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VALIDATION OF AN ANALYTICAL METHOD TO DETERMINE THE CONTENT OF SUCRALOSE IN BEVERAGES

REPORT ON A METHOD VALIDATION BY COLLABORATIVE STUDY

I. DONCHEVA and JOERG STROKA

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VALIDATION OF AN ANALYTICAL METHOD TO DETERMINE THE CONTENT OF SUCRALOSE IN BEVERAGES

REPORT ON A METHOD VALIDATION BY COLLABORATIVE STUDY

Determination of Sucralose in Beverages by
Thin Layer Chromatography in Combination with
Reagent-free Derivatisation and
Ultraviolet/Fluorescence Detection

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Abstract

An inter-laboratory comparison was carried out to evaluate the performance characteristics of a method for the determination of sucralose in beverages, which was developed at the JRC-IRMM. The method is based on high-performance thin layer chromatography (HPTLC), and reagent-free derivatisation followed by ultraviolet/fluorescence detection. It was tested for the determination of Sucralose ($C_{12}H_{19}Cl_3O_8$; (2R,3R,4R,5S,6R)-2-[(2R,3S,4S,5S)-2,5-bis(chloromethyl)-3,4-dihydroxy-oxolan-2-yl]oxy-5-chloro-6-hydroxymethyl)oxane-3,4-diol; CAS No: 56038-13-2) in carbonated and still alcoholic and non-alcoholic beverages at proposed European regulatory limits according to Directive 2003/115/EC (1). Precise determination of Sucralose levels in some of the matrices for which European legislative limits apply, required a robust and reliable analytical method. HPTLC employing reagent-free derivatisation offered such a reliable but simple, fast, cost-effective and environment friendly method (very limited quantities of organic solvents methanol and acetonitrile were used). Separation of Sucralose was performed by direct application of samples (diluted, degassed and/or filtered, if necessary) on amino-bonded silica gel HPTLC plates without prior cleanup and development with acetonitrile:water. The sweetener was determined after heating of the developed plate to 190°C and quantified both in ultraviolet absorption and fluorescence measurement mode. Beverages spiked with sucralose as well as beverages taken from the market and labelled to contain sucralose, were sent to 14 laboratories in five different countries following IUPAC guidelines. A sample that did not contain measureable amounts of sucralose was spiked at levels of 30.5 mg/L, 100.7 mg/L and 299 mg/L. Recoveries ranged from 104 – 125 % with an average of 112 % for ultraviolet detection and from 98 – 101 % with an average of 100 % for fluorescent detection. Based on results for spiked samples (blind duplicates at three levels), as well as samples containing Sucralose (blind duplicates at three levels and one split level), the relative standard deviation for repeatability (RSD_r) ranged from 10 – 31 % for ultraviolet detection and from 9 – 16 % for fluorescence detection. The relative standard deviation for reproducibility (RSD_R) ranged from 14 – 31 % for ultraviolet detection and from 9 – 21 % for fluorescence detection. The limit of quantification on the basis of 10x the baseline noise was 6 mg/L and response was linear in the range between 30 – 150

ng/spot. The method is therefore considered suitable for the determination of Sucralose in beverages at the proposed European legislative limits.

Introduction

Sucralose was approved in the EU as a sweetener in food products according to Directive 2003/115/EC (1). It is a non-volatile substance, which does not contain a chromophore to facilitate detection. Methods elaborated for its determination involved high performance liquid chromatography with refractive index detection (2), high performance anion exchange chromatography with pulsed amperometric detection (3), and capillary electrophoresis with indirect ultraviolet absorbance (4). Another approach by high performance liquid chromatography with evaporative light scattering detection for the determination of multiple sweeteners, including Sucralose, had been described (5). That method was recently collaboratively tested. A promising alternative was the simple and fast high performance thin layer chromatographic method (6). It required very little or no sample preparation. Sucralose was separated on amino-HPTLC plates and heated at 190° C. At that temperature the substance reacted with the amino groups of the HPTLC layer (reagent free derivatisation) to form a brilliant yellow coloured spot of unknown structure that could be measured both in ultraviolet absorption and fluorescence mode. The method allowed Sucralose determination at the levels of interest regarding European legislation.

