FAPAS® Report 3035

Acrylamide in Crispbread

July-August 2012

PARTICIPANT LABORATORY NUMBER

Participants can log in to FAPAS SecureWeb at any time to obtain their laboratory number for this proficiency test.

Laboratory numbers are displayed in SecureWeb next to the download link for this report.

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SUMMARY

1. The test material for FAPAS® proficiency test 3035 was dispatched in July 2012. Each participant received a crispbread test material to be analysed for acrylamide.

2. An assigned value (x_a) was determined for acrylamide and in conjunction with the standard deviation for proficiency (σ_p) was used to calculate a z-score for each result.

3. Results for this proficiency test are summarised as follows:

analyte	assigned value, x_a µg/kg	number of scores, |z| ≤2	total number of scores	% |z| ≤2
acrylamide	299.2	48	53	91

4. Surplus test materials are available for sale, see APPENDIX II.

CONTENTS

1. INTRODUCTION

1.1. Proficiency Testing

Proficiency testing aims to provide an independent assessment of the competence of participating laboratories. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

Further details of the FAPAS® proficiency testing scheme are available in our protocols [3, 4].

2. TEST MATERIAL

2.1. Preparation

Preparation of the samples for this proficiency test was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [2].

The test material was prepared from a commercially available dark rye crispbread.

All analytes were present at natural levels in the test material.

Samples were stored at -20°C until dispatch.

2.2. Homogeneity

To test for homogeneity, randomly selected test materials were analysed in duplicate. Testing was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [2].

These data showed sufficient homogeneity and were not included in the subsequent calculation of the assigned values.

2.3. Dispatch

The start date was 10 July2012. Test materials were sent to 61 participants.

3. RESULTS

The instructions for reporting results were as follows:

Determine the level of acrylamide present in the test material, in **µg/kg**, as received.
PLEASE NOTE:

- Indicate whether or not you added a **labelled internal standard** at the outset (Y/N).
- If you have NOT added a labelled internal standard at the outset please report your **recovery (%)** AND indicate whether or not the recovery figure was **applied** to the result (Y/N).

Results were submitted by 53 participants (87%) before the closing date for this test, 21 August 2012.

Each participant was given a laboratory number, assigned in order of receipt of results. The reported analyte concentrations are given in Table 1.

Participants' comments are given in Table 2.

The analytical methods used by each participant are summarised in APPENDIX I.

4. STATISTICAL EVALUATION OF RESULTS

The results submitted by participants were statistically analysed in order to provide an assigned value for each analyte. The assigned values were then used in combination with the standard deviation for proficiency, σ_p, to calculate a z-score for each result. The procedure follows that recommended in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

Further details on the procedure followed can be found in the relevant protocols [3, 4].

4.1. Calculation of the Assigned Value, x_a

The assigned value, x_a, for acrylamide was derived from the consensus of the results submitted by participants.

The following results were excluded from the calculation of the assigned value:

i) results reported as approximately 10, 100 or 1000 × greater or smaller than the majority of submitted results (as these were considered to be reporting errors),

ii) results uncorrected for recovery.

For acrylamide, this procedure was straightforward and the robust mean was chosen as the assigned value.

The assigned values for all analytes are shown in Table 3.

4.2. Standard Deviation for Proficiency, σ_p

The standard deviation for proficiency, σ_p, was set at a value that reflects best practice for the analyses in question.

For analytes, σ_p was derived from the appropriate form of the Horwitz equation [6].

The values for σ_p used to calculate z-scores from the reported results of this test are given in Table 3.

4.3. Individual z-Scores

Participants' z-scores were calculated as:

$$z = \frac{(x - x_a)}{\sigma_p}$$

where x = the participant's reported result,
x_a = the assigned value
and σ_p = the standard deviation for proficiency.

Participants' z-scores for acrylamide are given in Table 1 and shown as a histogram in Figure 1. It is possible for the z-scores published in this report to differ slightly from the z-score that can be calculated using the formula given above. These differences arise from the necessary rounding of the actual assigned values and standard deviations for proficiency prior to their publication in Table 3.

The number and percentage of z-scores in the range $-2 \leq z \leq 2$ for all analytes are given in Table 4.

