

ABS Laboratories Ltd
BioPark
Broadwater Road
Welwyn Garden City
Herts AL7 3AX
United Kingdom
www.abslabs.com
Tel: +44 (0) 1707 358666
Fax: +44 (0) 1707 358667

Analytical Report

Study Number: ABS/10/18

**Determination of Nicotine in Human Plasma Samples from Swedish Match
Clinical Protocol No. SM 17-03**

Sponsor: Swedish Match
Sponsor's Address: SE-Box 17037
104 62 Stockholm,
Sweden

Sponsor's Study Number: SM 17-03, Final 1.0 05Oct2017

Report Issue Date: 08 May 2018

Title: Determination of Nicotine in Human Plasma Samples from Swedish Match Clinical Protocol No. SM 17-03

ABS Report No: ABS/10/18

ABS Study No: ABS/10/18

Electronic filename: ABS_10_18 Final Analytical Report.docx

Sponsor Study No: SM 17-03

Analytical Laboratory: ABS Laboratories Ltd
BioPark
Broadwater Road
Welwyn Garden City
Herts
AL7 3AX

Sponsor: Swedish Match
SE-Box 17037
104 62 Stockholm
Sweden

Clinical Study Site: CTC Clinical Trial Consultants AB
Dag Hammarskjölds Väg 13, 2trp
Uppsala
75237 Sweden

Study Director: (b) (4), (b) (6)

Bioanalyst: (b) (4), (b) (6)

Report Author: (b) (4), (b) (6)

Sponsor's Study Monitor: (b) (4), (b) (6)

Experimental Phase Began: 8 February 2018

Experimental Phase Ended: 21 February 2018

No. of samples analysed: 860

STUDY DIRECTOR'S STATEMENT

This study was conducted to the standards described in the United Kingdom Good Laboratory Practice Regulations (SI 1999 No. 3106 as amended SI 2004 No. 994) and the OECD Principles of Good Laboratory Practice 1997 (ENV/MC/CHEM(98)17).

This study was also conducted in compliance with the United Kingdom "The Medicines for Human Use (Clinical Trials) Regulations", (SI 2004 No. 1031 and subsequent amendments).

I declare that this report fully reflects the raw data generated during this study.

Signature

(b) (4)

(Study Director)

Date: 08-MAY-2018

QA STATEMENT

QA personnel have examined the raw data related to the analysis of the samples from study protocol number ABS/10/18 and their findings are detailed in ABS/10/18 QA-01 to 05. Their findings were reported to the Study Director and management on the following dates:

Dates of Audit	Date findings reported to the Study Director	Date findings reported to Management	Audit description
23 & 25-Jan-2018	26-Jan-2018	26-Jan-2018	Review of draft analytical protocol
26-Jan-2018	26-Jan-2018		Review of final analytical protocol
18-Jan-2018	26-Jan-2018		Review of method SOP 5-85.4
11 to 13-Feb-2018	13-Feb-2018	02-May-2018	Reviewed sample extraction and processing of Batch 6.
23-Feb-2018	26-Feb-2018	02-May-2018	Review of raw data to final results
29-Mar & 11-Apr-2018	09-Apr-2018	02-May-2018	Review of draft report to raw data
02-May-2018	04-May-2018	02-May-2018	Review of final report after receipt of sponsor's comments on 26-Apr-2018

In addition to the detailed study-based inspections a series of routine facility and processed-based inspections were also being conducted and reported to management during the course of this study. A full facility audit is conducted once a year whilst specified facilities are audited on a rolling schedule.

The raw data and the study report have been audited and the report accurately reflects the raw data.