Test materials for the collaborative study

For this inter-laboratory comparison exercise the following products were purchased from food supermarkets or kindly provided by Sucralose producer Tate & Lyle Specialty Sweeteners (Reading, United Kingdom): various brands of energy-reduced water based flavoured drinks, juice drinks, spirit drinks containing less than 15% alcohol by volume, and food supplements in liquid form. All were labelled to contain Sucralose. Coke® Light was used as a blank material.
The type of the test materials is given in Table 1.

Table 1: Type of test materials

Test Material	Type
Blank	Energy-Reduced Beverage
Low Level	Energy-Reduced Food Supplement
Medium Level 1	Energy-Reduced Flavoured Water
Medium Level 2	Energy-Reduced Cloudy Fruit Juice
High Level	Energy-Reduced Low Alcohol Spirit Drink

Original products were degassed, if necessary, and similar products were mixed to obtain the levels as stated in Table 2.

Table 2: Target levels of Sucralose, mg/L, in test samples

Test Material	Sample	Sucralose, [mg/L]
Low Level (Blind Duplicates)	04; 08	42
Medium Level 1 (Split Level)	01; 03	69
Medium Level 2 (Blind Duplicates)	02; 06	105
High Level (Blind Duplicated)	05; 07	112

As spiking by each participant would require dispatching large volumes of blank material, the procedure was performed at the Institute of Reference Materials and Measurements. The Spiking Protocol can be found in the Annex.
Spiked levels are shown in Table 3.

Table 3: Levels of Sucralose, mg/L, in spiked samples

Test Material	Sample	Sucralose, [mg/L]
Low Level	A; E	30.5
Medium Level	B; D	100.7
High Level	C; F	299

The materials were subsequently filled into 4 mL glass vials with screw caps (3.2 mL in each vial) and kept at 4° C until dispatch for collaborative trial testing.

Split level samples

The two split level test materials (Sample 01 and Sample 03), nearly identical but of slightly different composition (≤5% difference in composition), were obtained by diluting one portion of the material (Sample Medium level 1) with a small amount of diluent (5% of water by volume). Both portions were supplied to the participating laboratories as test samples, each under a random code number, and each test sample was analyzed only once. These 2 test samples constitute a split level sample and should be considered Youden matched pairs (YP).

Table 4: Sucralose results from analysis of split level test material

Measurement Mode	Sample	Sucralose, [mg/L]	$y_c \geq 0.95x_c$ YES/NO
UV	Sample 01	73	YES
	Sample 03	71	
FL	Sample 01	69	YES
	Sample 03	66	

Results from Table 4 identify Sample 01 and Sample 03 as a Youden matched pair (YP).

Organisation of the collaborative study

The instructions for participants in the inter-laboratory comparison are given in the Annex of this report. A total of 14 collaborators from five different countries were invited to participate in the collaborative trial. 13 participants returned valid results. All names (in alphabetic order) and addresses of the participants are given in Table 5.

Table 5: List of participants who returned results (in alphabetic order) in the inter-laboratory comparison exercise for the determination of Sucralose