5. INTERPRETATION OF SCORES

In normal circumstances, over time, about 95% of z-scores will lie in the range $-2 \leq z \leq 2$. Occasional scores in the range $2 < |z| < 3$ are to be expected, at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

The consideration of a set or sequence of z-scores over time provides more useful information than a single z-score. Examples of suitable methods of comparison are provided in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

6. REFERENCES

1 Adobe Certified Document Services, http://www.adobe.com/security/partners_cds.html, accessed 08/05/2012.

2 ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing.

3 FAPAS, 2012, Protocol for Proficiency Testing Schemes, Part 1 – Common Principles, Version 3, Issued January 2012.

4 FAPAS, 2012, Protocol for Proficiency Testing Schemes, Part 2 – FAPAS®, Version 2, Issued January 2012.

5 Thompson, M., Ellison, S.L.R. and Wood, R., 2006, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, **78**, No. 1, 145–196.

6 Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, *Analyst*, **125**, 385-386.

Table 1: Results and z-Scores

laboratory number	analyte			
	acrylamide assigned value 299.2 µg/kg			
	result µg/kg	labelled internal standard used (Y/N)	if appropriate state % recovery & if applied (Y/N)	z-score
001	416.8	Y		2.0
002	323	N	98.7 %, N	0.4
003	280	Y	Y	-0.3
004	352	N	104 (N)	0.9
005	294	Y	N	-0.1
006	278	Y	100	-0.4
007	366	Y	140% Y	1.2
008	326	y	101 - N	0.5
009	314	Y	100% / N	0.3
010	293	Y	104% (N)	-0.1
011	325	Y	95/N	0.5
012	315	Y	N	0.3
013	322.2	y	n	0.4
014	274	Y	N	-0.4
015	266	y	N	-0.6
016	303	Y	Y	0.1
017	278	Y	N	-0.4
018	338	y		0.7
019	298.3	N	N	0.0
020	99.5	n	n	**-3.5**
021	550	n	y	**4.4**
022	314	Y	Y	0.3
023	279.4	Y		-0.3
024	301	Y	102%, N	0.0
025	326	Yes	No	0.5
026	229	Y		-1.2
027	290	Y	Y	-0.2
028	336.0	Y		0.6
029	288	Y	N	-0.2
030	310	yes		0.2

z-scores outside |z| >2 are shown in **bold,** see Section 5

Table 1 (continued): Results and z-Scores

laboratory number	analyte			
	acrylamide assigned value 299.2 µg/kg			
	result µg/kg	labelled internal standard used (Y/N)	if appropriate state % recovery & if applied (Y/N)	z-score
031	282.67	Y	N	-0.3
032	340.0	Y		0.7
033	294.7	Y	Y	-0.1
034	97	Y		**-3.5**
035	334	Y		0.6
036	275.57	Y		-0.4
037	320	Y	N	0.4
038	431	Y		**2.3**
039	200	y	106%, y	-1.7
040	303.88	Y	40	0.1
041	303.76	Y	N	0.1
042	280.0	Y		-0.3
043	286	Y	N	-0.2
044	300	yes	109	0.0
045	276	Y	N	-0.4
046	283.76	Y	N	-0.3
047	282	Y	N	-0.3
048	288	Y		-0.2
049	272.30	y	n	-0.5
050	293	Y	74 % (Y)	-0.1
051	255	Y	N	-0.8
052	460	Y	N	**2.8**
053	300	Y	89%, N	0.0

z-scores outside |z| >2 are shown in **bold,** see Section 5

Table 2: Participants' Comments

participant number	comments
025	No % recovery was calculated
031	Standard addition
039	was used kit Acrylamide ELISA Kit (Abraxis)

comments are as submitted by participants

Table 3: Assigned Values and Standard Deviations for Proficiency

analyte	data points, n	assigned value, x_a, µg/kg	uncertainty, u	standard deviation for proficiency, σ_p, µg/kg	
acrylamide	48	299.2	4.32	Horwitz [6]	57.4

Table 4: Number and Percentage of z-Scores where |z| ≤2

analyte	number of scores where |z| ≤2	total number of scores	% |z| ≤2
acrylamide	48	53	91