Signature: 
(b) (4)
(QA Manager)

Date: 8-May-2018

TABLE OF CONTENTS

STUDY DIRECTOR'S STATEMENT	3
QA STATEMENT	4
1. INTRODUCTION	7
2. EXPERIMENTAL	7
2.1. Method and Materials	7
2.1.1. Analytical Method	7
2.1.2. Reference Standard	7
2.1.3. Internal Standard	8
2.1.4. Chemical Structures	8
2.1.5. Biological Matrix	8
2.1.6. Calibration Standards and Quality Control Samples	8
2.2. Study Samples	8
2.2.1. Sample Source and Date of Receipt	8
2.2.2. Sample Storage	9
2.2.3. Sample Summary	9
2.2.4. Sample Analysis	9
2.2.5. Incurred Sample Reproducibility	9
3. RESULTS	9
3.1. Batch Acceptance Criteria	10
3.2. Quality Control Sample Analyses (Inter-Batch Precision and Accuracy)	10
3.3. Calibration Standard Concentrations	10
3.4. Standard Curve Parameters	10
3.5. Study Sample Concentrations	10
3.6. Reanalyses	10
3.6.1. Incurred Sample Reproducibility	10
3.6.2. Reanalyses for Analytical Reasons	11
4. RAW DATA AND CHROMATOGRAMS	11
5. COMMENTS AND NOTES	11
6. ARCHIVES	11
7. COMPUTER APPLICATION PROGRAMS	11
8. REFERENCES	11

TABLES

Table 1. Summary of batches performed for nicotine in human plasma	12
Table 2. Quality control sample data (inter-batch accuracy and precision) for nicotine in human plasma.....	13
Table 3. Back-calculated calibration standard concentrations for nicotine in human plasma.....	14
Table 4. Standard curve parameters for nicotine in human plasma	15
Table 5. Concentrations of nicotine in SM 17-03 study human plasma samples.....	16
Table 6. Incurred sample reproducibility for nicotine in human plasma	34
Table 7. Summary of reanalyses for analytical reasons for nicotine in human plasma	38

FIGURES

Figure 1. Representative standard curve for nicotine in human plasma from analysis batch 1; batch ID 20180208CF1	39
--	----

APPENDICES

Appendix 1. List of abbreviations.....	40
Appendix 2. Analytical protocol	41
Appendix 3. Certificate of analysis for nicotine (ABS CSR No. 18003)	49
Appendix 4. Certificate of analysis for nicotine-d ₄ (ABS CSR No. 18001).....	56
Appendix 5. Result tables.....	65
Appendix 6. Chromatograms.....	102

1. INTRODUCTION

ABS Laboratories has determined the concentrations of nicotine in human plasma (lithium heparin) samples using high performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Study samples were received as part of Swedish Match clinical protocol number SM 17-03, entitled "Nicotine pharmacokinetics and subjective effects of a single dose of a non-tobacco-based nicotine pouch (ZYN®) compared with conventional, tobacco-based Swedish snus among current, daily snus users". This report provides the results and supporting documentation for the analysis of the study samples as well as standard curve and quality control data.

A list of standard abbreviations used in this report is presented in [Appendix 1](#).

2. EXPERIMENTAL

The support to be provided to the clinical study was described in an analytical protocol, reproduced in [Appendix 2](#).

2.1. Method and Materials

2.1.1. Analytical Method

The analytical method was validated at ABS Laboratories in previous studies^{1, 2} in accordance with the FDA Guidance for Industry³ and the EMA Guideline on bioanalytical method validation⁴. Samples (100 µL) of human plasma (lithium heparin) containing the analyte and internal standard were extracted by derivatisation followed by liquid-liquid extraction procedure. The extracted samples were derivatised and analysed by an HPLC interfaced with an AB Sciex 4000 mass spectrometer. Positive ions were monitored in the multiple reaction ion-monitoring (MRM) mode. Quantification was by peak area ratio. Full details of the analytical procedure are documented in the method SOP⁵.

All documents referenced are on file at ABS Laboratories.

2.1.2. Reference Standard

(b) (4)



The certificate of analysis is reproduced in [Appendix 3](#).

