD	0.29	5.86	1.26	7.76	13.64	1.08	6.73	17.86	1.67	0.42	N/AV****
					RSD%	**2.69**	**6.05**	**3.61**	**5.93**	**6.80**	**3.86**	**8.55**	**7.73**	**3.33**	**1.00**	**N/AV******
S-000028608	Plasma (Oxysterol)	921031	5/2/14 12:00 AM	15015		11.04	101.53	44.20	157.18	203.47	40.13	112.68	153.36	56.79	54.10	BLQ
					4.21	12.59	99.85	44.03	156.46	224.90	35.29	99.45	146.98	54.63	58.20	BLQ
						11.94	97.63	42.65	150.11	209.27	31.67	82.87	141.87	51.08	44.36	BLQ
					Median	**11.94**	**99.85**	**44.03**	**156.46**	**209.27**	**35.29**	**99.45**	**146.98**	**54.63**	**54.10**	**N/AV******
					Average	**11.86**	**99.67**	**43.63**	**154.58**	**212.55**	**35.70**	**98.34**	**147.40**	**54.17**	**52.22**	**N/AV******
					SD	0.78	1.96	0.85	3.89	11.08	4.24	14.93	5.76	2.88	7.11	N/AV****
					RSD%	**6.59**	**1.96**	**1.94**	**2.52**	**5.21**	**11.88**	**15.19**	**3.91**	**5.32**	**13.61**	**N/AV******
S-000028602	Plasma (Oxysterol)	920448	5/2/14 12:00 AM	15015		8.97	71.21	32.38	99.99	170.60	29.20	81.14	298.61	50.68	28.32	BLQ
					3.08	8.35	68.01	31.23	98.40	157.73	25.33	64.01	293.07	47.95	30.78	BLQ
						6.97	65.59	29.16	92.20	158.11	29.63	66.84	281.08	47.82	34.11	BLQ
					Median	**8.35**	**68.01**	**31.23**	**98.40**	**158.11**	**29.20**	**66.84**	**293.07**	**47.95**	**30.78**	**N/AV******
					Average	**8.10**	**68.27**	**30.92**	**96.86**	**162.14**	**28.05**	**70.66**	**290.92**	**48.81**	**31.07**	**N/AV******
					SD	1.03	2.82	1.63	4.12	7.32	2.37	9.19	8.96	1.62	2.91	N/AV****
					RSD%	**12.69**	**4.13**	**5.27**	**4.25**	**4.52**	**8.44**	**13.00**	**3.08**	**3.31**	**9.37**	**N/AV******
S-000028604	Plasma (Oxysterol)	920261	5/2/14 12:00 AM	15015		14.06	125.25	65.10	165.16	161.10	47.75	114.09	174.17	48.57	42.88	BLQ
					4.92	15.66	136.09	72.11	175.41	196.29	42.10	90.91	180.83	47.49	38.46	BLQ
						15.40	125.52	66.73	173.37	174.45	40.33	86.39	176.16	47.37	35.61	BLQ
					Median	**15.40**	**125.52**	**66.73**	**173.37**	**174.45**	**42.10**	**90.91**	**176.16**	**47.49**	**38.46**	**N/AV******
					Average	**15.04**	**128.95**	**67.98**	**171.31**	**177.28**	**43.39**	**97.13**	**177.05**	**47.81**	**38.98**	**N/AV******
					SD	0.86	6.18	3.67	5.43	17.77	3.88	14.86	3.42	0.66	3.66	N/AV****
					RSD%	**5.72**	**4.80**	**5.40**	**3.17**	**10.02**	**8.93**	**15.30**	**1.93**	**1.38**	**9.39**	**N/AV******
S-000028617	Plasma (Oxysterol)	920369	5/2/14 12:00 AM	15015		12.00	85.61	39.46	124.64	191.85	24.63	66.04	321.13	59.20	28.75	BLQ
					3.55	11.03	81.07	36.90	121.28	189.06	25.04	71.00	302.39	56.41	32.37	BLQ
						11.62	80.97	35.66	116.63	196.26	19.72	58.74	297.20	53.25	32.96	BLQ
					Median	**11.62**	**81.07**	**36.90**	**121.28**	**191.85**	**24.63**	**66.04**	**302.39**	**56.41**	**32.37**	**N/AV******
					Average	**11.55**	**82.55**	**37.34**	**120.85**	**192.39**	**23.13**	**65.26**	**306.91**	**56.28**	**31.36**	**N/AV******
					SD	0.49	2.65	1.94	4.02	3.63	2.96	6.17	12.59	2.98	2.28	N/AV****
					RSD%	**4.23**	**3.21**	**5.19**	**3.33**	**1.89**	**12.79**	**9.45**	**4.10**	**5.29**	**7.26**	**N/AV******
S-000028611	Plasma (Oxysterol)	921060	5/2/14 12:00 AM	15015		11.39	89.23	35.95	132.20	334.02	35.53	92.59	141.39	51.93	46.00	BLQ
					3.68	11.79	86.68	34.67	124.65	282.18	29.60	69.03	129.34	48.39	41.62	BLQ
						12.07	87.41	36.06	132.99	308.39	38.55	84.12	138.58	48.01	38.84	BLQ
					Median	**11.79**	**87.41**	**35.95**	**132.20**	**308.39**	**35.53**	**84.12**	**138.58**	**48.39**	**41.62**	**N/AV******
					Average	**11.75**	**87.77**	**35.56**	**129.95**	**308.20**	**34.56**	**81.92**	**136.43**	**49.44**	**42.15**	**N/AV******
					SD	0.34	1.31	0.77	4.61	25.92	4.55	11.94	6.30	2.16	3.61	N/AV****
					RSD%	**2.92**	**1.50**	**2.18**	**3.55**	**8.41**	**13.17**	**14.57**	**4.62**	**4.37**	**8.56**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028612	Plasma (Oxysterol)	920955	5/2/14 12:00 AM	15015	**4.70**	12.56	112.90	43.91	151.85	234.25	24.32	70.79	192.64	57.77	32.31	BLQ
						13.36	108.72	44.07	153.02	222.00	25.85	70.62	189.42	56.77	31.49	BLQ
						12.95	109.37	40.91	150.26	219.28	25.08	66.15	184.36	57.94	31.80	BLQ
					Median	**12.95**	**109.37**	**43.91**	**151.85**	**222.00**	**25.08**	**70.62**	**189.42**	**57.77**	**31.80**	**N/AV******
					Average	**12.96**	**110.33**	**42.96**	**151.71**	**225.18**	**25.08**	**69.19**	**188.81**	**57.49**	**31.86**	**N/AV******
					SD	0.40	2.25	1.78	1.39	7.97	0.77	2.63	4.17	0.63	0.41	N/AV****
					RSD%	**3.07**	**2.04**	**4.15**	**0.91**	**3.54**	**3.05**	**3.81**	**2.21**	**1.09**	**1.30**	**N/AV******
S-000028614	Plasma (Oxysterol)	921070	5/2/14 12:00 AM	15015	**4.05**	15.40	93.67	37.27	153.68	334.64	28.06	76.91	180.07	55.84	42.04	BLQ
						15.68	88.85	35.24	156.64	330.02	25.77	66.53	169.13	52.01	34.70	BLQ
						15.14	84.91	37.03	142.72	313.72	25.49	68.02	165.02	52.28	41.32	BLQ
					Median	**15.40**	**88.85**	**37.03**	**153.68**	**330.02**	**25.77**	**68.02**	**169.13**	**52.28**	**41.32**	**N/AV******
					Average	**15.41**	**89.14**	**36.51**	**151.01**	**326.13**	**26.44**	**70.49**	**171.41**	**53.38**	**39.35**	**N/AV******
					SD	0.27	4.39	1.11	7.33	10.99	1.41	5.61	7.78	2.14	4.05	N/AV****
					RSD%	**1.75**	**4.92**	**3.03**	**4.86**	**3.37**	**5.32**	**7.96**	**4.54**	**4.01**	**10.28**	**N/AV******
S-000028606	Plasma (Oxysterol)	921001	5/2/14 12:00 AM	15015	**7.98**	18.02	144.92	70.27	234.90	200.99	53.76	107.44	324.92	64.48	57.37	BLQ
						19.86	145.46	70.00	247.64	207.27	51.95	98.65	330.66	68.19	48.67	BLQ
						20.95	152.32	76.19	262.00	211.82	57.65	117.50	350.26	74.23	53.64	BLQ
					Median	**19.86**	**145.46**	**70.27**	**247.64**	**207.27**	**53.76**	**107.44**	**330.66**	**68.19**	**53.64**	**N/AV******
					Average	**19.61**	**147.57**	**72.15**	**248.18**	**206.69**	**54.45**	**107.86**	**335.28**	**68.96**	**53.23**	**N/AV******
					SD	1.48	4.12	3.50	13.56	5.44	2.91	9.43	13.28	4.92	4.36	N/AV****
					RSD%	**7.53**	**2.80**	**4.85**	**5.46**	**2.63**	**5.34**	**8.75**	**3.96**	**7.13**	**8.20**	**N/AV******
S-000028619	Plasma (Oxysterol)	920220	5/2/14 12:00 AM	15015	**3.99**	15.12	112.99	39.99	156.75	175.69	28.49	67.11	256.93	62.40	27.23	BLQ
						13.36	109.11	43.99	155.62	167.41	26.95	63.18	259.32	60.52	30.04	BLQ
						13.94	119.11	45.68	161.95	180.27	29.56	64.33	271.47	65.86	26.23	BLQ
					Median	**13.94**	**112.99**	**43.99**	**156.75**	**175.69**	**28.49**	**64.33**	**259.32**	**62.40**	**27.23**	**N/AV******
					Average	**14.14**	**113.74**	**43.22**	**158.11**	**174.45**	**28.33**	**64.87**	**262.57**	**62.93**	**27.83**	**N/AV******
					SD	0.89	5.04	2.92	3.38	6.52	1.31	2.02	7.80	2.71	1.97	N/AV****
					RSD%	**6.32**	**4.43**	**6.76**	**2.14**	**3.74**	**4.62**	**3.11**	**2.97**	**4.30**	**7.10**	**N/AV******
S-000028616	Plasma (Oxysterol)	920911	5/2/14 12:00 AM	15015	**3.61**	8.46	84.08	31.60	104.51	227.86	27.65	87.09	102.68	39.99	49.94	BLQ
						9.41	85.16	31.10	115.91	240.59	29.56	85.97	101.46	38.59	45.96	BLQ
						10.11	76.82	31.10	106.85	215.73	25.42	73.51	95.22	37.65	40.28	BLQ
					Median	**9.41**	**84.08**	**31.10**	**106.85**	**227.86**	**27.65**	**85.97**	**101.46**	**38.59**	**45.96**	**N/AV******
					Average	**9.32**	**82.02**	**31.27**	**109.09**	**228.06**	**27.54**	**82.19**	**99.79**	**38.74**	**45.39**	**N/AV******
					SD	0.83	4.53	0.29	6.02	12.43	2.07	7.54	4.00	1.18	4.86	N/AV****
					RSD%	**8.88**	**5.53**	**0.93**	**5.52**	**5.45**	**7.52**	**9.17**	**4.01**	**3.04**	**10.70**	**N/AV******
S-000028615	Plasma (Oxysterol)	920936	5/2/14 12:00 AM	15015	**3.76**	9.95	82.31	30.02	115.56	153.82	26.94	76.01	124.28	41.75	47.68	BLQ
						9.66	88.01	30.82	111.63	180.30	39.05	114.11	141.37	51.56	51.51	BLQ
						9.49	84.08	29.17	114.65	177.29	36.85	94.15	129.49	43.75	50.40	BLQ
					Median	**9.66**	**84.08**	**30.02**	**114.65**	**177.29**	**36.85**	**94.15**	**129.49**	**43.75**	**50.40**	**N/AV******
					Average	**9.70**	**84.80**	**30.01**	**113.95**	**170.47**	**34.28**	**94.76**	**131.71**	**45.69**	**49.86**	**N/AV******
					SD	0.23	2.92	0.82	2.05	14.49	6.45	19.06	8.76	5.18	1.97	N/AV****
					RSD%	**2.40**	**3.44**	**2.75**	**1.80**	**8.50**	**18.82**	**20.11**	**6.65**	**11.35**	**3.95**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028620	Plasma (Oxysterol)	920921	5/2/14 12:00 AM	15015		11.80	111.87	37.39	137.95	227.09	18.16	69.04	275.13	55.91	42.65	BLQ
					4.14	10.76	107.39	34.10	134.49	208.12	22.80	76.69	255.56	53.54	40.26	BLQ
						12.49	87.60	40.65	113.42	215.49	22.29	68.34	259.43	51.93	39.89	BLQ
					Median	**11.80**	**107.39**	**37.39**	**134.49**	**215.49**	**22.29**	**69.04**	**259.43**	**53.54**	**40.26**	**N/AV******
					Average	**11.68**	**102.29**	**37.38**	**128.62**	**216.90**	**21.08**	**71.35**	**263.37**	**53.79**	**40.93**	**N/AV******
					SD	0.87	12.92	3.27	13.28	9.57	2.54	4.63	10.36	2.00	1.50	N/AV****
					RSD%	**7.48**	**12.63**	**8.75**	**10.32**	**4.41**	**12.07**	**6.49**	**3.93**	**3.73**	**3.66**	**N/AV******
S-000028621	Plasma (Oxysterol)	920138	5/2/14 12:00 AM	15015		13.11	96.40	46.32	126.40	130.58	33.13	79.65	259.00	48.25	49.29	BLQ
					4.47	13.78	100.91	47.45	123.85	138.46	37.58	95.55	260.73	47.80	42.81	BLQ
						14.62	99.82	47.08	120.29	172.35	32.18	84.87	262.10	53.86	56.27	BLQ
					Median	**13.78**	**99.82**	**47.08**	**123.85**	**138.46**	**33.13**	**84.87**	**260.73**	**48.25**	**49.29**	**N/AV******
					Average	**13.83**	**99.04**	**46.95**	**123.51**	**147.13**	**34.29**	**86.69**	**260.61**	**49.97**	**49.46**	**N/AV******
					SD	0.76	2.35	0.58	3.07	22.19	2.88	8.11	1.55	3.37	6.73	N/AV****
					RSD%	**5.48**	**2.37**	**1.23**	**2.49**	**15.08**	**8.41**	**9.35**	**0.60**	**6.75**	**13.61**	**N/AV******
S-000028623	Plasma (Oxysterol)	920632	5/2/14 12:00 AM	15015		11.50	85.83	34.99	111.81	181.61	21.09	63.95	260.97	54.90	32.42	BLQ
					3.27	9.84	85.86	34.16	112.27	162.84	23.27	68.57	262.16	53.42	42.11	BLQ
						9.21	81.05	31.33	109.63	166.53	21.13	61.88	237.76	51.65	37.33	BLQ
					Median	**9.84**	**85.83**	**34.16**	**111.81**	**166.53**	**21.13**	**63.95**	**260.97**	**53.42**	**37.33**	**N/AV******
					Average	**10.18**	**84.24**	**33.49**	**111.24**	**170.33**	**21.83**	**64.80**	**253.63**	**53.32**	**37.29**	**N/AV******
					SD	1.18	2.77	1.92	1.41	9.95	1.25	3.43	13.76	1.63	4.85	N/AV****
					RSD%	**11.63**	**3.29**	**5.73**	**1.27**	**5.84**	**5.71**	**5.29**	**5.42**	**3.05**	**13.00**	**N/AV******
S-000028618	Plasma (Oxysterol)	920800	5/2/14 12:00 AM	15015		9.38	66.60	27.82	105.22	149.16	18.29	57.43	183.64	37.84	39.31	BLQ
					3.76	9.82	73.13	29.47	101.32	175.25	21.23	69.29	263.49	43.20	40.10	BLQ
						9.95	71.90	29.39	100.23	170.31	21.24	65.54	192.61	43.25	40.90	BLQ
					Median	**9.82**	**71.90**	**29.39**	**101.32**	**170.31**	**21.23**	**65.54**	**192.61**	**43.20**	**40.10**	**N/AV******
					Average	**9.72**	**70.54**	**28.89**	**102.26**	**164.91**	**20.26**	**64.09**	**213.25**	**41.43**	**40.11**	**N/AV******
					SD	0.30	3.47	0.93	2.63	13.86	1.70	6.06	43.74	3.11	0.80	N/AV****
					RSD%	**3.06**	**4.92**	**3.23**	**2.57**	**8.40**	**8.39**	**9.46**	**20.51**	**7.50**	**1.98**	**N/AV******
S-000028626	Plasma (Oxysterol)	920282	5/2/14 12:00 AM	15015		12.66	97.41	34.96	120.46	254.48	30.49	80.18	154.62	49.22	42.06	BLQ
					4.48	12.28	99.31	34.03	134.16	261.31	27.08	75.63	155.23	50.25	42.88	BLQ
						11.71	101.28	37.96	144.32	212.08	32.45	95.24	156.02	51.64	53.00	BLQ
					Median	**12.28**	**99.31**	**34.96**	**134.16**	**254.48**	**30.49**	**80.18**	**155.23**	**50.25**	**42.88**	**N/AV******
					Average	**12.22**	**99.33**	**35.65**	**132.98**	**242.62**	**30.01**	**83.68**	**155.29**	**50.37**	**45.98**	**N/AV******
					SD	0.48	1.93	2.05	11.97	26.67	2.71	10.26	0.70	1.22	6.09	N/AV****
					RSD%	**3.91**	**1.95**	**5.75**	**9.00**	**10.99**	**9.04**	**12.26**	**0.45**	**2.42**	**13.25**	**N/AV******
S-000028624	Plasma (Oxysterol)	920110	5/2/14 12:00 AM	15015		10.11	101.27	30.36	113.79	271.16	26.47	81.87	180.66	50.23	40.09	BLQ
					4.15	9.74	93.33	29.73	120.22	240.17	22.74	66.10	178.65	59.39	37.36	BLQ
						9.86	93.67	30.72	121.97	246.26	24.60	68.91	178.39	51.48	35.56	BLQ
					Median	**9.86**	**93.67**	**30.36**	**120.22**	**246.26**	**24.60**	**68.91**	**178.65**	**51.48**	**37.36**	**N/AV******
					Average	**9.90**	**96.09**	**30.27**	**118.66**	**252.53**	**24.60**	**72.29**	**179.23**	**53.70**	**37.67**	**N/AV******
					SD	0.18	4.49	0.50	4.31	16.42	1.86	8.41	1.24	4.97	2.28	N/AV****
					RSD%	**1.87**	**4.67**	**1.65**	**3.63**	**6.50**	**7.58**	**11.64**	**0.69**	**9.25**	**6.05**	**N/AV******