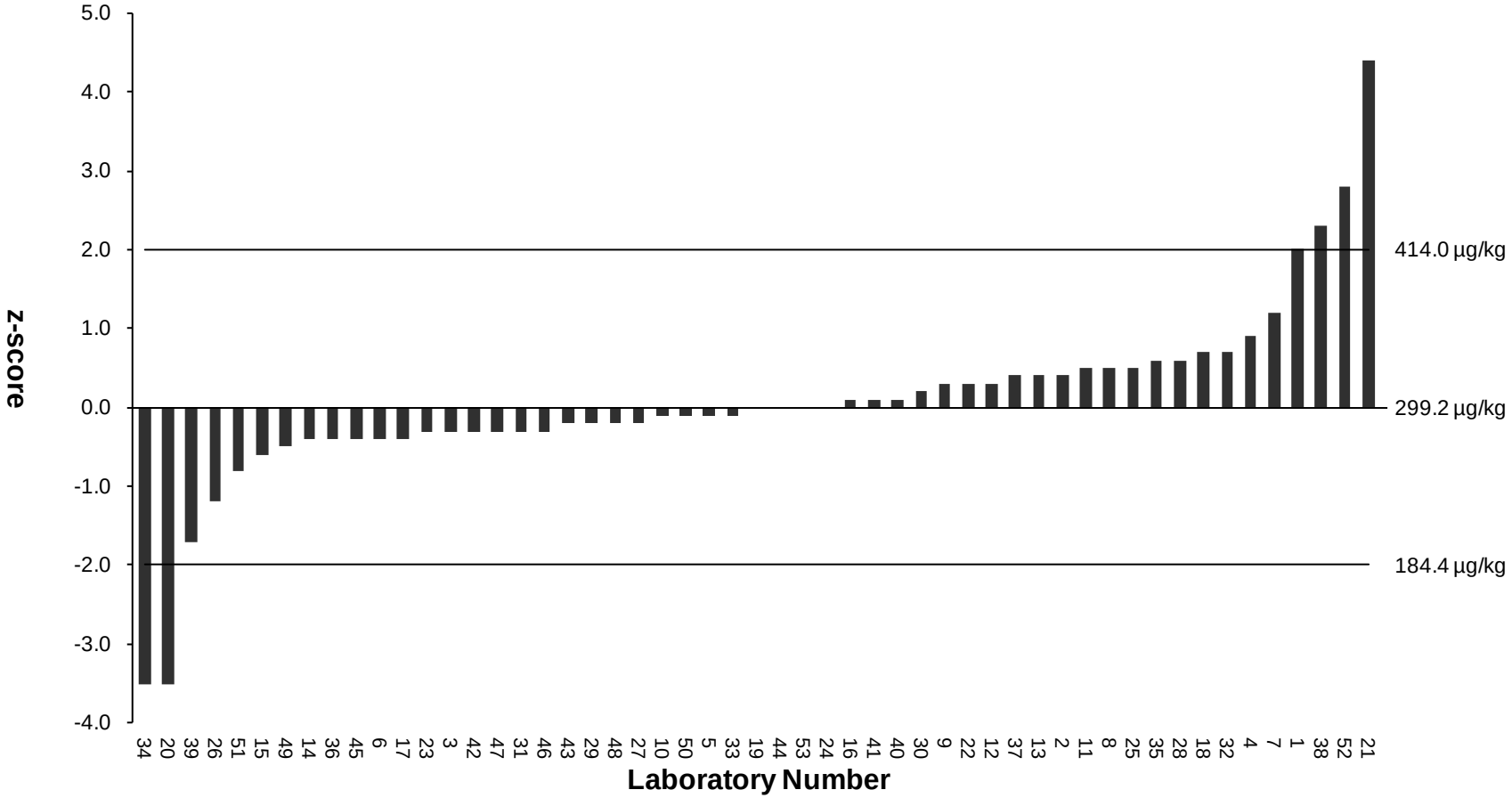

Figure 1: z-Scores for Acrylamide

APPENDIX I: Analytical Methods Used by Participants

Methods are tabulated according to the information supplied by participants, but some responses may have been combined or edited for clarity.