2.1.3. Internal Standard

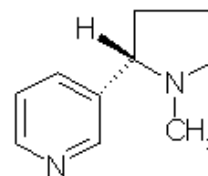
(b) (4)



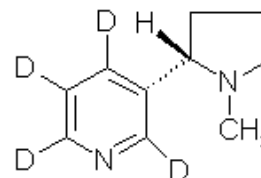
The certificate of analysis is reproduced in [Appendix 4](#).

2.1.4. Chemical Structures

Analyte: Nicotine
Formula: $C_{10}H_{14}N_2$
MW: 162.23 g/mol



Internal standard: Nicotine-D₄
Formula: $C_{10}H_{10}D_4N_2$
MW: 166.26 g/mol



2.1.5. Biological Matrix

Human plasma, with lithium heparin as anticoagulant, was obtained from healthy volunteers at ABS Laboratories. Human plasma (lithium heparin), free of significant interference, was used to prepare calibration standard and quality control (QC) samples.

2.1.6. Calibration Standards and Quality Control Samples

Calibration standards ranging from 0.500 to 50.0 ng/mL were prepared on 06 February 2018 and 12 February 2018 from standard spiking solutions which were prepared on 26 Jan 2018.

Quality Control (QC) samples at concentrations of 1.50, 15.0 and 40.0 ng/mL were used which were prepared on 06 February 2018. QC samples were stored at a nominal temperature of -20°C.

Copies of batch record forms, which document the preparation of these standard solutions and QC samples, are stored in the raw data for this study.

2.2. Study Samples

2.2.1. Sample Source and Date of Receipt

The first study samples were collected on 24 November 2017. Study samples were received at ABS Laboratories, with ice packs, on 19 January 2018 from CTC Clinical Trials Consultants AB, Dag Hammarskjölds Väg 13, 2trp, Uppsala, 75237 Sweden.

2.2.2. Sample Storage

After receipt, study samples were stored at a nominal temperature of -20°C, prior to analysis. All samples were analysed within 88 days of collection. The validation study¹ showed stability at -20°C for 246 days.

2.2.3. Sample Summary

The clinical protocol specified that a total of 18 subjects were to receive the following treatments in accordance to a pre-determined randomised order:

18 subjects were expected to receive the following:

1 = ZYN Smooth containing 3 mg nicotine per portion

2 = ZYN Smooth containing 6 mg nicotine per portion

3 = ZYN Smooth containing 3 mg nicotine per portion (alternative manufacturing process)

4 = ZYN Smooth containing 6 mg nicotine per portion (alternative manufacturing process)

5 = Swedish portion snus PSWL 1.0 g (8 mg nicotine/g)

Blood samples were scheduled to be taken at the following times:

Pre-dose (0), 5 mins, 10 mins, 15 mins, 30 mins, 60 mins, 90 mins, 2 hours, 4 hours and 6 hours post-dose.

Samples <i>(Identified as Subject, Time, Period)</i>	Subject Sample ID	No. of Samples
Specified in protocol to be received	-	900
Subject withdrew after 1 st dose so following samples not received	Subject 114	40
Total number of study samples received	-	860
Total number of study samples analysed		860

All study samples will be retained for at least three months after issue of this report. After this period the Sponsor will be contacted for further sample retention or disposal instructions.

2.2.4. Sample Analysis

All samples, for a given subject, were analysed together in a single batch except when samples had to be reanalysed. A batch, at a minimum, consisted of duplicate sets of calibration standards (consisting of a blank, a zero standard and at least 6 different non-zero standards) and duplicate low, medium and high concentration QC samples (equal to at least 5% of the number of unknown samples in the batch).

2.2.5. Incurred Sample Reproducibility

Approximately 10% of the analysed samples up to 1000 were selected for reanalysis. Half of the samples were randomly selected. The remainder were selected from the C_{max} region and from near the end of the elimination phase.

3. RESULTS

A summary of analysis batches performed in this study is presented ([Table 1](#)). There were no rejected batches.

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

All temperatures referenced in this report are nominal temperatures.