Table continued on next page

Table 13. (Continued)

Sample	Sample Type	CAN	Collection Date	Study	Total Cholesterol Conc. [mg/mL]	22-Hydroxycholesterol Conc. [ng/mL]	24-Hydroxycholesterol Conc. [ng/mL]	25-Hydroxycholesterol Conc. [ng/mL]	27-Hydroxycholesterol Conc. [ng/mL]	4β-Hydroxycholesterol Conc. [ng/mL]	5α6α-Epoxycholestanol Conc. [ng/mL]	5β6β-Epoxycholestanol Conc. [ng/mL]	7α-Hydroxycholesterol Conc. [ng/mL]	7β-Hydroxycholesterol Conc. [ng/mL]	7-Ketocholesterol Conc. [ng/mL]	6αOHSHydroxyCholestanol Conc. [ng/mL]
S-000028622	Plasma (Oxysterol)	920115	5/2/14 12:00 AM	15015	4.09	10.71	75.45	42.24	96.72	121.36	23.86	68.46	146.72	44.55	48.94	BLQ
						10.96	74.48	39.79	123.71	132.65	20.91	62.66	137.17	38.02	40.68	BLQ
						11.27	76.61	44.63	106.59	135.38	24.22	68.21	152.43	42.62	47.33	BLQ
					Median	**10.96**	**75.45**	**42.24**	**106.59**	**132.65**	**23.86**	**68.21**	**146.72**	**42.62**	**47.33**	**N/AV******
					Average	**10.98**	**75.52**	**42.22**	**109.01**	**129.80**	**22.99**	**66.44**	**145.44**	**41.73**	**45.65**	**N/AV******
					SD	0.28	1.07	2.42	13.66	7.43	1.82	3.28	7.71	3.35	4.38	N/AV****
					RSD%	**2.56**	**1.41**	**5.72**	**12.53**	**5.72**	**7.90**	**4.93**	**5.30**	**8.04**	**9.59**	**N/AV******
S-000028625	Plasma (Oxysterol)	920400	5/2/14 12:00 AM	15015	3.67	11.74	91.13	37.35	140.90	143.13	22.65	75.66	302.63	60.77	39.75	BLQ
						12.79	88.00	36.10	133.88	135.33	19.23	63.71	285.19	58.38	41.30	BLQ
						11.90	86.03	35.07	129.52	115.22	18.45	67.42	280.82	58.11	49.51	BLQ
					Median	**11.90**	**88.00**	**36.10**	**133.88**	**135.33**	**19.23**	**67.42**	**285.19**	**58.38**	**41.30**	**N/AV******
					Average	**12.14**	**88.39**	**36.17**	**134.76**	**131.23**	**20.11**	**68.93**	**289.55**	**59.09**	**43.52**	**N/AV******
					SD	0.56	2.57	1.14	5.74	14.40	2.23	6.11	11.54	1.47	5.24	N/AV****
					RSD%	**4.65**	**2.91**	**3.15**	**4.26**	**10.97**	**11.11**	**8.87**	**3.98**	**2.48**	**12.05**	**N/AV******

****N/AV - Not Available, no descriptive statistic could be performed due to single value determined concentrations are below limit of quantification (BLQ)

5.6.1.4 Month 8 Chromatograms Oxysterols

This section provides random selected example chromatograms of oxysterol analysis representing at least 5% of total sample chromatograms

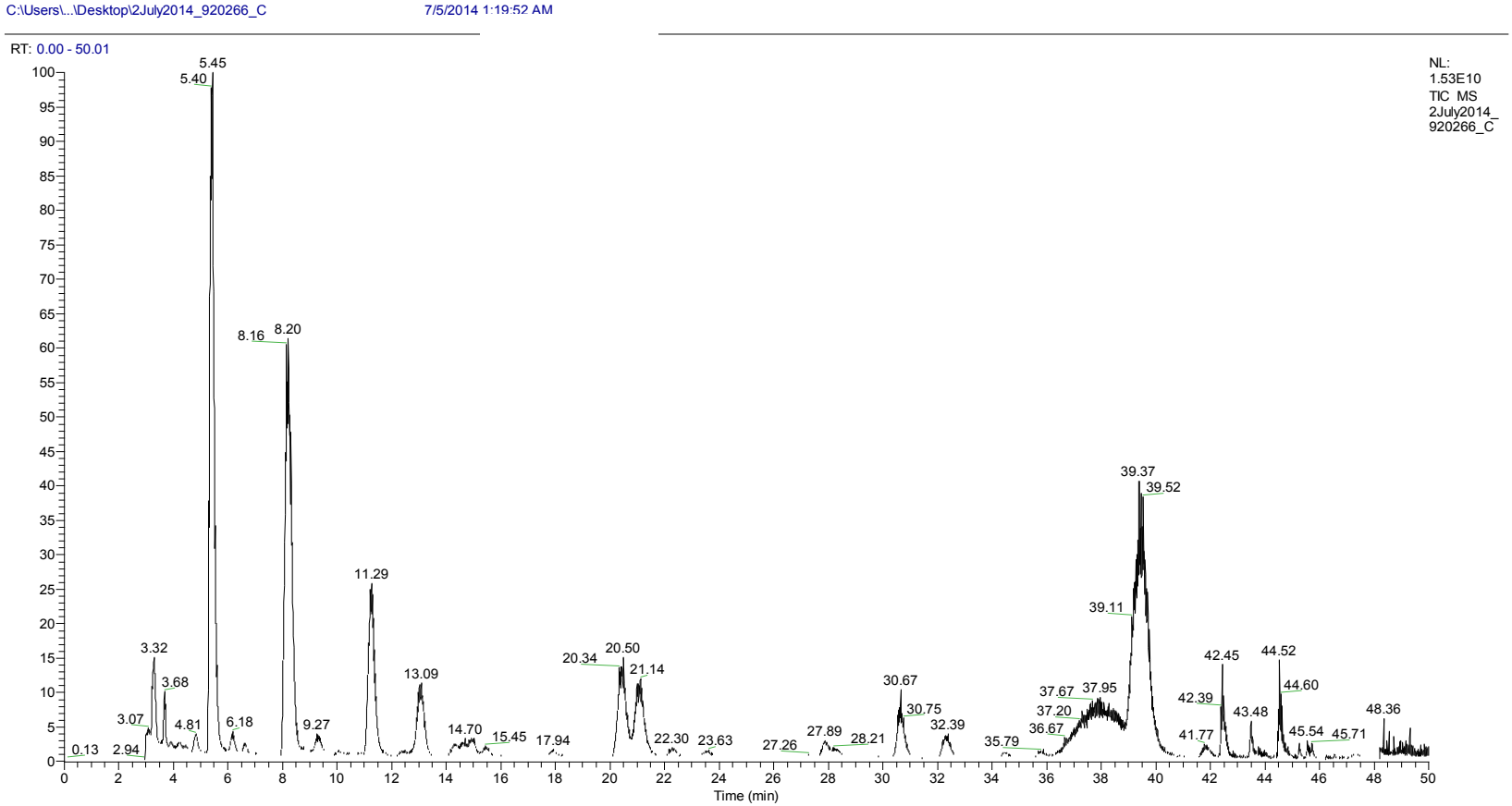


Figure 6. Oxysterols total ion chromatogram (TIC) example month 8 for sample 920266

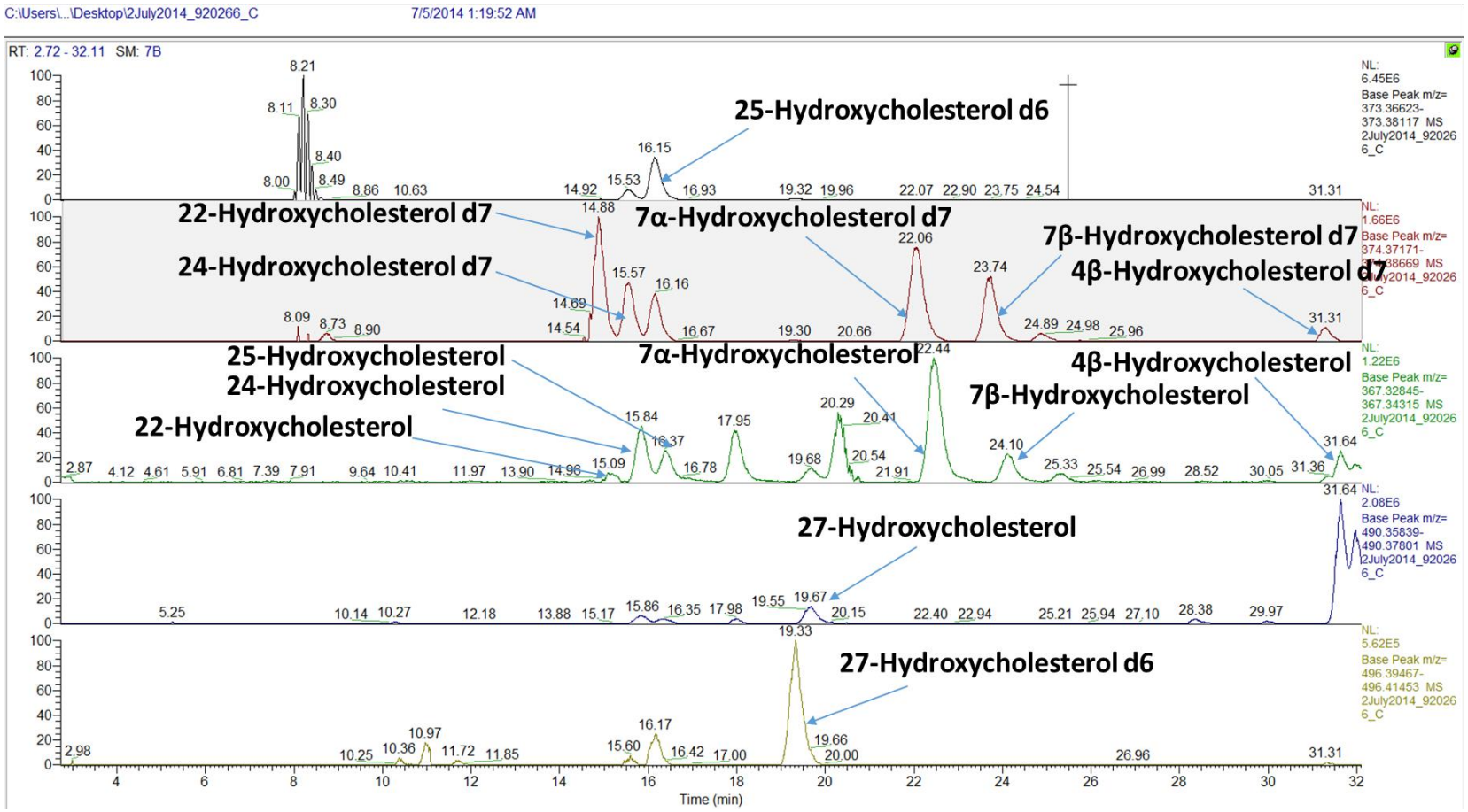


Figure 7. Oxysterols extracted ion chromatogram (XIC) example month 8 for sample 920266