Is The Method Used Accredited?	laboratory number
yes	001 002 006 007 009 010 011 012 013 014 023 024 026 027 028 029 032 033 035 036 037 040 041 043 044 045 047 048 049 050 052 053
no	004 015 016 017 018 020 031 038 039 051

Reference	laboratory number
Agro Food 2010 V21 n°5	017
Analytica- Chemica Acta 2004 520 207-215	027
Analytica Chimica Acta 2004 520 207-215	006
BfR 2002	047
Czech Journal of Food Science 2004 22 290	014
EPA Method 2005 10 860	048
FDA Draft 2004	015
FDA method	045
Food Additives and Contaminant 2003 20(3) 215-220	032
Food Additives and Contaminant 2003 20(3) 215-220	038
FSA-funded work conducted by CSL (GC-MS method) 2001	004
GIT Labor-Fachzeitschrift 2005 10 860	048
in house method	031
IRMM 2005 IRMM	033
J. Agric Food CHem 2006 54 7001-7008	028
J. Agric. Food Chem. 2003 51 7547	029
J. AOAC Int. 2004 87(1) 107-115	018
J. Chromatography 2008	044
J. Chromatography A 2006 1120 194-198	024
J. Chromatography A 2006 1132 211	001
journal of food composition and analysis 2009 22 142-147	052
KFDA Method(Republic of Korea) 2011	041
KFDA Notification No. 2007-10 2007 9-11	049
LMBG Method	026

Reference (continued)	laboratory number
Mitt. Gebiete Lebensm. Hyg. 2002 638-652	012
U.S Food and Drug Administration 2003 Detection and Quantitation of Acrylamide in Foods June 20, 2002; Updated July 23, 2002 and February 24, 2003. Draft	040
Waters 2002	007

Sample Weight (g)	laboratory number
<1	007 009 034 036
≥1 - <2	012 015 016 018 024 028 029 031 037 040 041 052
≥2 - <5	001 004 006 010 011 013 027 032 033 035 039 043 045 047 049 050 051 053
≥5 - <10	014 017 020 023 026 038 044 048
≥50	002

Internal Standard Added	laboratory number
1,2,3-13C-acrylamide	013 014 015 016 018 033 034 037 040 041 043 049 051
d3-acrylamide	001 006 007 009 010 011 017 023 024 026 027 028 029 031 032 035 036 038 044 045 048 050 052 053
labelled internal standard	012
methacrylamide	004 047
none	002
the kit includes internal control (solution of acrylamide)	039

Point at which internal standard was added	laboratory number
prior to extraction	001 006 007 009 011 012 013 014 016 017 018 023 024 026 027 028 029 031 032 033 035 036 037 038 040 041 043 044 045 047 048 049 051 052
ELISA method was used; internal control are derivatized and analysed together with samples and calibrate standarts	039
Prior to bromination	004
prior to injection into LC-MS/MS	050
prior to instrument analysis	053

Extraction Solvent Components	laboratory number
acetonitrile	020 023 028 031 047 051
hexane	031 051
methanol	012 024
sodium chloride solution	036
water	001 002 004 006 007 009 010 011 012 013 014 015 016 017 018 024 026 027 029 031 032 033 035 037 038 039 040 041 043 044 045 048 049 050 051 052 053
formic acid	010 018 052

Extraction Procedure	laboratory number
add NaCl	028 036 047
blend / homogenise with solvent	004 032 038 041
cold water extraction	002 004 010 015 047 048
hot water extraction	006 013 035
maceration / homogenisation	014 043
shake with solvent	007 016 017 024 028 031 037 039 040 047 049 051
shaking	001 009 012 015 053
sonicate/ultrasonic bath	006 009 026 035 045
Ultra Turrax	018 023 029 033 050
ultrasonic extraction	011 027 044
vortex mix	009 015 031 052

Sample Work Up	laboratory number
Carrez I & II	006 011 014 023 024 027 035 044 048 052
centrifuge	001 002 006 007 009 010 011 012 014 015 018 024 026 028 029 031 032 033 035 036 037 038 039 040 041 045 047 048 049 050 051
defatted with hexane	011 014 047 053
dry over Na_2SO_4	047 051
evaporate	031
filter	002 004 006 010 018 029 037 041 050
none	013 016
pH adjustment	014
defatted with heptane	017