3.1. Batch Acceptance Criteria

An analysis batch was considered acceptable if the following criteria were met:

- The standard curve was constructed from at least 11 of the standard samples, excluding the zero concentration standards. The back calculated concentrations for the standard samples must be within $\pm 15\%$ of the actual value, except at the lower limit of quantification where $\pm 20\%$ is acceptable.
- The accuracy of at least two thirds of the quality control concentrations had to be within $100 \pm 15\%$. Half of the quality control samples at each concentration had to be within $100 \pm 15\%$.
- At least half of the blank samples with internal standard and half of the blank samples without internal standard, placed immediately before the calibration standards, had to be free of interference. Overall two thirds of the total number of blank samples had to be free of interference. Interference is defined as a detectable response, at the retention time of the analyte, greater than 20% of the mean response of the lowest concentration (LLOQ) standards.

All 19 of the analysis batches performed in this study met the acceptance criteria.

3.2. Quality Control Sample Analyses (Inter-Batch Precision and Accuracy)

Inter-batch precision (CV) and accuracy results for QC samples prepared at low, medium and high QC concentrations are summarised in [Table 2](#). Precision was less than or equal to 3.8% and mean accuracy ranged from 98.9% to 107.2%.

3.3. Calibration Standard Concentrations

Back-calculated calibration curve standard concentrations are provided in [Table 3](#). Mean accuracy ranged from 97.6% to 100.9%.

3.4. Standard Curve Parameters

Standard curve parameters from 19 successful analytical batches are provided in [Table 4](#). The correlation coefficient (r) was greater than or equal to 0.9995. A representative calibration curve is shown in [Figure 1](#).

3.5. Study Sample Concentrations

Study samples with determined concentrations below that of the LLOQ of the standard curve are reported as <0.500 ng/mL as they were found to be BLQ ("Below the Limit of Quantification").

Study sample concentrations are provided in [Table 5](#).

3.6. Reanalyses

3.6.1. Incurred Sample Reproducibility

The incurred sample repeat analysis results are shown in [Table 6](#). The differences between the original and repeat results for all 88 of the samples reanalysed were within 20% of the mean of the original and repeat result. The acceptance criterion for incurred sample

reanalysis, which states that the differences for two thirds of the reanalysed samples have to be within 20% of the mean of the original and repeat result, was therefore met.

3.6.2. Reanalyses for Analytical Reasons

After initial analysis, study samples that were identified for reanalysis due to analytical reasons, were reanalysed singly if sufficient sample volume remained. These samples are identified in [Table 7](#).

4. RAW DATA AND CHROMATOGRAMS

The individual peak areas and result tables for all accepted analysis batches are presented in [Appendix 5](#). Chromatograms from a minimum of 5% of the subjects are provided in [Appendix 6](#) which contains all the chromatograms from analysis batch 1; batch ID 20180208CF1.

5. COMMENTS AND NOTES

There was no protocol or significant SOP deviations.

6. ARCHIVES

All raw data, associated data, and a copy of the final report and study file are archived at ABS Laboratories according to the SOP in effect during the conduct of the study.

7. COMPUTER APPLICATION PROGRAMS

Computer application programs used to acquire and derive data for this study included NuGenesis 7.1 (Waters), AB Sciex Analyst[®] 1.6.1 (for API 4000), Waters Vision Publisher 7.1 SR6 and Microsoft[®] Excel.

8. REFERENCES

1. V/Nic/HP/A: The Partial Validation of an LC-MS/MS Method for the Determination of Nicotine in Human Plasma using Microtitre Injection Plates. Study Director: Paul Baker.
2. V/NIC/HP: The Validation of an Analytical Method for the Determination of Nicotine in Human Plasma using LC-MS/MS. Study Director: Laura McMeekin.
3. Guidance for Industry – Bioanalytical Method Validation (CDER, May 2001).
4. Guideline on bioanalytical method validation. 21 July 2011. EMEA/CHMP/EWP/192217/2009. Committee for Medical Products for Human Use (CHMP).
5. SOP No. 5-85.4: Determination of Nicotine in Human Plasma by LC-MS/MS.