Figure continues on next page

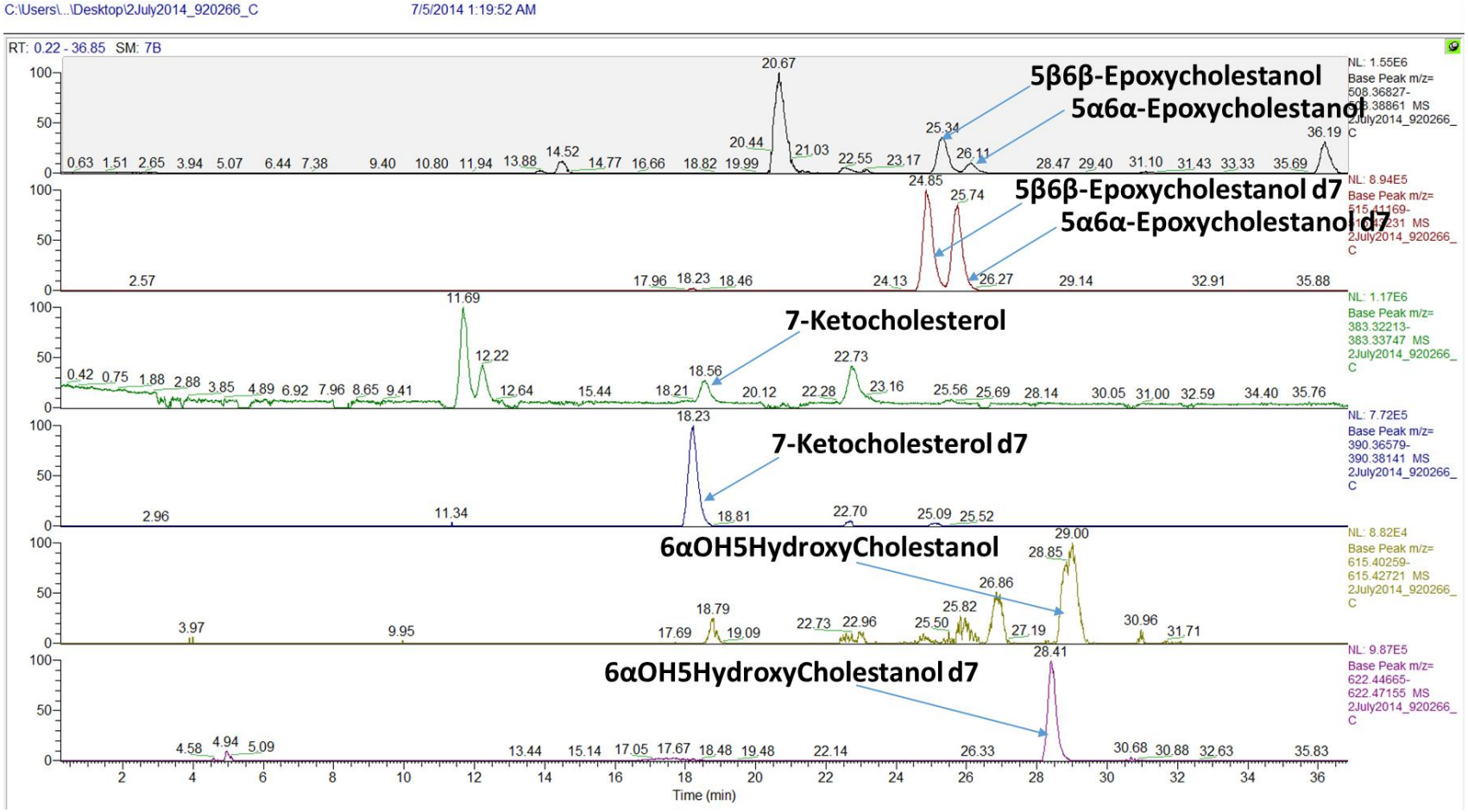


Figure 7. (Continued)

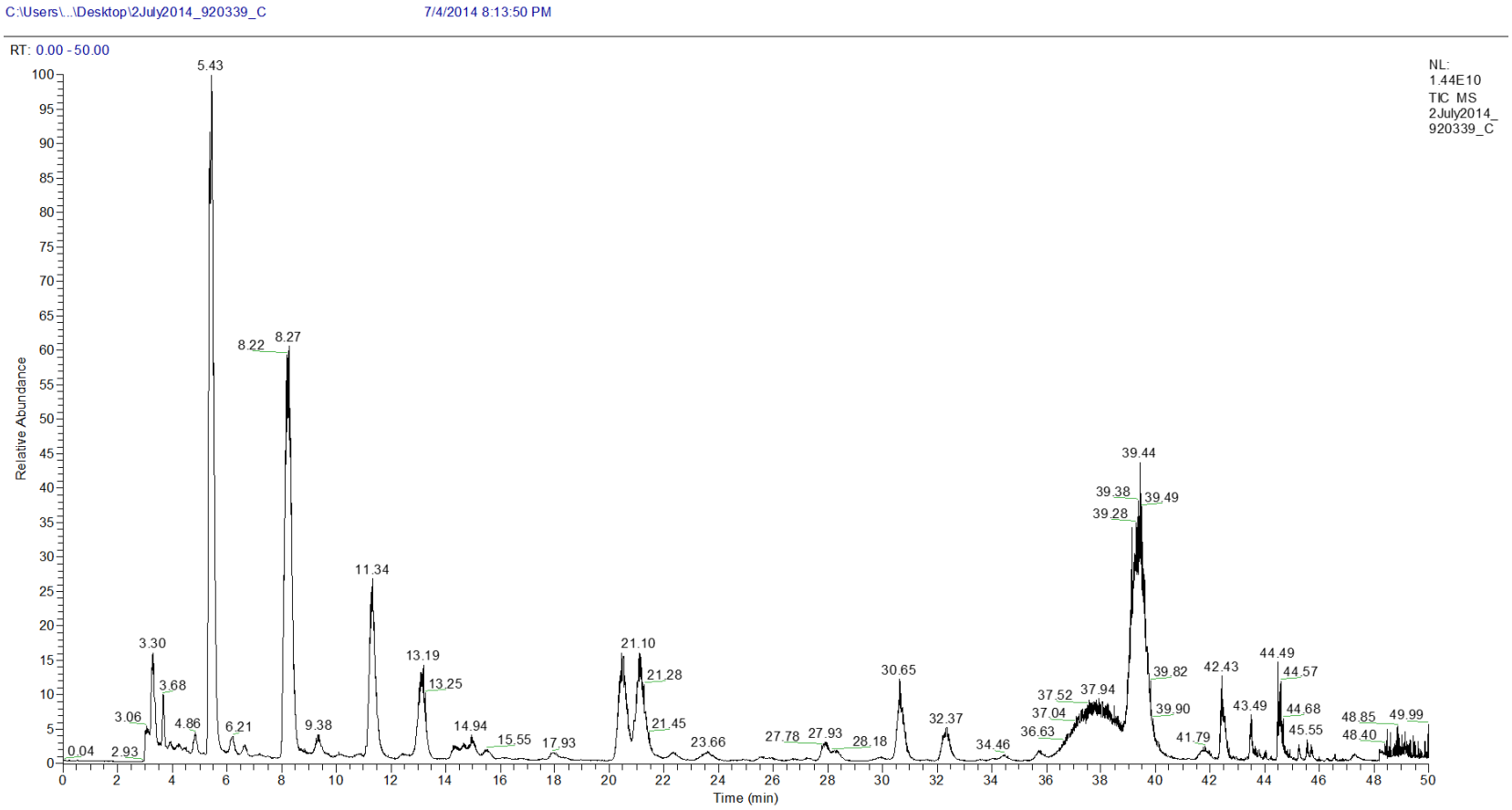


Figure 8. Oxysterols total ion chromatogram (TIC) example month 8 for sample 920339

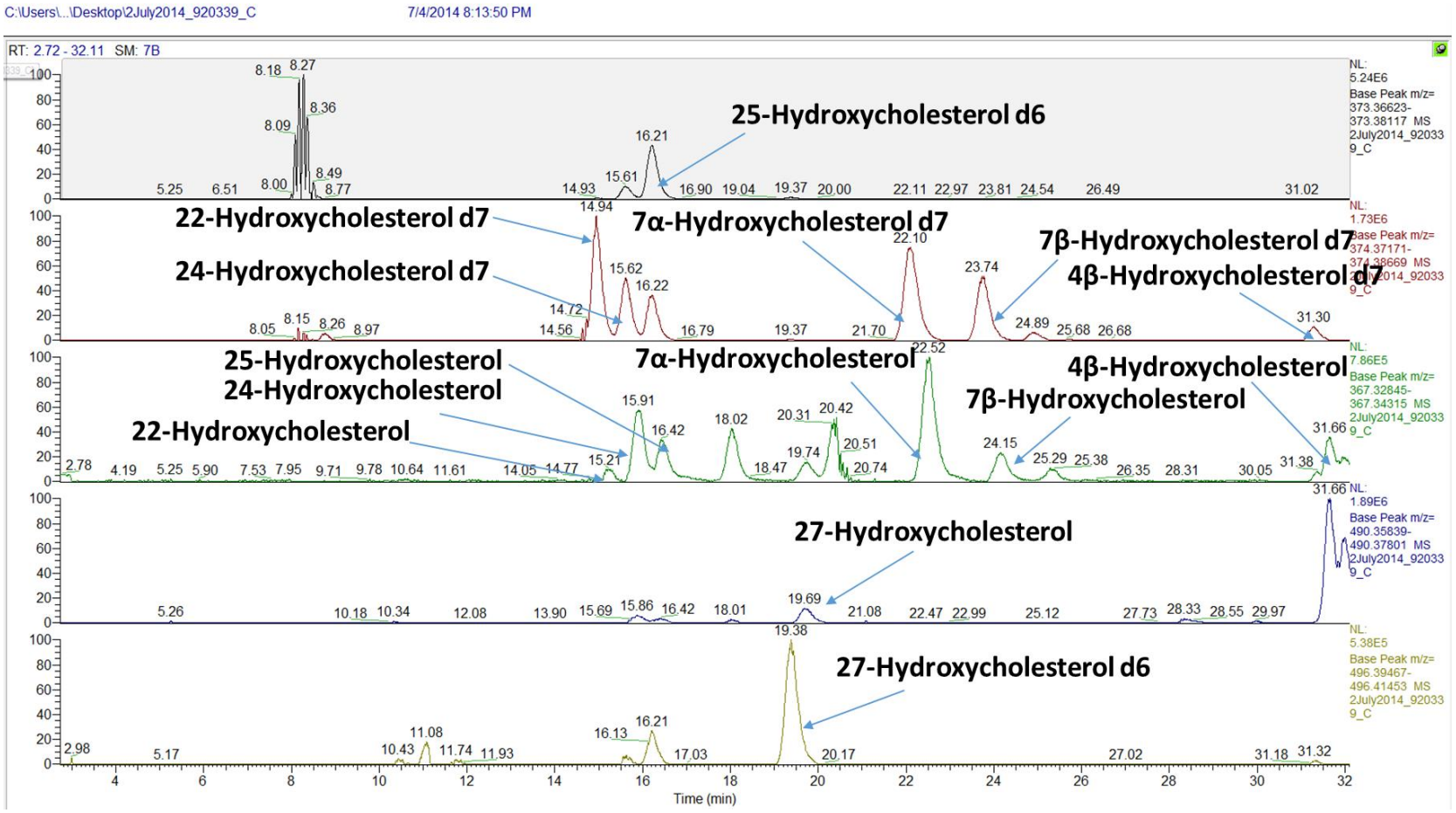


Figure 9. Oxysterols extracted ion chromatogram (XIC) example month 8 for sample 920339

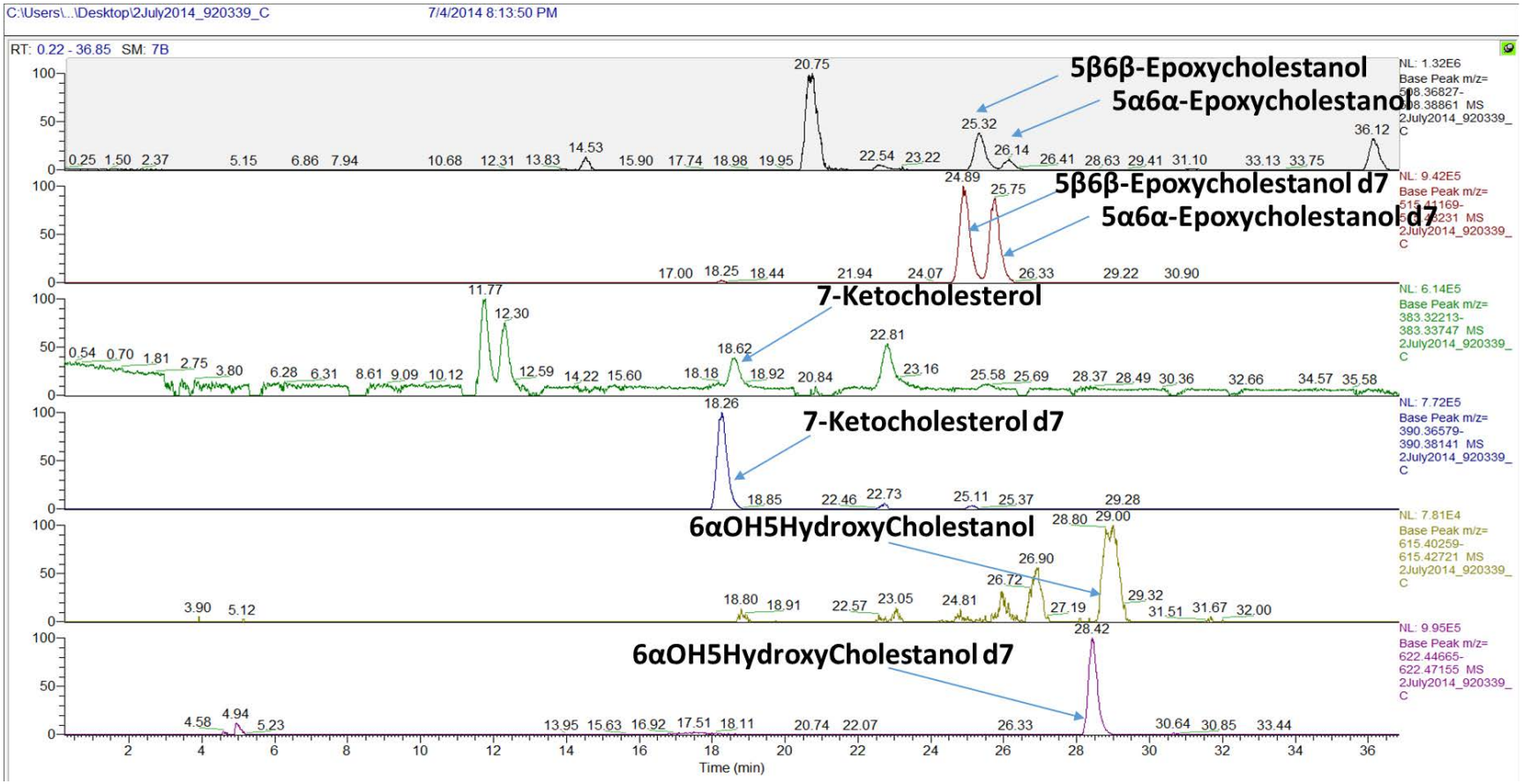


Figure 9. (Continued)