Participant	Institution	Address
A. Koch	FROHME-APOTHEKE	Frohmestrasse 14, D-22457 Hamburg, Germany
B. Caemmerer	TU Berlin, Institut für Lebensmittelchemie	Gustav-Mayer-Allee 25, D-13355 Berlin, Germany
B. Spangenberg	University of Applied Science Offenburg, Fachbereich Verfahrenstechnik	Badstrasse 24, D-77652 Offenburg, Germany
G. Morlock	University of Hohenheim	Garbenstrasse 28, D-70599 Stuttgart, Germany
HHAC	HHAC Labor Dr. Heusler GmbH	Hindenburgstrasse 33, D-76297 Stutensee, Germany
J. Große-Damhues	Gemeinsames Chemisches und Lebensmitteluntersuchungsamt für den Kreis Recklinghausen (CEL)	Kurt-Schumacher-Allee 1, D-45657 Recklinghausen, BGermany
J. Kemme	Landesbetrieb Hessisches Landeslabor, Standort Kassel Druseltalstraße	Druseltalstrasse 67, D-34131 Kassel, Germany
K. Bouten	JRC-IRMM	Retieseweg 111, B-2440 Geel, Belgium
M. Schulz	Merck KGaA	Frankfurter Strasse 250, D-64293 Darmstadt, Germany
M. Vega	University of Concepcion, Faculty of Pharmacy	Barrio Universitario, CL-4089100 Concepcion, Chile
R. Schneider	CVUA Karlsruhe	Weissenburgerstrasse 3, D-76187 Karlsruhe, Germany
T. Dzido	Medical University of Lublin	Staszica 6, PL-20-081 Lublin, Poland
V. Widmer, M. Steiner	CAMAG Laboratory	Sonnenmattstrasse 11, CH-4132 Muttenz, Switzerland

Prior to the trial, each participant received a questionnaire to evaluate the type of equipment available. Then a pre-trial was conducted to familiarize the participants with the method and to report back any problems experienced. The pre-trial involved the analysis of a blank and a spiked sample. Remarks and comments were received from seven participants, the most important ones being (**a**) to use 10 mL instead of 5 mL volumetric flasks for diluting the test material, (**b**) to mention explicitly in the procedure that the TLC chamber should not be saturated (provided a chamber for TLC and not for HPTLC is used), (**c**) to put a clear sign on the parcel with the samples upon receipt to be stored in a refrigerator. These comments were taken into account for the trial. In addition, four participants observed cracks in the layer of the HPTLC plates just after delivery. As a result additional shipping precautions were taken to ensure safe shipment of the NH_2-HPTLC glass plates.

For the collaborative trial each participant received:

1. Eight coded test materials (blind duplicates at three concentration levels and one split level).
2. One vial marked 'Sucralose Standard' containing Sucralose, which was to be employed as the calibrant, as described in the method.
3. Six vials marked 'Spike solution A, B, C, D, E, and F'.
4. Eight NH_2-HPTLC glass plates, 10 x 10 cm.
5. Five HPLC syringe filters, 0.45 µm.
6. One 3 mL syringe.
7. A copy of the collaborative study method.
8. A 'Collaborative Study Materials Receipt' form.
9. Report forms.

Each participant was required to dilute the test solutions and the spiked ones and to perform a single analysis of each material by HPTLC.

Method of analysis

The method of analysis that was used in this study can be found in the Annex.

Results and Discussion

Collaborative trial results

All data submitted for the study is presented in Table 6 & Table 7. The data is given as individual pairs of results for each laboratory (identified by the laboratory identification codes). Blank samples spiked at levels of 30.5 mg/L, 100.7 mg/L, and 299 mg/L of Sucralose are identified as sample '30.5', '100.7' and '299'. Samples 'Low level', 'Medium level 2' and 'High level' were blind duplicates (BD), and sample 'Medium level 1' was a split level (YP) of Sucralose containing energy-reduced beverages. The results for duplicate determinations of Sucralose by UV-absorption and fluorescence are shown in chart form (Mean & Range and Youden Plots) in Annex II and III.

Three laboratories that participated in the study reported zero or "< XX" values for some of the test samples.

Laboratory 08 reported several zero and "< XX" values, indicating a consistent and laboratory inherent bias.

Laboratory 17 reported results from ultraviolet absorption measurement mode at λ = 310 nm only due to lack of equipment to measure fluorescence intensity.