Sample Clean-up Technique	laboratory number
Extrelut	017
filter	006 041 044 050
florisil column	043
liquid/liquid extraction	013 014 023 028
solid phase extraction (SPE) (column/cartridge)	001 007 009 012 015 016 026 029 032 033 035 036 037 038 039 040 045 049 052
solid phase extraction (SPE) (dispersive)	020
none	002 004 011 018 024 027 048 053
magnesium sulphate/sodium chloride	031
PSA	047

Acrylamide Determination	laboratory number
GC (derivatised)	004 013 014 038 048
GC (underivatised)	017 035 047
LC	001 002 006 007 009 010 011 012 015 016 018 020 023 024 026 027 028 029 031 032 033 036 037 040 041 044 045 049 050 051 052 053
ELISA (with derivatization procedure)	039
LC/MS/MS	034

Acrylamide Detection	laboratory number
MS	002 004 013 017 035 038 043 047 048
MS-MS	001 006 007 009 010 011 012 014 015 016 018 020 023 024 026 027 028 029 031 032 033 034 036 037 040 041 044 045 049 050 051 052 053
ELISA (with derivatization procedure)	039

GC Column Packing	laboratory number
50% methyl 50% phenyl polysiloxane	004 013 014 038
95% methyl 5% phenyl polysiloxane	048
polyethyleneglycol (PEG) (C2H6O2)	017
FFAP	035
Innowax	047
not applicable (ELISA was used)	039

GC Column Length (capillary) (m)	laboratory number
≥25 - <30	013
≥30 - <50	004 014 038 043 047 048
≥50 - <100	017 035

GC Column Film Thickness (µm)	laboratory number
≥0.25 - <0.75	004 013 014 017 035 038 043 047 048

GC Injector Temperature (°C)	laboratory number
≥100 - <150	038
≥200 - <250	004 013 014
≥250 - <300	017 035 043 047 048

GC Transfer Line Temperature (°C)	laboratory number
230	013
250	014 035
250° C	047
260 °C	048
275°C	017
280	038
300	004

GC Injection Volume (µL)	laboratory number
≥1 - <2	004 014 017 035 038 043 047
≥2 - <5	048
≥5 - <10	013

GC Injection Mode	laboratory number
splitless	004 013 014 017 035 038 043 047 048

GC Oven Temperature (°C)	laboratory number
programmed	004 013 014 017 035 038 043 047 048

Ions Monitored, m/z	laboratory number
106, 108, 150 and 152	004
106, 108, 150, 152, 155	013
149	043
150 / 152 and 153 / 155 (D3)	048
150, 152 (internal standard 153, 155)	038
152 -> 73, 154 -> 73, 149 -> 70, 151 -> 70	014
71, 85	047
72	002
72, 75, 55, 58	017
72, 89, 106; 75, 92, 109	035
72,55,27	040
72.1 75.1	023
72>55	016

HPLC Guard Column Used?	laboratory number
yes	001 007 011 018 020 026 027 028 029 031 032 033 037 041 045 051 052
no	002 009 010 012 015 016 023 024 036 040 044 047 049 050 053

HPLC Column Packing	laboratory number
C18	006 007 010 011 012 016 018 020 023 024 026 028 032 033 036 040 041 044 045 049 050 052 053
endcapped	018 050
ACQUITY UPLC HSS T3	051
Carbon	029
CN	027
HYPERCARB	001
Ion Exclusion	002
phenyl	031
synergi Hydro RP	009

HPLC Column Length (cm)	laboratory number
10	001 007 012 015 026 029 040 050 052 053
100	031
15	006 011 018 028 032 036 044 051
150	010 016 033
20	027
25	002 024 041 045 049
250	009
5	037
50	023

HPLC Column Diameter (mm)	laboratory number
0.46	045
2	010 018 024 036 040 041 044 049
2,1	037 051
2.1	001 012 016 023 029 032 050
3	006 011 015 027 028 031 033
4	002
4.6	007 009 026 053

HPLC Particle Size (µm)	laboratory number
1,8	051
1.7	040
1.8	012 023 050
3	001 006 007 010 016 026 028 031 032 033 036
3.5	053
4	009 011 018
5	015 024 027 029 037 041 044 045 049

HPLC Column Temperature (°C)	laboratory number
ambient	011 018 027 028 037 040 041 044 052
>ambient - <50	001 002 006 007 009 010 012 016 020 024 026 029 031 032 033 034 036 045 049 050 051 053
≥50	015 023