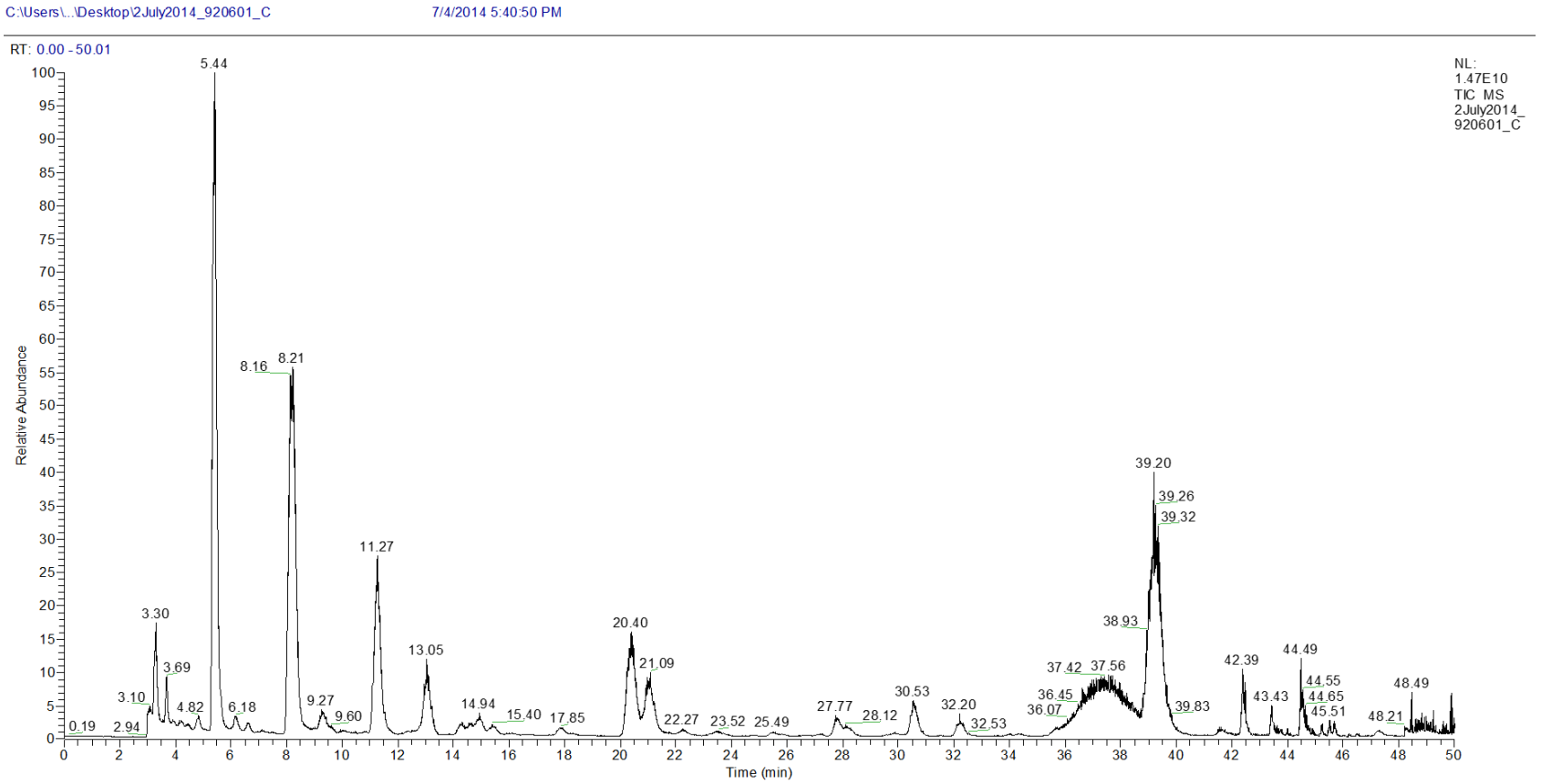


Figure 10. Oxysterols total ion chromatogram (TIC) example month 8 for sample 920601

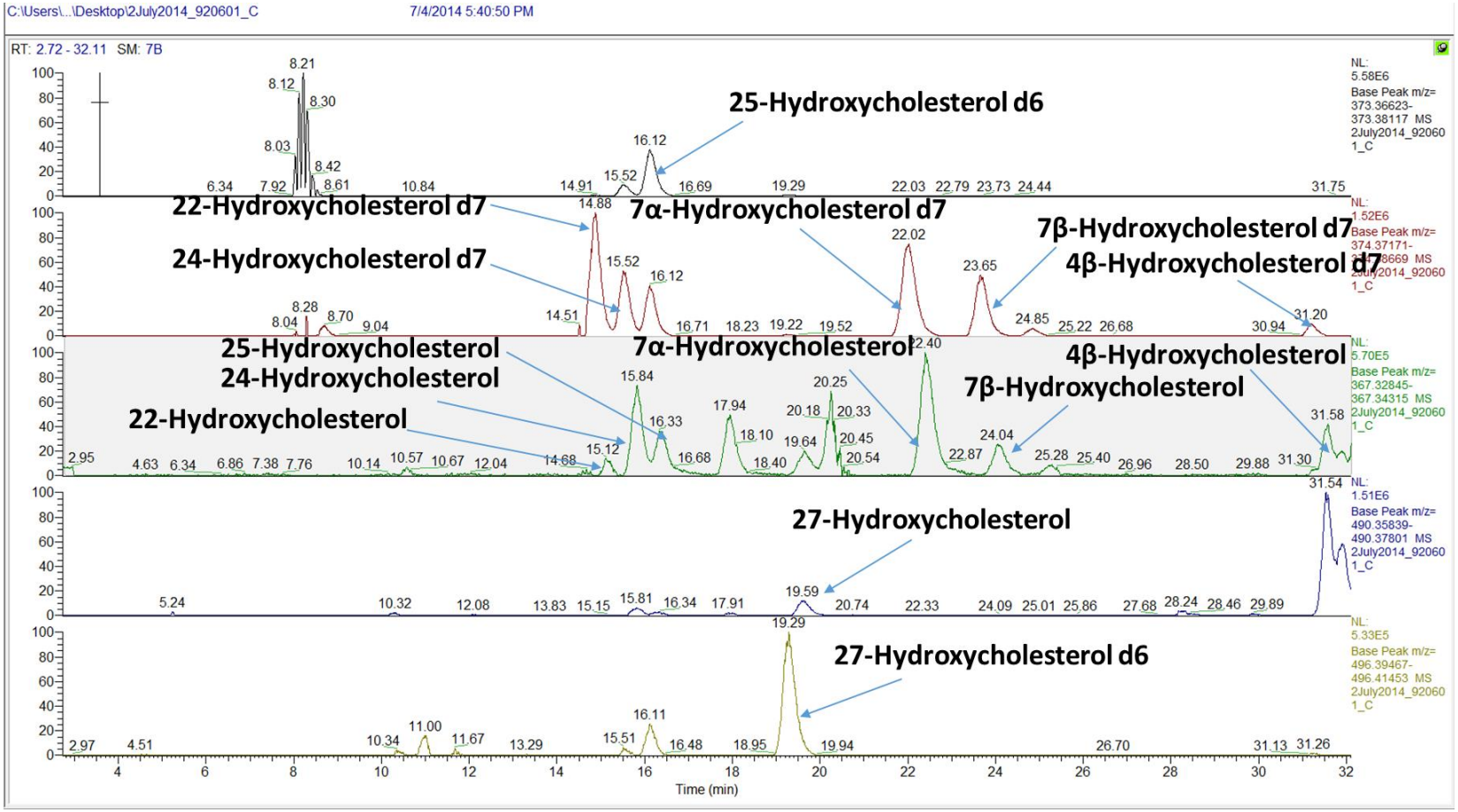


Figure 11. Oxysterols extracted ion chromatogram (XIC) example month 8 for sample 920601

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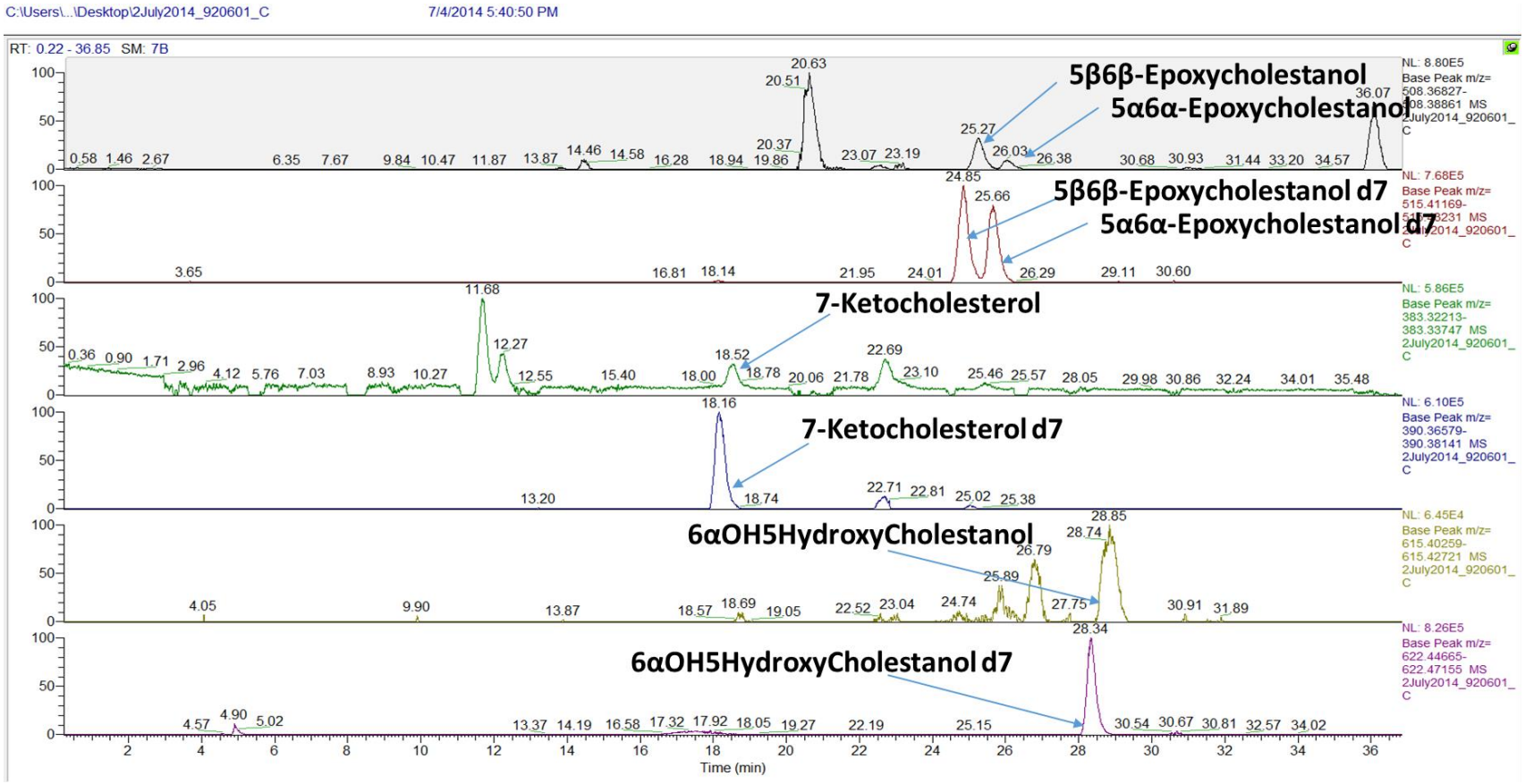


Figure 11. (Continued)

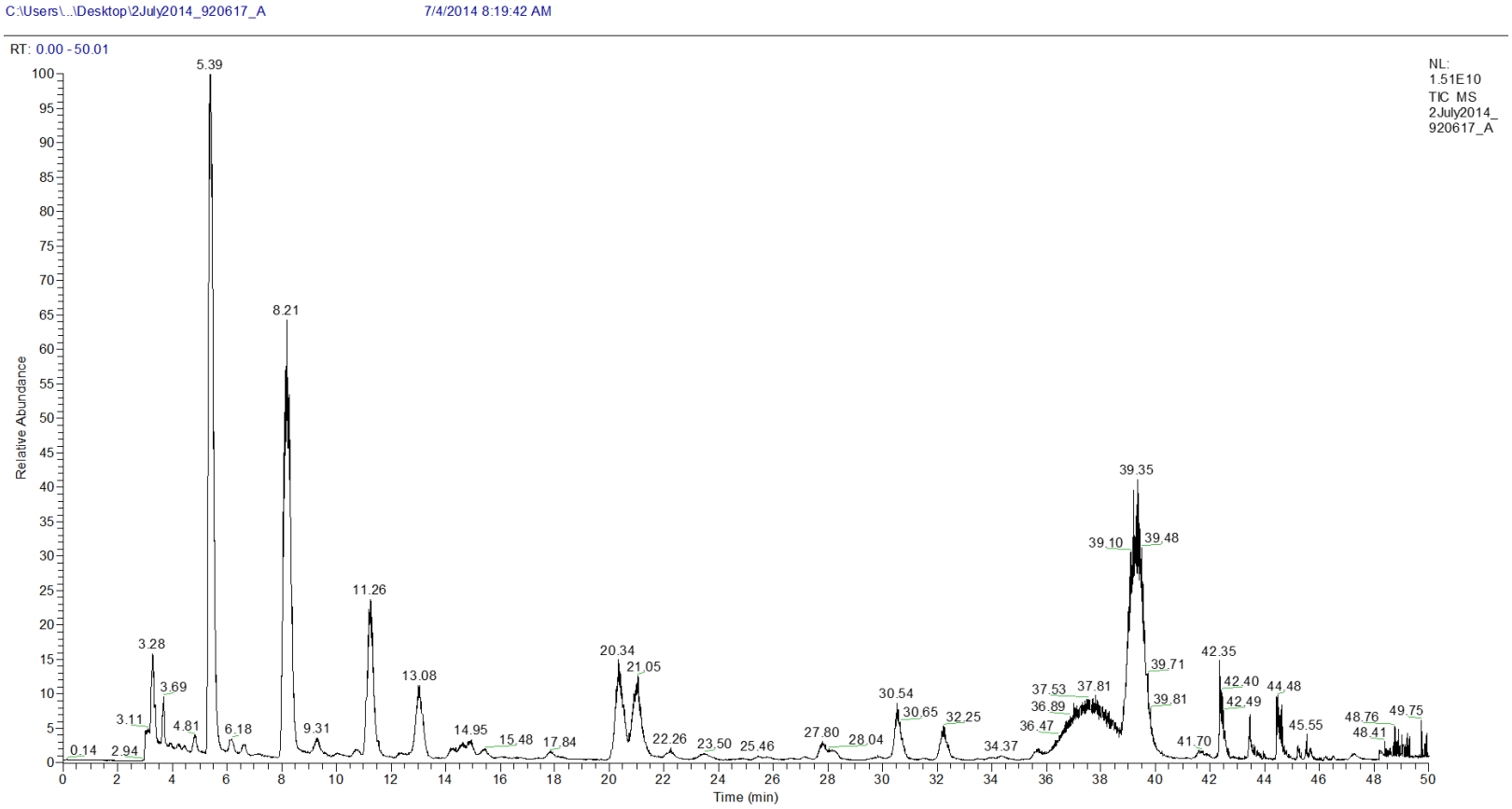


Figure 12. Oxysterols total ion chromatogram (TIC) example month 8 for sample 920617