Table 6: Individual results of Sucralose in spiked beverage determined using UV and FL quantification

Sucralose concentration in spiked beverage, [mg/L]												
	30.5				**100.7**				**299**			
Lab Code	**UV**	**UV**	**FL**	**FL**	**UV**	**UV**	**FL**	**FL**	**UV**	**UV**	**FL**	**FL**
Lab 01	41	33	29	32	69	70	91	67	328	313	330	290
Lab 02	41	53	30	32	116	66	107	86	330	330	328	326
Lab 03	30	32	30	30	111	114	107	102	300	309	308	314
Lab 04	31	45	30	27	110	117	109	113	321	302	284	292
Lab 06	27	55	32	28	119	103	114	93	296	389	285	348
Lab 08	33	58	32	57	105		98	205		0	403	0
Lab 09	36	36	28	32	121	121	112	104	310	328	299	296
Lab 10	28	57	29	61			171	144			299	230
Lab 11	29	52	21	53	60	102	82	116	291	287	293	299
Lab 16	45	43	38	38	106	146	112	119	293	231	277	253
Lab 18	43	26	32	25	118	99	108	65	386	259	352	269
Lab 17	21	43			135	135			279	370		
Lab MYC	30	26	31	28	118	127	112	111	289	322	299	300

Data sets in shaded fields were not taken for statistical analysis

Non-compliant data[1] Outlier

Table 7: Individual results of Sucralose in energy-reduced beverages determined using UV and FL quantification

Sucralose concentration in energy-reduced beverages, [mg/L]																
	Low level (BD)				**Medium level (YP)**				**Medium level (BD)**				**High level (BD)**			
Lab Code	**UV**	**UV**	**FL**	**FL**	**UV**	**UV**	**FL**	**FL**	**UV**	**UV**	**FL**	**FL**	**UV**	**UV**	**FL**	**FL**
Lab 01	40		38		72	88	64	64	119	105	104	97	137	127	129	125
Lab 02	43	44	49	42	<58	67	62	66	80	121	85	124	150	102	124	132
Lab 03	46	46	41	46	80	68	77	58	139	117	142	123	117	125	114	115
Lab 04	41	42	40	48	63	58	66	79	125	134	117	116	170	130	127	100
Lab 06	32	39	38	37	97	74	65	80	154	167	147	123	173	130	147	113
Lab 08	<30	67	54	49	<60	83	<60	60	<60	142	<60	131	96	151	114	138
Lab 09		82	57	34	56	74	67	59		137	44	139	139	158	119	131
Lab 10	89	108	58	99			106	102	154		160	154		232	99	131
Lab 11	56	79	60	80	38	68	50	62	117	126	116	136	96	124	98	111
Lab 16	61	49	36	44	97	67	79	58	128	135	86	118	111	137	109	152
Lab 17	51	40				56			114	131			137	139		
Lab 18	47	37	44	39	161	45	76	49	121	109	115	101	116	94	108	109
Lab MYC	46	41	48	49	73	73	70	73	120	112	106	112	117	120	113	118

Data sets in shaded fields were not taken for statistical analysis

Non-compliant data[1] Outlier

[1] Where only 1 set of results was available statistical analysis was not possible, the whole set was rejected as non-compliant data.

Statistical analysis of results

In some cases data was excluded from the statistical analysis, e.g. when statistical evaluation was impossible (values reported as zero or as “< XX”), or on basis of problems that were identified by the organiser (e.g. calibration problems).
On basis of the latter case, all results submitted from laboratory 08 were classified as non compliant prior statistical analysis.

The collaborative trial results were evaluated according to the IUPAC Harmonised Protocol (7, 8). Outlying results were identified by applying Cochran’s and Grubbs' tests ($p<0.025$). Pairs of results that were identified as outliers are indicated with shaded background in Table 6 and Table 7. The maximum numbers of outliers identified were two laboratories giving acceptable data ranging from eight to 12 laboratories for ultraviolet absorption and fluorescence measurement mode. Precision estimates were obtained using the one-way analysis of variance approach according to the IUPAC Harmonised Protocol (7, 8). Details of the average analyte concentration, the standard deviations for repeatability (RSD_r) and reproducibility (RSD_R), the number of statistical outlier laboratories, the HORRAT ratio and the percentage recovery for the individual samples are presented in Table 9 at the end of this document section.

The performance parameters of the method are summarised in Table 8.