Mobile Phase Components	laboratory number
acetate	029 036
acetic acid	007 011 024 033 041 050
acetonitrile	001 002 009 010 011 023 024 033 051
formic acid	001 002 006 010 015 018 029 045 051 053
methanol	006 007 011 015 018 020 026 029 032 033 036 041 045 053
water	001 002 006 007 009 010 011 015 016 018 026 029 033 036 037 041 045 049 050 051 052 053
Aqueous 0.1% acetic acid, 0.5 % methanol	040
FA in water : FA in methanol	031
propan-2-ol	050
Water, 0.1% acetic acid	012
water/ Acetonitrile	027

Isocratic Mobile Phase?	laboratory number
yes	001 002 006 007 009 015 016 018 023 024 027 029 032 036 037 040 041 049 050 052
no (gradient)	011 012 020 026 031 033 044 045 051 053

Mobile Phase Flow Rate (mL/min)	laboratory number
<0.25	001 002 007 010 011 016 018 023 029 032 033 036 037 041 044 045 049
≥0.25 - <0.75	006 009 012 015 020 024 026 027 028 031 040 050 051 052 053

HPLC Injection Volume (µL)	laboratory number
<5	023
≥5 - <10	016 031 040 044 050 051
≥10 - <25	001 006 007 009 010 011 012 015 018 020 024 026 029 032 036 037 045 049 053
≥25 - <50	002 027 033 041 052
≥50 - <100	028

Acquisition Mode	laboratory number
electrospray positive	053
ES+	010 015
ESI	050
ESI pos.	011
ESI+	023 029 033
LC-MS/MS(Multi range ion monitor)	040
MRM	007 009 012 020 024 032 037 045 049 052
MRM ESI positiv	027
MRM positiv	006
MRM(+VE)	031
MS MS	051
MS/MS	018 036
POS	016
positive EI	044
SRM	001 041

Transitions Monitored	laboratory number
55	045
55,44	015
71.6>54.6 and 71.6>26.3	007
71.7>43.8, 71.7>54.7,71.7>71.7,75>58,75>75	029
71.80 > 27.00; 71.80 > 43.90; 71.80 > 55.10	033
71.9>55, 71.9>72.0	009
71>54(Target), 74>57(ISTD)	041
72 - 55; 75 - 58 (D3)	044
72 > 55	010
72 > 55 72>44	051
72 > 55 ,27	040
72 -> 55; 72 -> 71.8; 75 -> 58	050
72,55 75,58	052
72.0-54.9, 72.0-54.9, 72.0-44.0	011
72.07-55.13; 75.07-58.08	012
72.1>54.9, 72.1>72.1	053
72.1-55.1 72.1-44.2 75.1-58.1 75.1 29.9	023
72.2>55.1	001
72/55 72/44	006

Transitions Monitored (continued)	laboratory number
72/55 72/44 72/54	020
72/55 72/54	037
72/55, 72/44, 72/75	024
72/55; 72/72	027
72>55	031 032
72->55,1	026
72-55 75-58(IS)*	018
Acrylamide: m/z 72?55, IS: m/z 75?58	049
Internal Standard 74.8 - 58, Acrylamide 72 - 55	036
m/z:72,55;75,58 DP:51;46 CE:17;17	016

APPENDIX II: FAPAS SecureWeb, Protocol and Contact Details

1. FAPAS SECUREWEB

Access to the secure area of our website is only available to participants in our proficiency tests. Please contact us if you require a UserID and Password. FAPAS SecureWeb allows participants to:

- Obtain their laboratory numbers for the proficiency tests in which they have participated.
- View the results they submitted in past and current proficiency tests.
- Submit their results and methods for current tests.
- Review future tests they have ordered.
- Order proficiency tests and quality control materials.
- Freely download copies of reports (PDF file), of proficiency tests in which they have participated.

2. PROTOCOL

The Protocols [3, 4] set out how FAPAS® is organised. Copies can be downloaded from our website.

3. CONTACT DETAILS

This report was prepared and authorised on behalf of FAPAS by Dominic Anderson (Round Coordinator). Participants with any comments or concerns about this proficiency test should contact:

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