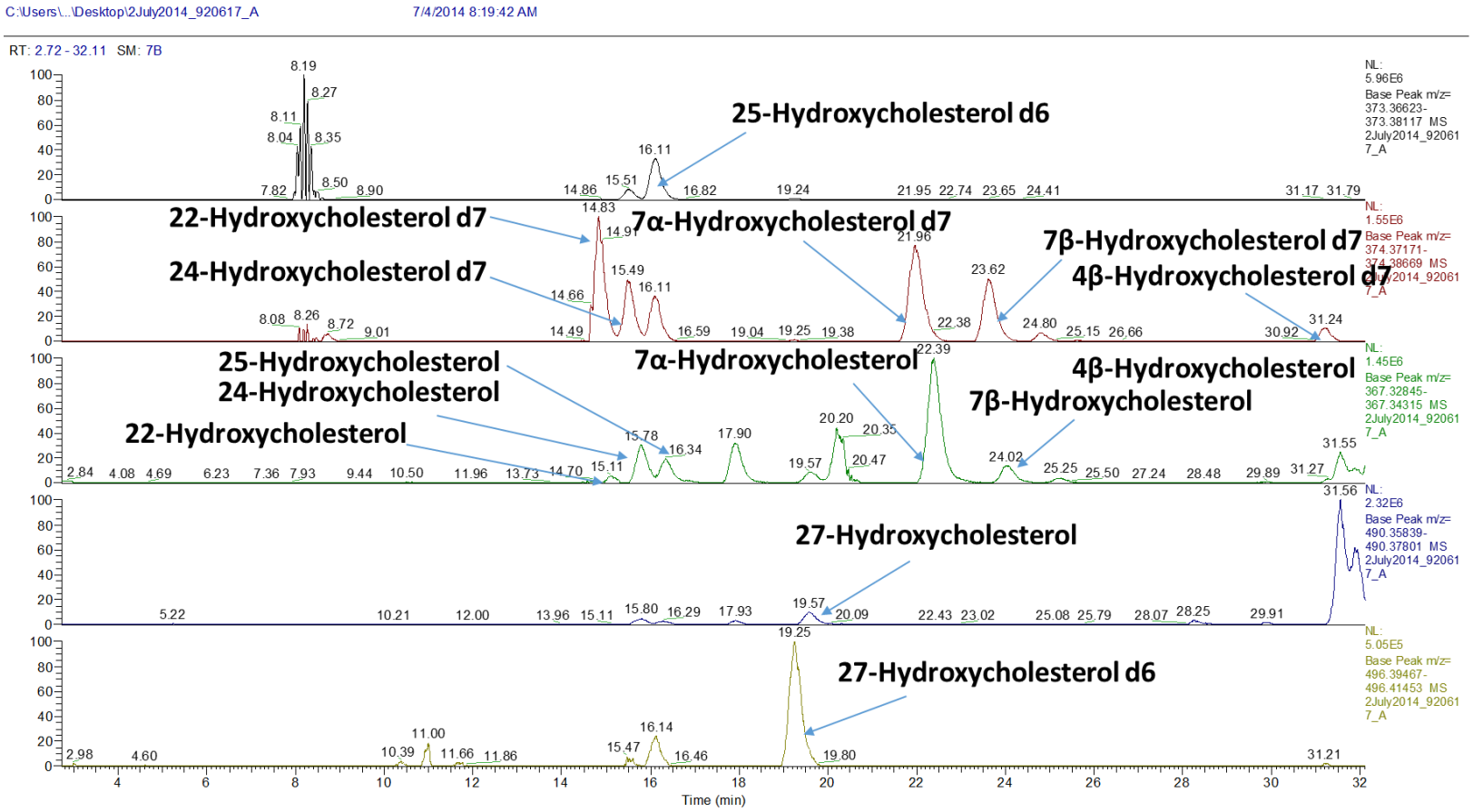


Figure 13. Oxysterols extracted ion chromatogram (XIC) example month 8 for sample 920617

Figure continues on next page

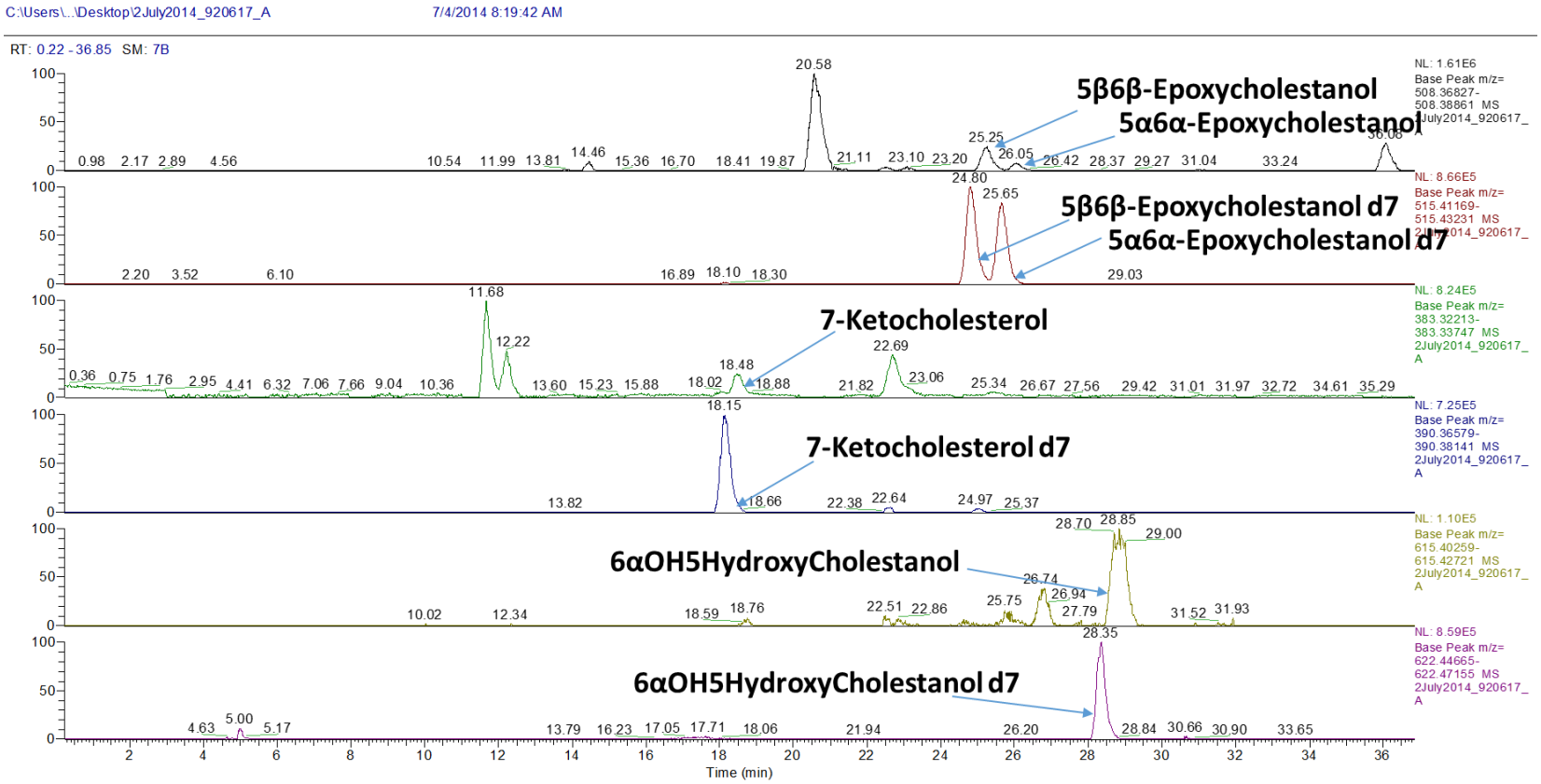


Figure 13. (Continued)

5.6.1.5 Month 8 Chromatograms Cholesterol

This section provides random selected example chromatograms of total cholesterol analysis representing at least 5% of total sample chromatograms