TABLES

Table 1. Summary of batches performed for nicotine in human plasma

(b) (4)



Table 2. Quality control sample data (inter-batch accuracy and precision) for nicotine in human plasma

(b) (4)



Failed acceptance criteria, included in statistics

Acceptance criteria for individual QCs:
Accuracy for QCs within $100 \pm 15\%$

Table 3. Back-calculated calibration standard concentrations for nicotine in human plasma

(b) (4)

A large black rectangular redaction box covers the majority of the page, obscuring the data for Table 3. The text "(b) (4)" is written in red at the top left corner of this redacted area.

Acceptance criteria for individual calibration standards:

Accuracy at LLOQ within 100 ±20%

Accuracy at all other concentrations within 100 ±15%

Table 4. Standard curve parameters for nicotine in human plasma

(b) (4)



Table 5. Concentrations of nicotine in SM 17-03 study human plasma samples

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



Table 6. Incurred sample reproducibility for nicotine in human plasma

(b) (4)

A large black rectangular redaction box covers the majority of the page content below the caption. The text "(b) (4)" is written in red at the top left corner of this redacted area.

(b) (4)



(b) (4)



(b) (4)

Acceptance criterion

The differences for two thirds of the reanalysed samples must be within $\pm 20\%$ of the mean of the original and repeat result.

$$\text{Difference} = \frac{\text{Repeat} - \text{Original}}{\text{Mean of Repeat and Original}} \times 100\%$$

Table 7. Summary of reanalyses for analytical reasons for nicotine in human plasma

(b) (4)



Total number of reanalyses for analytical reasons: 13

(b) (4)



APPENDICES

Appendix 1. List of abbreviations

<i>List of Abbreviations</i>	
ABS	Advanced Bioanalytical Service
AB	Applied Biosystems
API	atmospheric pressure ionisation
°C	degree Celsius (centigrade)
CDER	Center for Drug Evaluation and Research
CHEM	Testing of Chemicals
CHMP	Committee for Medical Products for Human Use
CSR	Compound storage record
CV	coefficient of variation (relative standard deviation)
EDTA	ethylenediaminetetraacetic acid
EMA/EMEA	European Medicines Agency
EWP	Ethics Working Party
ENV	Environment
FDA	US Department of Health and Human Services Food and Drug Administration
g	gram
GLP	Good Laboratory Practice
HP	Human Plasma
HPLC	high performance liquid chromatography
ID	identification
ISR	incurred sample reanalysis
L	litre
LC	liquid chromatography
LLOQ	lower limit of quantification
MC	Member Country
mg	milligram
mins	minutes
mL	millilitre
MRM	multiple reaction monitoring
MS	mass spectrometry
MW	molecular weight
n	number of data
ng	Nanogram
NIC	Nicotine
No.	number
OECD	Organization for Economic Cooperation and Development
QC	quality control
r	correlation coefficient
SI	Statutory Instrument
SOP	standard operating procedure
STD/Stds	standard
Stds	calibration standards
U.S./US	United States of America
V	Validation

Appendix 2. Analytical protocol



ABS Laboratories Ltd
BioPark
Broadwater Road
Welwyn Garden City
Herts AL7 3AX
United Kingdom
www.abslabs.com
Tel: +44 (0) 1707 358666
Fax: +44 (0) 1707 358667

Analytical Protocol

Study Number ABS/10/18

**Determination of Nicotine in Human Plasma Samples from Swedish Match Clinical
Protocol No. SM 17-03**

Sponsor: Swedish Match
SE-Box 17037
104 62 Stockholm,
Sweden

Sponsor's Clinical Protocol Number: SM 17-03, Final 1.0 05Oct2017

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18



SIGNATURE PAGE

ABS Laboratories

Approved by the Study Director, (b) (4), (b) (6)

Signature..... (b) (4), (b) (6)

Date 26-Jan-2018

Approved by the Laboratory & QA Manager, (b) (4), (b) (6)

Signature..... (b) (4), (b) (6)

Date 26-JAN-2018

Swedish Match

Only subjects who give their informed consent will participate in the clinical trial. ABS Laboratories will be informed, as soon as practicable, of any subjects who withdraw their consent and do not wish any samples taken so far to be analysed.