Table 8: Method performance parameters obtained in the collaborative trial

Method	Measurement Mode	Mean Level [mg/L]	Performance parameter		
			RSD_r %	RSD_R %	Recovery %
HPTLC	UV	38	31.4	31.4	124.6
		44	11.9	15.0	—
		72	20.7	21.5	—
		108	16.0	21.6	107.2
		124	10.3	14.8	—
		130	14.9	16.3	—
		312	13.5	13.5	104.3
	FL	30	8.9	8.9	98.4
		43	15.9	15.9	—
		70	14.6	20.7	—
		102	15.0	15.6	101.3
		119	12.2	17.6	—
		119	12.9	12.9	—
		299	9.6	9.6	100

Materials for which no recovery data is given (marked with '–') were labelled to contain Sucralose.

Comments from collaborative trial participants

Comments were received from three collaborative trial participants. They regarded the method description as being clear and adequate. Laboratory 02 reported a different aliquotation scheme used with the intention to save time for calculations. This change had apparently no effect on the results and was not further regarded for the evaluation of the end result.

Furthermore several comments were made on the mechanical stability and homogeneity of the coating of the commercial HPTLC glass plates.

Laboratory 02 reported deviation from the procedure due to instability of the mercury lamp (too many burning hours) and an urgent need to replace it. For that reason fluorescence measurement was performed four days after plate development. No outliers were identified from that lab, indicating that these deviations had no observable effect on the results while showing the robustness of the method principle.

Laboratory 17 reported deviation from the procedure measuring UV absorption at wavelength λ = 310 nm. This change had apparently no effect on the results and was not further regarded for the evaluation of the end result.

Based on the comments from the participants the method description was adjusted at some points and small editorial additions were made. As the main result, the dilution procedure was adapted.

Precision characteristics of the method

The precision data for all samples is summarised in Table 9. Based on results for spiked samples (blind duplicates at three levels), as well as samples taken from the market and labelled to contain Sucralose (blind duplicates at three levels and one at split level), the relative standard deviation for repeatability (RSD_r) ranged from 10.3 – 32 % for UV detection and from 9.6 – 26 % for FL detection. The relative standard deviation for reproducibility (RSD_R) ranged from 13.5 – 32 % for UV detection and from 9.6 – 26 % for FL measurement mode.

The recovery values for Sucralose derived from the spiked samples were found to range from 104.3 – 126.3 % with an average of 112.7 % for ultraviolet detection and from 99.9 – 100.8 % with an average of 100.3 % for fluorescent detection.

Ultraviolet absorption has previously shown by one of the participants to offer a detection limit of 5 ng/spot in the plate, resulting in a Sucralose concentration of 1 mg/L in the tested solution, taking into account that 5 µL were applied on the plate (6).

The limit of quantification on the basis of 10x the baseline noise was found as 6 mg/L and a linear calibration was applicable in the range of 10 – 380 ng of Sucralose for the ultraviolet absorption measurement mode (6) and 30 – 150 ng/spot for the fluorescent measurement mode.

Conclusions

The results of this inter-laboratory comparison show satisfying precision characteristics (RSD_r, RSD_R and recovery) at the levels of interest that have been stipulated by European legislation (1) in order to support Commission regulations on legislative limits. Fluorescence measurement mode turned out to be superior compared with ultraviolet absorption measurement mode as far as robustness, precision and recovery were concerned.

HORRAT values are in a few cases slightly higher than 2. This should however not be considered as unsatisfactory, as the method showed for most levels sufficient HORRAT values. According to Linsinger and Josephs (9) HORRAT values are also influenced by the type of method applied and the obtained performance should be seen with respect to the simplicity of the analytical procedure such as no need for clean-up and reagent free and simple derivatisation.

Based on the precision values alone, thus recovery, reproducibility and repeatability, the here described method in both measurement modes can compare with methods for the determination of food contaminants that qualify for official food control (10).

In conclusion, the method qualified for food beverages at levels equal and above 30 mg/L. The JRC is currently transforming this method into a format suitable for CEN and will submit it to CEN TC.