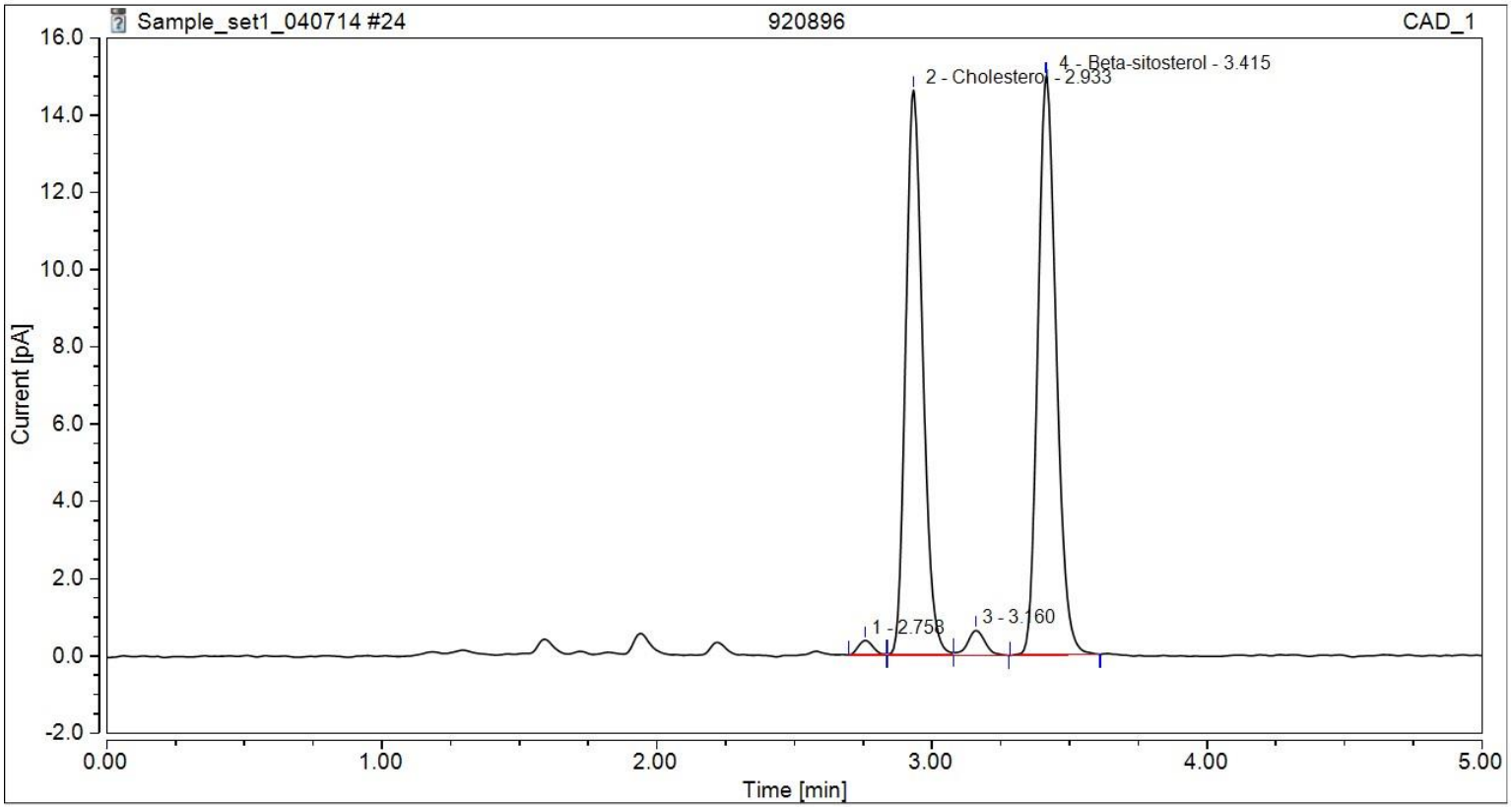


Figure 14. Total cholesterol chromatogram example month 8 for sample 920896

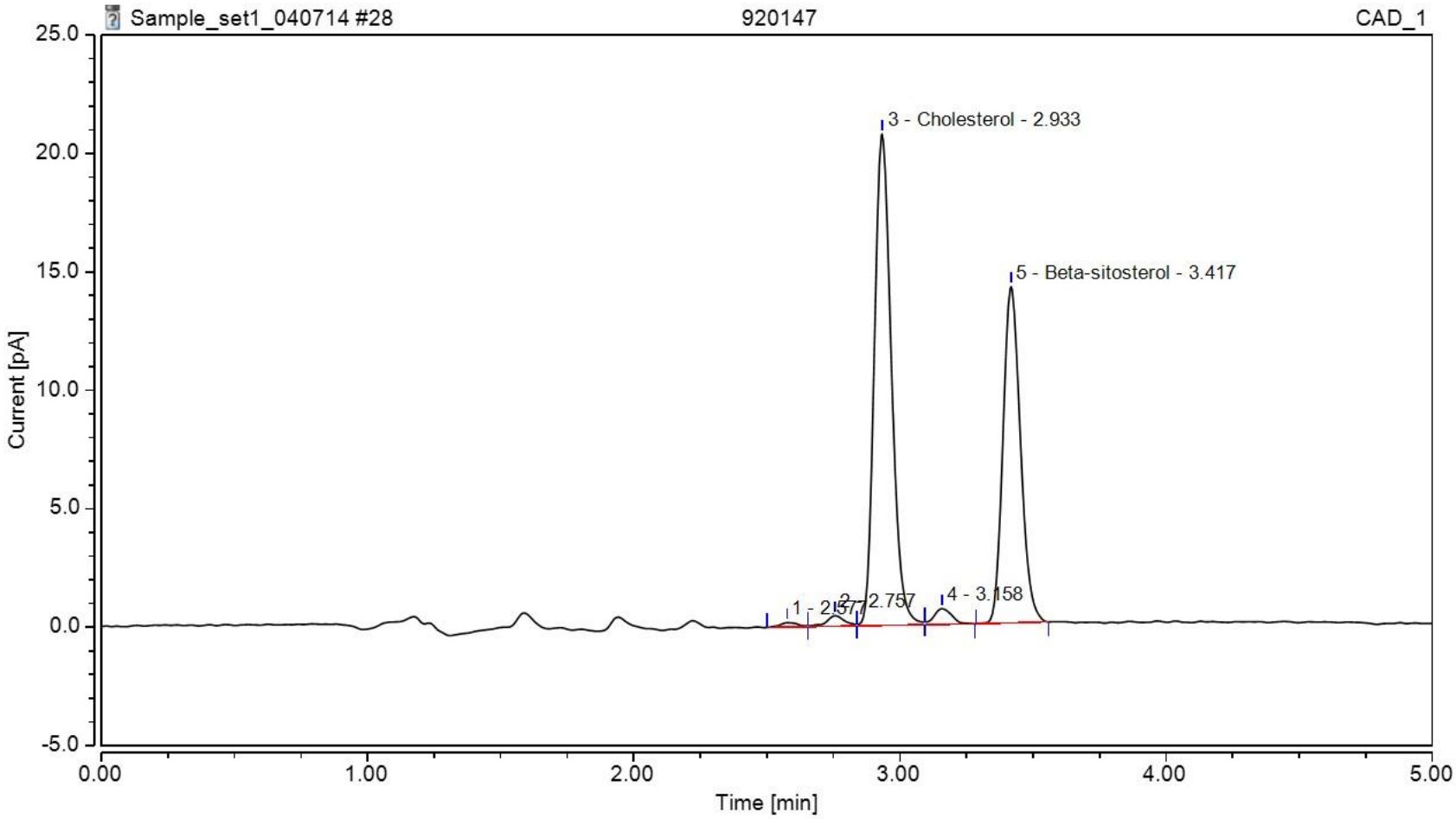


Figure 15. Total cholesterol chromatogram example month 8 for sample 920147

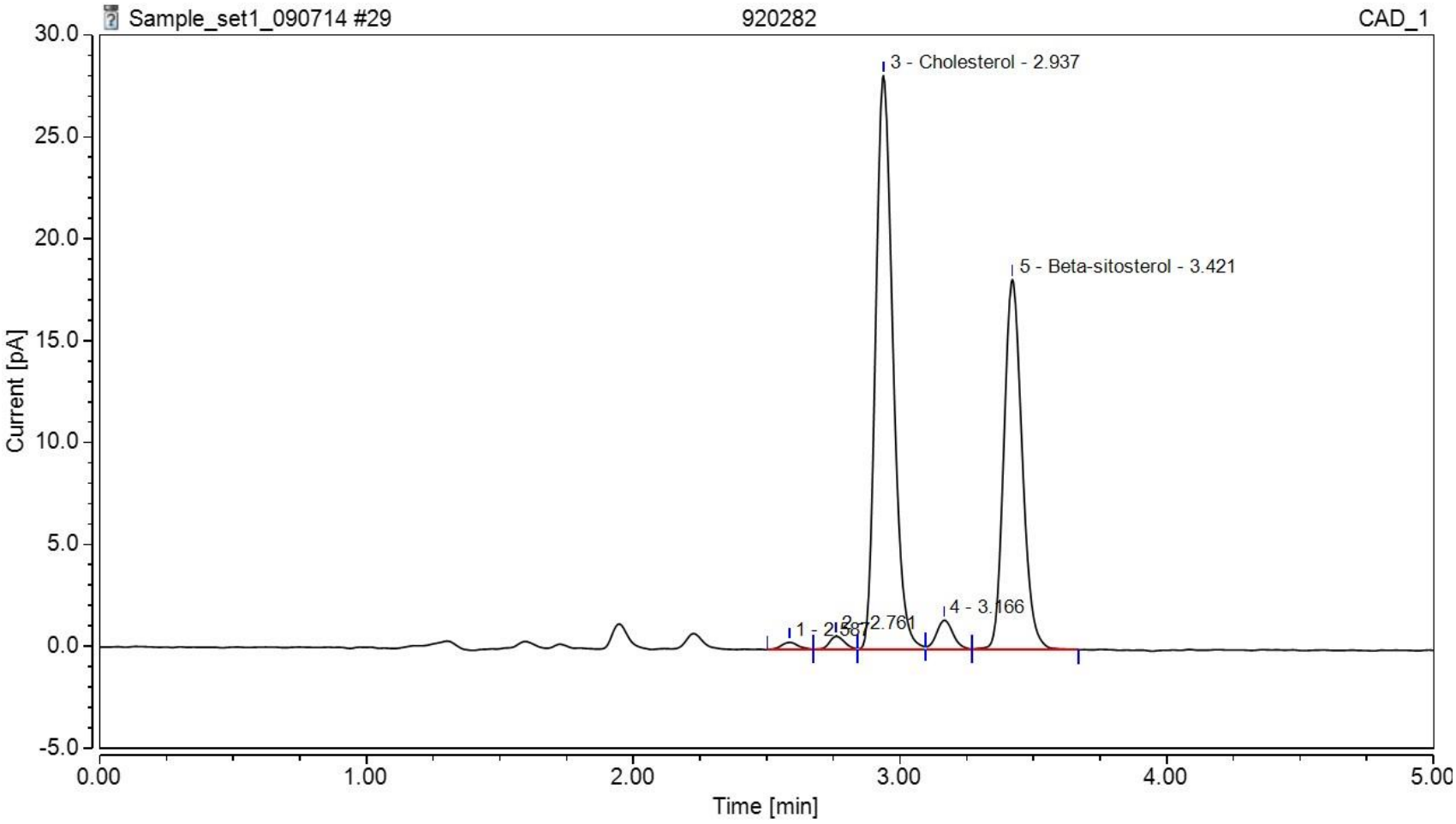


Figure 16. Total cholesterol chromatogram example month 8 for sample 920282

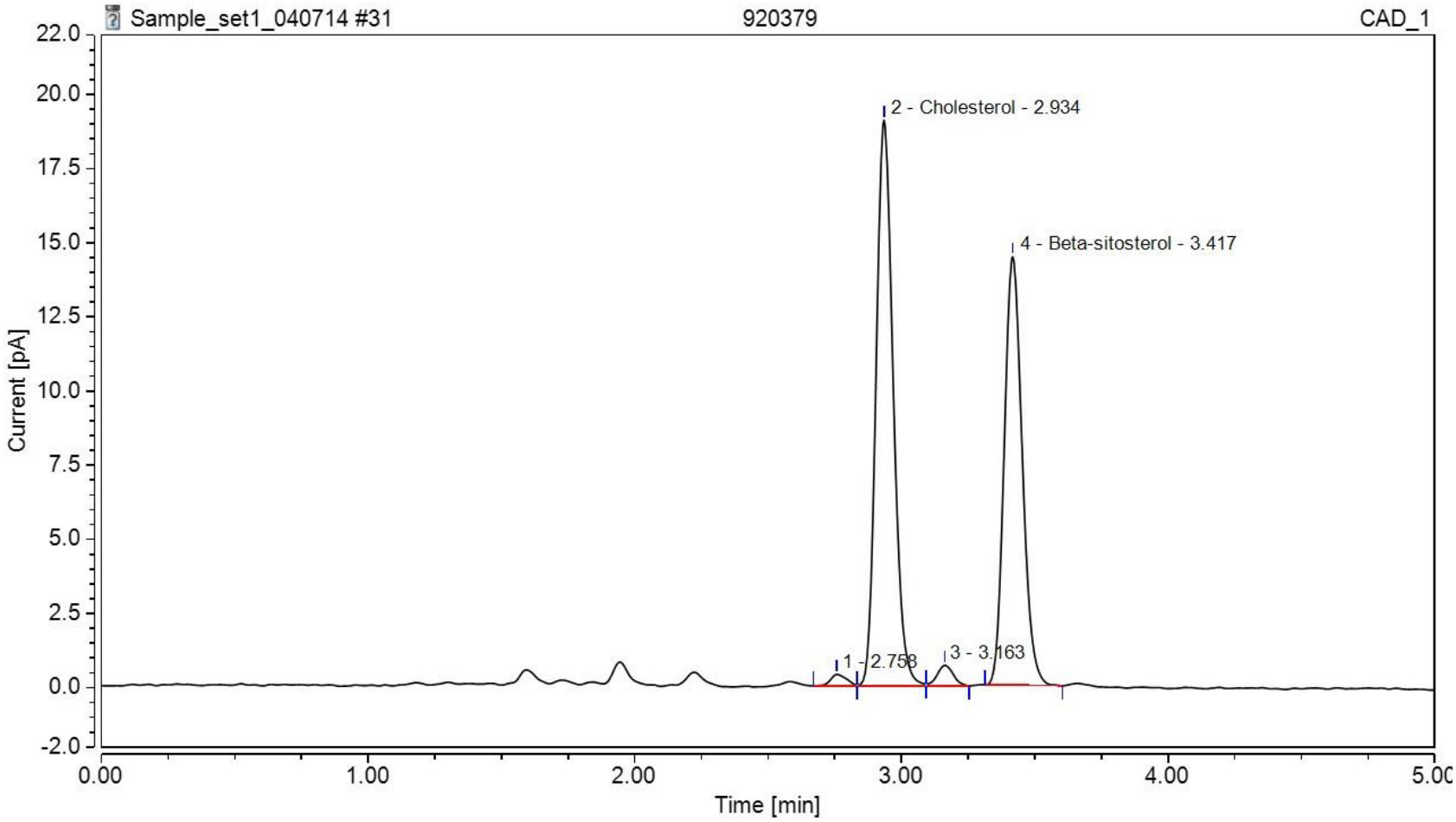


Figure 17. Total cholesterol chromatogram example month 8 for sample 920379

5.6.1.6 Oxysterol QC Sequence Results Month 3 samples

Acceptance Criteria:

Passed - ≤± 15% Accuracy

Failed - >±15% Accuracy

Table 14. Oxysterol QC Samples (20Dec2013) for 22-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	6.48	7.03	8.50	x	
QC Level 1_2	6.48	7.17	10.69	x	
QC Level 2_1	51.82	54.43	5.04	x	
QC Level 2_2	51.82	55.45	7.01	x	
QC Level 3_1	829.12	789.12	-4.83	x	
QC Level 3_2	829.12	764.98	-7.74	x	

Table 15. Oxysterol QC Samples (20Dec2013) for 24-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	9.18	10.31	12.26	x	
QC Level 1_2	9.18	10.22	11.28	x	
QC Level 2_1	73.48	74.41	1.28	x	
QC Level 2_2	73.48	79.42	8.09	x	
QC Level 3_1	1175.63	1107.40	-5.80	x	
QC Level 3_2	1175.63	1088.85	-7.38	x	

Table 16. Oxysterol QC Samples (20Dec2013) for 25-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	10.34	11.08	7.16	x	
QC Level 1_2	10.34	10.84	4.83	x	
QC Level 2_1	82.69	84.56	2.27	x	
QC Level 2_2	82.69	86.29	4.36	x	
QC Level 3_1	1323.00	1275.61	-3.58	x	
QC Level 3_2	1323.00	1197.39	-9.49	x	

Table 17. Oxysterol QC Samples (20Dec2013) for 27-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	6.24	6.95	11.40	x	
QC Level 1_2	6.24	6.51	4.32	x	
QC Level 2_1	49.92	49.33	-1.19	x	
QC Level 2_2	49.92	54.84	9.85	x	
QC Level 3_1	798.75	804.62	0.73	x	
QC Level 3_2	798.75	760.80	-4.75	x	

Table 18. Oxysterol QC Samples (20Dec2013) for 4β-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	5.11	4.41	-13.67	x	
QC Level 1_2	5.11	4.56	-10.78	x	
QC Level 2_1	40.85	46.59	14.06	x	
QC Level 2_2	40.85	46.55	13.95	x	
QC Level 3_1	653.62	714.58	9.33	x	
QC Level 3_2	653.62	678.65	3.83	x	

Table 19. Oxysterol QC Samples (20Dec2013) for 5α6α-Epoxycholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	7.91	8.82	11.56	x	
QC Level 1_2	7.91	9.09	14.88	x	
QC Level 2_1	63.28	66.76	5.49	x	
QC Level 2_2	63.28	69.57	9.94	x	
QC Level 3_1	1012.50	995.36	-1.69	x	
QC Level 3_2	1012.50	926.34	-8.51	x	

Table 20. Oxysterol QC Samples (20Dec2013) for 5β6β-Epoxycholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	6.21	4.64	-25.25		x*BLQ
QC Level 1_2	6.21	7.40	19.26		x*BLQ
QC Level 2_1	49.64	53.22	7.21	x	
QC Level 2_2	49.64	52.91	6.59	x	
QC Level 3_1	794.25	838.99	5.63	x	
QC Level 3_2	794.25	791.29	-0.37	x	

*BLQ – Below Limit of Quantification; actual LLOQ is 16 ng/mL for 5β6β-Epoxycholestanol. However values are acceptable due to the fact that all determined values for Month 3 QC check samples are on average 90.72 ng/mL, median 86.51 ng/mL, min. 44.97 ng/mL and max 140.40ng/mL.

Therefore QC Level 2 and QC Level 3 adequately control the linear range used for quantification.

Table 21. Oxysterol QC Samples (20Dec2013) for 7α-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	6.19	7.02	13.39	x	
QC Level 1_2	6.19	7.07	14.27	x	
QC Level 2_1	49.50	54.83	10.76	x	
QC Level 2_2	49.50	54.84	10.78	x	
QC Level 3_1	792.00	823.45	3.97	x	
QC Level 3_2	792.00	775.42	-2.09	x	

Table 22. Oxysterol QC Samples (20Dec2013) for 7β-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	7.30	7.66	4.84	x	
QC Level 1_2	7.30	8.17	11.84	x	
QC Level 2_1	58.43	61.71	5.62	x	
QC Level 2_2	58.43	64.27	9.99	x	
QC Level 3_1	934.87	979.39	4.76	x	
QC Level 3_2	934.87	914.98	-2.13	x	

Table 23. Oxysterol QC Samples (20Dec2013) for 7-Ketocholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	7.87	7.12	-9.50	x	
QC Level 1_2	7.87	8.71	10.70	x	
QC Level 2_1	62.93	62.58	-0.55	x	
QC Level 2_2	62.93	68.49	8.83	x	
QC Level 3_1	1006.88	967.37	-3.92	x	
QC Level 3_2	1006.88	916.84	-8.94	x	

Table 24. Oxysterol QC Samples (20Dec2013) for 6α-hydroxy-5α-cholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
QC Level 1_1	9.17	9.20	0.31	x	
QC Level 1_2	9.17	8.95	-2.35	x	
QC Level 2_1	73.34	75.47	2.91	x	
QC Level 2_2	73.34	83.01	13.20	x	
QC Level 3_1	1173.38	1274.21	8.59	x	
QC Level 3_2	1173.38	1109.85	-5.41	x	

5.6.1.7 Oxysterol QC Sequence Results Month 8 samples

Acceptance Criteria:

Passed - ≤± 15% Accuracy

Failed - >±15% Accuracy

Table 25. Oxysterol QC Samples Month 8 for 22-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	13.37	13.77	2.94	x	
4July2014_QC_1	13.37	13.57	1.44	x	
7July2014_QC_1	13.37	16.83	25.86		x*UISR
8July2014_QC_1	13.37	17.70	32.35		x*UISR
9July2014_QC_1	13.37	15.90	18.89		x*UISR
2July2014_QC_2	53.50	57.07	6.67	x	
4July2014_QC_2	53.50	53.87	0.69	x	
7July2014_QC_2	53.50	56.43	5.49	x	
8July2014_QC_2	53.50	ISP	ISP		x**ISP
9July2014_QC_2	53.50	52.57	-1.74	x	
2July2014_QC_3	427.96	448.53	4.81	x	
4July2014_QC_3	427.96	395.26	-7.64	x	
7July2014_QC_3	427.96	445.33	4.06	x	
8July2014_QC_3	427.96	421.50	-1.51	x	
9July2014_QC_3	427.96	409.80	-4.25	x	

*UISR - Unacceptable Internal Standard Response; however obtained study values are considered acceptable due to the fact that determined sample concentrations for Month 8 are:

- in the sequence of 07 July 2014 on average 14.22 ng/mL, median 13.65 ng/mL, min. 8.39 ng/mL and max 23.40 ng/mL with an average RSD% for all analyzed samples in triplicate of 6.79%.
- in the sequence of 08 July 2014 on average 13.12 ng/mL, median 12.58 ng/mL, min. 6.97 ng/mL and max 20.95 ng/mL with an average RSD% for all analyzed samples in triplicate of 5.35%.