The version of the clinical protocol specified on the title page is the most recent version and ABS Laboratories will be supplied with any updates or amendments and that these amendments will have received the necessary regulatory approvals (where applicable). I have reviewed this analytical protocol and confirm that the work described does not exceed or contradict the requirements set out in the clinical protocol. I will inform ABS Laboratories if relevant sections in the clinical protocol are updated or amended.

Sponsor, (b) (4), (b) (6)

Signature..... (b) (4), (b) (6)

Date 26-Jan-2018

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18

 **ABS**
LABORATORIES

1 CONTACT DETAILS

Study Director

Test Facility Management

Sponsor's Study Monitor

Receipt of Data

(b) (4), (b) (6)

2 QUALITY STATEMENT

This study will be conducted to the standards described in the United Kingdom Good Laboratory Practice Regulations (SI 1999 No. 3106 as amended SI 2004 No. 994) and the OECD Principles of Good Laboratory Practice 1997 (ENV/MC/CHEM(98)17).

This study will also be conducted in compliance with the United Kingdom "The Medicines for Human Use (Clinical Trials) Regulations", (SI 2004 No. 1031 and subsequent amendments).

Study based inspections will be carried out on this study by ABS Laboratories Quality Assurance as follows:

1. The experimental phase inspection for this study will be the analysis of one batch of samples.
2. Raw data to final results tables will be audited prior to the issue of any final results to the sponsor.
3. The analytical report and the study file will be audited.
4. Various aspects of this study may also be audited during process audit inspections.
5. Inspections and audits will be carried out by personnel independent of the staff involved in this study.

3 OBJECTIVE

The determination of nicotine in human plasma samples from Swedish Match Protocol No. SM 17-03 entitled "Nicotine pharmacokinetics and subjective effects of a single dose of a non-tobacco-based nicotine pouch (ZYN®) compared with conventional, tobacco-based Swedish snus among current, daily snus users."

4 SCHEDULE

Anticipated Experimental Start Date: January 2018

Anticipated Experimental Completion Date: February 2018

Anticipated Draft Report Date: March 2018

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18

 **ABS**
LABORATORIES

5 EXPERIMENTAL

5.1 Test Method

Concentrations of nicotine will be determined in the samples using an analytical method developed and validated by ABS Laboratories^{1,2}. The method is documented in SOP No. 5-85.4³ (and subsequent amendments).

5.2 Reference Compounds

(b) (4)



Other appropriate in-date batches of reference compounds may be used. Full details including certificates of analysis of the actual reference compounds used will be included in the final report.

5.3 Internal Standard

(b) (4)



Other appropriate in-date batches of compound may be used. Full details including certificates of analysis of the actual reference compounds used will be included in the final report.

5.4 Biological Matrix

Blank human plasma, with lithium heparin as anticoagulant, will be supplied from healthy volunteers by ABS Laboratories. Human plasma (lithium heparin), free of significant interference, will be used to prepare calibration standards and quality control (QC) samples.

5.5 Test Samples

This is not a blinded study.

Number of samples expected: Approximately 900 human plasma samples.

Study design: 18 subjects are expected to receive the following:
1 = ZYN Smooth containing 3 mg nicotine per portion

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18



2 = ZYN Smooth containing 6 mg nicotine per portion

3 = ZYN Smooth containing 3 mg nicotine per portion
(alternative manufacturing process)

4 = ZYN Smooth containing 6 mg nicotine per portion
(alternative manufacturing process)

5 = Swedish portion snus PSWL 1.0 g (8 mg nicotine/g)

Blood samples are scheduled to be taken at the following times:

Pre-dose (0), 5 mins, 10 mins, 15 mins, 30 mins, 60 mins, 90 mins, 2 hours, 4 hours
and 6 hours post-dose.