- in the sequence of 09 July 2014 on average 10.97 ng/mL, median 10.73 ng/mL, min. 8.46 ng/mL and max 14.62 ng/mL with an average RSD% for all analyzed samples in triplicate of 5.19%.

QC Level 1 (QC_1) values for sequences performed on 07th July, 08th July and 09th July were outside the accepted bias limits (≤±15%). However for all study samples on these days, the average precision values (RSD%) for triplicate measurements were 6.79% on 07th July, 5.35% on 08th July and 5.19% on 09th July, demonstrating excellent reproducibility. The estimated bias for concentrations determined around the QC_1 level was approx. ≤±32% (≤±15% normal acceptance). Only QC Level 3 was valid for the analysis performed on 08th July for this analyte.

Table 26. Oxysterol QC Samples Month 8 for 24-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	15.43	16.73	8.48	x	
4July2014_QC_1	15.43	14.20	-7.94	x	
7July2014_QC_1	15.43	16.73	8.48	x	
8July2014_QC_1	15.43	16.87	9.34	x	
9July2014_QC_1	15.43	15.43	0.05	x	
2July2014_QC_2	61.70	61.57	-0.22	x	
4July2014_QC_2	61.70	56.83	-7.89	x	
7July2014_QC_2	61.70	64.13	3.94	x	
8July2014_QC_2	61.70	ISP	ISP		x
9July2014_QC_2	61.70	58.50	-5.19	x	
2July2014_QC_3	493.59	493.53	-0.01	x	
4July2014_QC_3	493.59	427.73	-13.34	x	
7July2014_QC_3	493.59	497.73	0.84	x	
8July2014_QC_3	493.59	454.53	-7.91	x	
9July2014_QC_3	493.59	474.73	-3.82	x	

Table 27. Oxysterol QC Samples Month 8 for 24-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	15.57	16.67	7.04	x	
4July2014_QC_1	15.57	14.60	-6.24	x	
7July2014_QC_1	15.57	16.73	7.46	x	
8July2014_QC_1	15.57	14.20	-8.80	x	
9July2014_QC_1	15.57	16.90	8.54	x	
2July2014_QC_2	62.29	63.60	2.11	x	
4July2014_QC_2	62.29	55.93	-10.20	x	
7July2014_QC_2	62.29	70.57	13.30	x	
8July2014_QC_2	62.29	ISP	ISP		x
9July2014_QC_2	62.29	57.90	-7.04	x	
2July2014_QC_3	498.28	494.86	-0.69	x	
4July2014_QC_3	498.28	443.06	-11.08	x	
7July2014_QC_3	498.28	512.96	2.95	x	
8July2014_QC_3	498.28	475.86	-4.50	x	
9July2014_QC_3	498.28	460.03	-7.68	x	

Table 28. Oxysterol QC Samples Month 8 for 27-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	14.59	15.23	4.41	x	
4July2014_QC_1	14.59	13.90	-4.73	x	
7July2014_QC_1	14.59	14.90	2.12	x	
8July2014_QC_1	14.59	13.60	-6.79	x	
9July2014_QC_1	14.59	15.40	5.55	x	
2July2014_QC_2	58.36	61.10	4.70	x	
4July2014_QC_2	58.36	53.63	-8.10	x	
7July2014_QC_2	58.36	60.80	4.18	x	
8July2014_QC_2	58.36	ISP	ISP		x
9July2014_QC_2	58.36	62.63	7.32	x	
2July2014_QC_3	466.87	466.13	-0.16	x	
4July2014_QC_3	466.87	402.53	-13.78	x	
7July2014_QC_3	466.87	497.83	6.63	x	
8July2014_QC_3	466.87	448.90	-3.85	x	
9July2014_QC_3	466.87	414.73	-11.17	x	

Table 29. Oxysterol QC Samples Month 8 for 4β-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	14.58	13.60	-6.69	x	
4July2014_QC_1	14.58	15.50	6.35	x	
7July2014_QC_1	14.58	14.80	1.54	x	
8July2014_QC_1	14.58	14.97	2.69	x	
9July2014_QC_1	14.58	12.50	-14.24	x	
2July2014_QC_2	58.30	62.03	6.40	x	
4July2014_QC_2	58.30	58.67	0.63	x	
7July2014_QC_2	58.30	62.80	7.72	x	
8July2014_QC_2	58.30	ISP	ISP		x
9July2014_QC_2	58.30	58.53	0.40	x	
2July2014_QC_3	466.40	473.00	1.41	x	
4July2014_QC_3	466.40	397.40	-14.80	x	
7July2014_QC_3	466.40	492.43	5.58	x	
8July2014_QC_3	466.40	441.70	-5.30	x	
9July2014_QC_3	466.40	459.26	-1.53	x	

Table 30. Oxysterol QC Samples Month 8 for 5α6α-Epoxycholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	11.68	21.67	85.58		x***UCR
4July2014_QC_1	11.68	9.00	-22.91		x***UCR
7July2014_QC_1	11.68	19.90	70.45		x***UCR
8July2014_QC_1	11.68	16.17	38.48		x***UCR
9July2014_QC_1	11.68	14.70	25.91		x***UCR
2July2014_QC_2	46.70	46.23	-1.00	x	
4July2014_QC_2	46.70	38.20	-18.20		x***UCR
7July2014_QC_2	46.70	55.47	18.77		x***UCR
8July2014_QC_2	46.70	ISP	ISP		x
9July2014_QC_2	46.70	45.33	-2.93	x	
2July2014_QC_3	373.59	366.60	-1.87	x	
4July2014_QC_3	373.59	322.40	-13.70	x	
7July2014_QC_3	373.59	388.00	3.86	x	
8July2014_QC_3	373.59	355.40	-4.87	x	
9July2014_QC_3	373.59	330.46	-11.54	x	

*** UCR - Unacceptable Chromatography, however obtained study values are considered acceptable due to the fact that all determined samples for Month 8 were:

- in the sequence of 02 July 2014 on average 39.93 ng/mL, median 39.96 ng/mL, min. 22.77 ng/mL and max 60.34 ng/mL with an average RSD% for all analyzed samples in triplicate of 11.42%.
- in the sequence of 04 July 2014 on average 33.17 ng/mL, median 32.38 ng/mL, min. 19.71 ng/mL and max 45.96 ng/mL with an average RSD% for all analyzed samples in triplicate of 7.64%.
- in the sequence of 07 July 2014 on average 42.42 ng/mL, median 40.60 ng/mL, min. 27.95 ng/mL and max 65.55 ng/mL with an average RSD% for all analyzed samples in triplicate of 9.59%.
- in the sequence of 08 July 2014 on average 32.72 ng/mL, median 29.18 ng/mL, min. 19.72 ng/mL and max 57.65 ng/mL with an average RSD% for all analyzed samples in triplicate of 7.74%.
- in the sequence of 09 July 2014 on average 25.70 ng/mL, median 24.04 ng/mL, min. 18.16 ng/mL and max 39.05 ng/mL with an average RSD% of all analyzed samples in triplicate of 9.65%.

Therefore QC Level 2 and QC Level 3 are considered to adequately control the linear range representative for acceptable quantification of study samples. The majority of study sample values were closer to the values of QC_2 rather than to QC_1. The bias for concentrations determined around the QC_2 level was estimated to be approx. ≤±19% (≤±15% accepted). Only QC level 3 was valid for analyses performed on 04th July, 07th July and 08th July.

Table 31. Oxysterol QC Samples Month 8 for 5β6β-Epoxycholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	11.29	23.23	105.71		x***UCR
4July2014_QC_1	11.29	8.03	-28.87		x***UCR
7July2014_QC_1	11.29	24.43	116.34		x***UCR
8July2014_QC_1	11.29	22.60	100.11		x***UCR
9July2014_QC_1	11.29	17.90	58.49		x***UCR
2July2014_QC_2	45.18	47.90	6.03	x	
4July2014_QC_2	45.18	35.90	-20.53		x***UCR
7July2014_QC_2	45.18	61.27	35.62		x***UCR
8July2014_QC_2	45.18	ISP	ISP		x
9July2014_QC_2	45.18	45.00	-0.39	x	
2July2014_QC_3	361.40	368.56	1.98	x	
4July2014_QC_3	361.40	316.96	-12.30	x	
7July2014_QC_3	361.40	404.03	11.79	x	
8July2014_QC_3	361.40	360.80	-0.17	x	
9July2014_QC_3	361.40	352.30	-2.52	x	

*** UCR - Unacceptable Chromatography, however obtained study values are considered acceptable due to the fact that all determined samples for Month 8 were:

- in the sequence of 02 July 2014 on average 117.07 ng/mL, median 110.63 ng/mL, min. 70.28 ng/mL and max 177.40 ng/mL with an average RSD% for all analyzed samples in triplicate of 17.99%.
- in the sequence of 04 July 2014 on average 85.74 ng/mL, median 85.60 ng/mL, min. 57.18 ng/mL and max 117.98 ng/mL with an average RSD% for all analyzed samples in triplicate of 7.07%.
- in the sequence of 07 July 2014 on average 122.96 ng/mL, median 108.57 ng/mL, min. 84.58 ng/mL and max 204.44 ng/mL with an average RSD% for all analyzed samples in triplicate of 18.27%.
- in the sequence of 08 July 2014 on average 80.44 ng/mL, median 74.80 ng/mL, min. 58.74 ng/mL and max 117.50 ng/mL with an average RSD% for all analyzed samples in triplicate of 9.97%.
- in the sequence of 09 July 2014 on average 75.52 ng/mL, median 71.40 ng/mL, min. 57.43 ng/mL and max 114.11 ng/mL with an average RSD% for all analyzed samples in triplicate of 9.76%.

Therefore QC Level 2 and QC Level 3 are considered to adequately control the linear range representative for acceptable quantification of study samples. All study sample values fell between the concentration values for QC_2 and QC_3. The bias for concentrations determined around the QC_2 was estimated to be approx. ≤±35% (≤±15% accepted). Only QC level 3 was valid for analyses performed on 04th July, 07th July and 08th July.

Table 32. Oxysterol QC Samples Month 8 for 7α-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	12.80	14.17	10.65	x	
4July2014_QC_1	12.80	13.03	1.80	x	
7July2014_QC_1	12.80	11.77	-8.09	x	
8July2014_QC_1	12.80	11.83	-7.58	x	
9July2014_QC_1	12.80	11.10	-13.30	x	
2July2014_QC_2	51.21	55.73	8.83	x	
4July2014_QC_2	51.21	44.93	-12.26	x	
7July2014_QC_2	51.21	50.90	-0.61	x	
8July2014_QC_2	51.21	ISP	ISP		x
9July2014_QC_2	51.21	47.43	-7.38	x	
2July2014_QC_3	409.68	412.96	0.80	x	
4July2014_QC_3	409.68	363.00	-11.40	x	
7July2014_QC_3	409.68	433.50	5.81	x	
8July2014_QC_3	409.68	390.50	-4.68	x	
9July2014_QC_3	409.68	377.53	-7.85	x	

Table 33. Oxysterol QC Samples Month 8 for 7β-Hydroxycholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	14.50	16.43	13.32	x	
4July2014_QC_1	14.50	12.33	-14.96	x	
7July2014_QC_1	14.50	15.73	8.49	x	
8July2014_QC_1	14.50	12.87	-11.27	x	
9July2014_QC_1	14.50	15.07	3.90	x	
2July2014_QC_2	58.01	60.93	5.04	x	
4July2014_QC_2	58.01	51.37	-11.45	x	
7July2014_QC_2	58.01	64.40	11.02	x	
8July2014_QC_2	58.01	ISP	ISP		x
9July2014_QC_2	58.01	56.97	-1.79	x	
2July2014_QC_3	464.06	470.30	1.34	x	
4July2014_QC_3	464.06	406.16	-12.48	x	
7July2014_QC_3	464.06	469.76	1.23	x	
8July2014_QC_3	464.06	443.76	-4.37	x	
9July2014_QC_3	464.06	443.30	-4.47	x	

Table 34. Oxysterol QC Samples Month 8 for 7-Ketocholesterol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	14.46	15.17	4.90	x	
4July2014_QC_1	14.46	14.03	-2.94	x	
7July2014_QC_1	14.46	15.70	8.59	x	
8July2014_QC_1	14.46	13.30	-8.01	x	
9July2014_QC_1	14.46	14.37	-0.63	x	
2July2014_QC_2	57.83	53.30	-7.84	x	
4July2014_QC_2	57.83	57.63	-0.34	x	
7July2014_QC_2	57.83	59.03	2.08	x	
8July2014_QC_2	57.83	ISP	ISP		x
9July2014_QC_2	57.83	51.23	-11.41	x	
2July2014_QC_3	462.65	493.76	6.72	x	
4July2014_QC_3	462.65	403.60	-12.76	x	
7July2014_QC_3	462.65	450.10	-2.71	x	
8July2014_QC_3	462.65	473.70	2.39	x	
9July2014_QC_3	462.65	465.10	0.53	x	

Table 35. Oxysterol QC Samples Month 8 for 6α-hydroxy-5α-cholestanol

Sample Name	Expected Conc. [ng/mL]	Detected Conc. [ng/mL]	Bias [%]	Passed	Failed
2July2014_QC_1	14.43	15.18	5.20	x	
4July2014_QC_1	14.43	14.03	-2.77	x	
7July2014_QC_1	14.43	15.44	7.00	x	
8July2014_QC_1	14.43	15.30	6.03	x	
9July2014_QC_1	14.43	14.98	3.81	x	
2July2014_QC_2	57.71	56.95	-1.32	x	
4July2014_QC_2	57.71	51.71	-10.40	x	
7July2014_QC_2	57.71	63.41	9.88	x	
8July2014_QC_2	57.71	ISP	ISP		x
9July2014_QC_2	57.71	52.29	-9.39	x	
2July2014_QC_3	461.72	450.45	-2.44	x	
4July2014_QC_3	461.72	439.11	-4.90	x	
7July2014_QC_3	461.72	465.58	0.84	x	
8July2014_QC_3	461.72	436.05	-5.56	x	
9July2014_QC_3	461.72	413.73	-10.39	x	

5.6.1.8 Cholesterol QC Sequence Results Month 8 samples

Acceptance Criteria:

Passed - ≤± 15% Accuracy

Failed - >±15% Accuracy

Table 36. Cholesterol Sequence 1 (03Jul2014)

Sample Name	Expected Conc. [mg/mL]	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1	1.57	1.58	0.63	x	
QC Level 1_2	1.57	1.53	-2.74	x	
QC Level 1_3	1.57	1.54	-2.23	x	
QC Level 2_1	2.62	2.65	1.18	x	
QC Level 2_2	2.62	2.58	-1.49	x	
QC Level 2_3	2.62	2.61	-0.34	x	
QC Level 3_1	4.20	4.02	-4.36	x	
QC Level 3_2	4.20	4.02	-4.24	x	
QC Level 3_3	4.20	4.01	-4.50	x	

Table 37. Cholesterol Sequence 2 (04Jul2014)

Sample Name	Expected Conc. (mg/mL)	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1	1.57	1.58	0.57	x	
QC Level 1_2	1.57	1.56	-0.70	x	
QC Level 1_3	1.57	1.57	-0.25	x	
QC Level 2_1	2.62	2.66	1.45	x	
QC Level 2_2	2.62	2.63	0.42	x	
QC Level 2_3	2.62	2.65	0.99	x	
QC Level 3_1	4.20	4.06	-3.43	x	
QC Level 3_2	4.20	4.09	-2.71	x	
QC Level 3_3	4.20	4.00	-4.71	x	

Table 38. Cholesterol Sequence 3 (07Jul2014)

Sample Name	Expected Conc. (mg/mL)	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1	1.57	1.60	1.33	x	
QC Level 1_2	1.57	1.57	0.00	x	
QC Level 1_3	1.57	1.56	-0.57	x	
QC Level 2_1	2.62	2.67	1.95	x	
QC Level 2_2	2.62	2.68	2.18	x	
QC Level 2_3	2.62	2.68	2.37	x	
QC Level 3_1	4.20	4.07	-3.12	x	
QC Level 3_2	4.20	4.01	-4.45	x	
QC Level 3_3	4.20	4.00	-4.83	x	

Table 39. Cholesterol Sequence 3 (07Jul2014)

Sample Name	Expected Conc. (mg/mL)	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1	1.57	1.59	1.01	x	
QC Level 1_2	1.57	1.53	-2.61	x	
QC Level 1_3	1.57	1.55	-1.53	x	
QC Level 2_1	2.62	2.65	1.03	x	
QC Level 2_2	2.62	2.59	-0.99	x	
QC Level 2_3	2.62	2.63	0.50	x	
QC Level 3_1	4.20	4.01	-4.62	x	
QC Level 3_2	4.20	4.02	-4.33	x	
QC Level 3_3	4.20	4.03	-4.14	x	

Table 40. Cholesterol Sequence 5 (09Jul2014)

Sample Name	Expected Conc. (mg/mL)	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1	1.57	1.58	0.50	x	
QC Level 1_2	1.57	1.54	-1.91	x	
QC Level 1_3	1.57	1.55	-1.34	x	
QC Level 2_1	2.62	2.61	-0.23	x	
QC Level 2_2	2.62	2.61	-0.34	x	
QC Level 2_3	2.62	2.58	-1.56	x	
QC Level 3_1	4.20	4.02	-4.19	x	
QC Level 3_2	4.20	4.03	-4.14	x	
QC Level 3_3	4.20	4.07	-3.19	x	

Table 41. Cholesterol Sequence Diluted (28Jul2014)

Sample Name	Expected Conc. (mg/mL)	Detected Conc. [mg/mL]	Bias [%]	Passed	Failed
QC Level 1_1_Dil	1.57	1.57	-0.32	x	
QC Level 1_2_Dil	1.57	1.59	1.21	x	
QC Level 1_3_Dil	1.57	1.55	-1.02	x	
QC Level 2_1_Dil	2.62	2.57	-1.79	x	
QC Level 2_2_Dil	2.62	2.66	1.41	x	
QC Level 2_3_Dil	2.62	2.63	0.42	x	
QC Level 3_1_Dil	4.20	4.12	-1.86	x	
QC Level 3_2_Dil	4.20	4.08	-2.93	x	
QC Level 3_3_Dil	4.20	4.12	-1.98	x	

6 Archiving

After finalization of the study, the study plan, raw data, reference items, the report, and all study-related records will be stored for a maximal period of one year at the archive at Philip Morris Products S.A, Research & Development. Thereafter, all records and materials will be transferred to the external GLP-certified service archive Iron Mountain Deutschland GmbH, Harpener Hellweg, 44805 Bochum, Germany.

Electronic records will be archived on the central archiving server at Philip Morris Products S.A, Research & Development, which is managed by the local IT specialists.

Electronic and paper records will be archived for at least 10 years after finalization of the final report according to the specific archiving SOPs.

7 References

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8 Abbreviations

Table 42 Table of Abbreviations

Abbreviation	
AAR	Above the accepted range
BLQ	Below limit of quantification
CAD	Charged Aerosol Detector
CC	Conventional Cigarette
CI	Confidence Interval
CV	Coefficient Interval
DCU	Diluted Concentration Unreliable
DFNR	Dilution Factor Not Reliable
EDMS	Electronic Document Management System
GLP	Good Laboratory Practice
HPLC	High Performance liquid Chromatography
HSR	Highest Standard Removed
IAA	Incomplete Instrument Analysis
ID	Identifier
IS	Internal Standard
ISA	Insufficient Sample for Analysis
ISP	Incomplete Sample Processing
ISR	Insufficient Sample Reproducibility
IVR	Insufficient Sample Volume for Reanalysis
LC-HR-MS	Liquid Chromatography with High Resolution Mass Spectrometric Detection
LLOQ	Lower Limit Of Quantification
LOT	Lot Number

Table continued on next page

Abbreviation	
LSR	Lowest Standard Removed
N/AP	Not Applicable
N/AV	Not Available
NR	Not Reportable
QA	Quality Assurance
QC	Quality Control
RE	Relative Error
RSD	Relative Standard Deviation
RT	Room Temperature
SD	Standard Deviation
SDMS	Scientific Data Management System
SOP	Standard Operating Protocol
STD	Calibration Standard
THS	Tobacco Heating System
TIC	Total Ion Chromatogram
UCR	Unacceptable Chromatography
UISR	Unacceptable Internal Standard Response
ULOQ	Upper Limit Of Quantification
XIC	Extracted Ion Chromatogram

For complete definition, refer to PMI OPS Glossary and PMI RD Glossary.

9 Appendix

9.1 Deviations from Analytical Analysis Plan 150156

The validation of the assays “Determination of Oxysterols in Plasma using LC-HR-MS” with the corresponding WKI (PMI_RD_WKI_01241) and the “Determination of Cholesterol in Plasma using LC-CAD” with the corresponding WKI (PMI_RD_WKI_01240) was ongoing during this explorative study. Assay validation were finalized after the end of the experimental part of this explorative study. Therefore it was necessary to apply some unintended changes.

All unintended deviations from the Analytical Analysis Plan 150156 are summarized here. Changes are marked in red font and the consequence is described.

9.1.1 Objective

The purpose of this study was to determine the concentrations of 11 Oxysterols (5α,6α-epoxycholestanol, 5ß,6ß-epoxycholestanol, 6α-hydroxy-5α-cholestanol, 7-ketocholesterol, 4β-hydroxycholesterol, 7α-hydroxycholesterol, 7ß-hydroxycholesterol, 22-hydroxycholesterol, 24(R)-hydroxy-cholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol) in plasma samples derived from ApoE-/- mice by LC-HR-MS. Total Cholesterol was also determined in the same samples using LC-CAD.

↓

- During the ongoing method development throughout the experimental part of this study 2 additional Oxysterol species, namely 4β-hydroxycholesterol and 22-hydroxycholesterol, could be included to the assay and validated.
  - ➢ As a consequence two additional Oxysterols are reported in order to increase the comprehensive overview of metabolic fade based on lipid profile during this study.

9.1.2 Reference Items and Internal Standards

Table 43. Deviations from Reference Items and Internal Standards

Identity	Specification	Storage	Supplier	Product Nr.	Lot-Nr.
4ß-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700036P-5mg	4βOHCH-11
4ß-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700042P-5mg	4βOHCH(D7)-14
6α-hydroxy-5α-cholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700030P-5mg	6aOHCHL-11
6α-hydroxy-5α-cholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700045P-5mg	6aOH5aCHL(D7)-12
7α-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700034P-5mg	7aOHCH-12
7α-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700043P-5mg	7aOHCH(D7)-11
7ß-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700035P-5mg	7βOHCH-12
7ß-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700044P-5mg	7βOHCH(D7)-11
5α,6α-epoxycholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700032P-5mg	5a,6a-EPCHOL-10
5α,6α-epoxycholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700047P-5mg	5a,6a-EPCHOL(D7)-11
5ß,6ß-epoxycholestanol	Purity >99% powder	-20°C	Avanti Polar Lipids	700033P-5mg	5β,6β-EPCHOL-11
5ß,6ß-epoxycholestanol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700047P-5mg	5β,6β-EPCHOL(D7)-13
7-ketocholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700015P-5mg	7-KETOCH-14
7-ketocholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700046P-5mg	7-KETOCH(D7)-11
22-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700058P-5mg	22(R)OHCH-12
22-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700052P-5mg	22(R)OHCHD7-10
24(R)-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700071P-5mg	24(R)OHCH-10
24(R/S)-hydroxycholesterol (d7)	Purity >99% powder	-20°C	Avanti Polar Lipids	700018P-5mg	24OHCH(D7)-10
25-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700019P-5mg	25OHCH-13
25-hydroxycholesterol (d6)	Purity >99% powder	-20°C	Avanti Polar Lipids	700053P-5mg	25OHCHD6-10
27-hydroxycholesterol	Purity >99% powder	-20°C	Avanti Polar Lipids	700021P-5mg	27OHCH-13
27-hydroxycholesterol (d6)	Purity >99% powder	-20°C	Avanti Polar Lipids	700059P-5mg	27OHCHD6-10
Cholesterol	Purity >99% powder	-20°C	Sigma Aldrich	C8667	080M5304V
β-sitosterol	Purity >85% powder	-20°C	Sigma Aldrich	43623	BCBM2889V

↓

- As described in Section 9.1.1 during the ongoing method development throughout the experimental part of this study 2 additional Oxysterol species, namely 4β-hydroxycholesterol and 22-hydroxycholesterol, incl. their corresponding deuterated species as internal standard, could be

included to the assay and validated. These two Oxysterols were initially not in scope of the Analysis Plan 150156.

- As a consequence two additional Oxysterols are quantified and reported in order to increase the comprehensive overview of metabolic fade based on lipid profile during this study.

9.1.3 Procedure and Instrumentation - Cholesterol

Sample Preparation	Liquid-liquid extraction
Chromatography Type	HPLC
Instrument Platform	Charged Aerosol Detection (Dionex Corona Veo ultra RS™)
Regression Type	Quadratic (no weighting)
Quantitation Method	Peak Area Ratio
Assay Volume	5 µL

Table 44 Deviations for Procedure and Instrumentation for the determination of Cholesterol

↓

- During the ongoing method development throughout the experimental part of this study the extraction procedure was changed from Liquid Extraction to Liquid-liquid Extraction.
  - As a consequence the updated sample preparation procedure lead to more robust and reliable results and could be successful validated.

- The Regression Type of the assay was changed to Quadratic without weighting due to fact that the CAD is a nonlinear detection system.
  - As a consequence the updated Regression Type procedure lead to more robust and reliable results and could be successful validated. Calibration curves of cholesterol could maintained in linear regression of R^2=0.999.

9.1.4 Computer Application Software

LC-HR-MS Software	Thermo Fisher Scientific Xcalibur® version 2.2 SP1.48
Quantification Software (Oxysterols)	Thermo LC Quan® for Month 3 only Thermo Fisher Scientific Trace Finder™ 3.1
Quantification Software (Cholesterol)	Dionex Chromeleon™ version 7.2 ~~3~~
Sample Management	Labvantage®
Office Applications	Microsoft Word® 2013, Microsoft Excel® 2013

Table 45 Deviations from Computer Application Software

↓

- During the experimental of this study the new version of Dionex Chromeleon™ 7.3 was not available.
  - ➢ No consequence

9.1.5 Description of an Analytical Run

An analytical run, as a minimum contained:

- a sufficient number of blank samples to reflect at least 2% of the study samples (with at least one blank sample positioned after a known high concentration sample),
- two standard zero samples (blank sample with internal standard (IS) added),
- one set of at least 6 non-zero calibration standards at the beginning of a sample run,
- one set of at least 6 non-zero calibration standards at the end of a sample run,
- a minimum number of three QCs (one replicate at three concentration levels) to reflect at least 5% of the number of study samples,
- if study samples were diluted: three dilution QC (DQC) replicates diluted by the same factor as used for the dilution of the study samples were included.

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- Due to enormous time constrains during the sample preparation phase the minimum number of QC samples was reduced to one replicate per QC level (for the determination of Oxysterols of Month 8 only).
  - As a consequence the low concentration QC series failed for 5α6α-Epoxycholestanol and 5β6β-Epoxycholestanol on three consecutive days of Month 8 samples with the predefined expected concentrations. For 22-Hydroxycholesterol the low concentration QC series failed on one day. The LLOQs had to be adjusted and explanation was given in the respective section of the affected QC sequence reports (Section 5.6.1.7). The gained knowledge was implemented in the assay validation.

9.1.6 Analytical Run Acceptance Criteria

An analytical run was acceptable if all of the following criteria were met:

- at least 75% of the non-zero calibration standards are within ± 15% (± 20% for the lower limit of quantification (LLOQ) calibration standard) of their nominal concentration,
- at least two-thirds of the QC samples are within ± 15% 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,
- 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 fulfil the above described interference criteria.

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- Due to enormous time constrains during the sample preparation phase the minimum number of QC samples was reduced to one replicate per QC level (for the determination of Oxysterols of Month 8 only).
  - ➢ As a consequence the low concentration QC series failed for 5α6α-Epoxycholestanol and 5β6β-Epoxycholestanol on three consecutive days of Month 8 samples with the predefined expected concentrations. For 22-Hydroxycholesterol the low concentration QC series failed on one day. The LLOQs had to be adjusted and explanation was given in the respective section of the affected QC sequence reports (Section 5.6.1.7). The gained knowledge was implemented in the assay validation.