All samples received will be analysed.

Samples were received with frozen ice packs and are stored at a nominal
temperature of -20°C, until analysis.

5.6 Calibration Standards and Quality Control (QC) Samples

Calibration standards will be prepared and used which contain the following nominal
concentrations, in pooled human plasma:

Concentration of nicotine, ng/mL							
0.00	0.500	1.00	2.00	5.00	10.0	20.0	50.0

Calibration standards may be prepared in bulk and stored in portions under
conditions of known stability. Calibration standards at each concentration will be
analysed in duplicate in each analysis batch.

Quality control (QC) samples will be used which contain the following nominal
concentrations, in pooled plasma:

Concentration of nicotine, ng/mL		
1.50	15.0	40.0

If the concentrations of the QC samples prove to be inappropriate, then QC samples
at additional concentrations will be prepared, including a dilution QC sample if
required. Quality control samples will be stored in portions at a nominal temperature
of -20°C. Duplicate quality control samples will be included in each analysis batch.

5.7 Analysis Batches

Study samples will be analysed in separate uniquely labelled batches. All the
samples from a subject will be analysed in the same analysis batch in time profile
order. Each analysis batch will include the following:

1. A blank sample without IS (double blank)
2. A set of calibration standards (including single blank)
3. A carry-over blank
4. A QC sample
5. Study samples with QC samples interspersed
6. A QC sample
7. A blank sample without IS (double blank)
8. A set of calibration standards (including single blank)
9. A carry-over blank

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18



Initially the samples will be analysed once. If the sample volume is small or the sample concentration is believed to be over-range, the initial analysis may be done on diluted sample.

Samples may be re-analysed for the reasons listed in SOP 1-14.

5.8 Incurred Sample Reproducibility

To investigate incurred sample reproducibility 10% of the analysed study samples up to 1000 will be reanalysed. As 860 samples were received, 86 samples will be reanalysed. These samples will be selected and reported according to ABS SOP 1-14.

5.9 Data Handling

Applied Biosystems Analyst 1.6.1 will be used for peak integration and for calculation of concentrations. The concentrations of the analyte in the samples will be determined using a weighted least squares ($1/x^2$) linear regression on the peak area ratios from the calibration standards. The purity of the test material will be taken into account. Zero concentration calibration standards will not be included in the construction of the standard curve. Concentrations will be reported to 3 significant figures.

6 ANALYSIS BATCH ACCEPTANCE CRITERIA

The calibration standards must have a back-calculated accuracy within $100 \pm 15\%$, except at the lower limit of quantification (LLOQ) where it must be within $100 \pm 20\%$. The standard curve must be constructed from at least three quarters (i.e. 12) of the calibration standards, excluding the zero concentration calibration standards. If both of the calibration standards at the lower limit of quantification are rejected then this particular analytical batch will have a raised LLOQ, corresponding to the lowest acceptable calibration standard. Samples with determined concentrations below the raised LLOQ will be re-analysed, if sufficient sample remains.

Duplicate quality control samples at low, medium and high concentrations will be included in each analysis batch. The accuracy of at least two thirds of the quality control samples must be within $100 \pm 15\%$. Half of the quality control samples at each concentration must be within $100 \pm 15\%$.

At least half of the blank samples with internal standard and half of the blank samples without internal standard, placed immediately before the calibration standards, must be free of interference. Overall, two thirds of the total number of blank samples must be free of interference. Interference is defined as a detectable response, at the retention time of the analyte, greater than 20% of the mean response of the lowest concentration (LLOQ) standards.

7 RECORDS

Documentation at the test facility will include, but not be restricted to, the following records:

1. Sample receipt records
2. Weighing and solution preparation records
3. LC-MS/MS conditions
4. Chromatograms
5. Raw data entered on study specific proforma
6. Derived data

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18



7. Plasma control data
8. Calibration data

8 REPORTING

The sponsor will be provided with a draft report in order to make comments. The report will include, but will not be restricted to, the following:

1. The title of the study
2. The objectives stated in the approved protocol
3. The identity of the reference compounds including details of their origin, purity and stability
4. The name and address of the sponsor
5. The name and address of the test facility and the dates on which the study was started and completed
6. The signature of the Study Director accepting responsibility for the validity of the report
7. Any unforeseen circumstances, which may have affected the quality or integrity of the study
8. A description of the method and materials used
9. A summary of results
10. Results including QC data, calibration data and determined concentrations in the study samples
11. A discussion of the results
12. The location of all the raw data and the final report
13. Data on the analytical method conditions used including representative chromatograms from a minimum of 5% of the subjects
14. A Quality Assurance statement

When the sponsor's comments are received a final report will be produced. The sponsor will receive one bound and one electronic (PDF) copy of the final report. Although procedures are in place to make sure that the PDF copy of the final report is a true copy the insertion of the signatures does not comply with 21CFR.11.

9 PATIENT SAFETY AND EXPEDITED REPORTING OF ANOMALOUS RESULTS

Since the samples are being analysed after the administration to each subject is complete, it is not considered necessary to expedite the reporting of any anomalous results.

If urgent analysis of any samples is required due to an unexpected patient safety issue, then such samples may be analysed outside of the requirements of this protocol, if meeting the requirements of the protocol would cause an unacceptable delay. The requirements for such analysis and the reporting of results will be agreed with the sponsor when the request for the urgent analysis is made.

10 ARCHIVE

The study file, containing all the study raw data and a copy of the final report, will be archived within 3 months of issuing the final report. After approximately two years, the study file will be transferred to the sponsor for storage. Some of the items will be supplied electronically on CD in PDF format. When the study file has been transferred to the sponsor, ABS Laboratories will only have a copy of the clinical protocol and the final analytical report in its GLP archive.

Determination of nicotine in human plasma samples
ABS Analytical Protocol ABS/10/18



The remaining test samples and stock solutions will be stored under appropriate conditions and retained for 3 months after submission of the final report. At this time the sponsor will be contacted to ascertain whether continued storage is necessary. ABS Laboratories reserves the right to charge for sample storage after this time.

11 REFERENCES

- 1 Validation Report: V/NIC/HP/A - The Partial Validation of an LC-MS/MS Method for the Determination of Nicotine in Human Plasma using Microtitre Injection Plates. Study Director: Paul Baker.
- 2 Validation Report: V/NIC/HP – The Validation of an Analytical Method for the Determination of Nicotine in Human Plasma using LC-MS/MS. Study Director: Laura McMeekin.
- 3 SOP-5-85.4: Determination of Nicotine in Human Plasma by LC-MS/MS.
- 4 SOP 1-14: Guidelines for Performing Repeat Analyses.

Appendix 3. Certificate of analysis for nicotine (ABS CSR No. 18003)

(b) (4)

A large, solid black rectangular redaction box covers the majority of the page content, starting below the section header and ending above the footer. The text "(b) (4)" is written in red at the top left corner of this redacted area.

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



Appendix 4. Certificate of analysis for nicotine-d₄ (ABS CSR No. 18001)

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



Appendix 5. Result tables

The batch ID is given at the top left hand corner of the page for each table.

(b) (4)

A large, solid black rectangular redaction box covers the majority of the page content, starting below the text '(b) (4)' and extending nearly to the bottom and right edges of the page.

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



Appendix 6. Chromatograms

Chromatograms from batch 1 batch ID 20180208CF1 of ABS Laboratories study ABS/10/18.

Within each pair of chromatograms, the analyte is shown on the left and the internal standard is shown on the right. Each chromatogram has a sample name and sample ID at the top left hand side. The result table for these chromatograms is shown on pages [65](#) and [66](#).

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)