Table 9: Results for spiked and test samples, calculated by conventional statistics

UV	low (BD) nc[2]	medium (YP) nc[2]	medium (BD) nc[2]	high (BD) nc[2]	spike 30.5	spike 100.7	Spike 299
# of labs[3]	8 (3) [2]	8 (4) [1]	10 (3)	11 (2)	12 (1)	11 (2)	11 (2)
Mean mg/L	44	72	124	130	38	108	312
S_r mg/L	5.3	14.8	12.7	19.3	11.8	17.3	42.1
RSD_r %	11.9	20.7	10.3	14.9	31.4	16.0	13.5
S_R mg/L	6.6	15.4	18.3	21.1	11.8	23.4	42.1
RSD_R %	15.0	21.5	14.8	16.3	31.4	21.6	13.5
HORRAT[4]	1.7	2.6	1.9	2.1	3.4	2.7	2.0
Recovery %	n.a.	n.a.	n.a.	n.a.	124.6	107.2	104.3
FL	low (BD) nc[2]	medium (YP) nc[2]	medium (BD) nc[2]	high (BD) nc[2]	spike 30.5	spike 100.7	Spike 299
# of labs[3]	8 (2) [2]	11 (1)	10 (1) [1]	11 (1)	8 (2) [2]	10 (1) [1]	11 (1)
Mean mg/L	43	70	119	119	30	102	299
S_r mg/L	6.9	10.2	14.5	15.3	2.6	15.2	28.6
RSD_r %	15.9	14.6	12.2	12.9	8.9	15.0	9.6
S_R mg/L	6.9	14.4	20.9	15.3	2.6	15.8	28.6
RSD_R %	15.9	20.7	17.6	12.9	8.9	15.6	9.6
HORRAT[4]	1.8	2.4	2.3	1.7	0.9	1.9	1.4
Recovery %	n.a.	n.a.	n.a.	n.a.	98.4	101.3	100

n.a. = not applicable; S_r = Standard deviation for repeatability; S_R = Standard deviation for reproducibility; RSD_r = Relative standard deviation for repeatability (%); RSD_R = Relative standard deviation for reproducibility (%); BD = Blind duplicates; YP = Youden matched pair; UV = Ultraviolet detection mode; FL = Fluorescence detection mode

[2] nc = (labelled to contain Sucralose)
[3] Number in round brackets (-) indicate the number of outliers, numbers in straight brackets [-] indicate non compliant data.
[4] HORRAT values are calculated by the modified function, as proposed by Thompson [ref].

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Annex Ia

Spiking Protocol

SPIKING PROTOCOL

In order to calculate the recovery of the method each participant will be supplied with 6 different vials marked Spike solution A, Spike solution B, Spike solution C, Spike solution D, Spike solution E and Spike solution F. These vials contain 3.2 mL spiked sucralose solutions of unknown concentrations. Store vials at 4 °C. Prior to analysis let them reach room temperature.

Dilute 1000 µL of spiked solutions B, C, D and F to exactly 10.00 mL (e.g. in a 10 mL volumetric flask) with methanol-water [50+50 v/v] and shake well to mix thoroughly.

Dilute 2000 µL of spike solutions A and E to exactly 10.00 mL (e.g. in a 10 mL volumetric flask) with methanol-water [50+50 v/v] and shake well for complete mixing.

These solutions can directly be used for spotting on the NH2-HPTLC plate for determination of sucralose, no further cleanup is required.

SPIKING PROCEDURE (done in IRMM):

Coke Light (used as 'blank') was fortified with sucralose to give three spike solutions (each in duplicate):

Exactly 1.0 mL, 3.3 mL and 9.8 mL of the sucralose stock standard solution (concentration 6.1 [g/L]) were added to Coke Light to give 200 mL spike solutions with concentrations of 31 [mg/L], 101 [mg/L] and 299 [mg/L].

Annex Ib

Instructions for Participants

VALIDATION OF A HPTLC ANALYTICAL METHOD TO DETERMINE THE CONTENT OF THE INTENSE SWEETENER SUCRALOSE IN BEVERAGES

Draft of a method for the determination of sucralose by HPTLC in beverages in a suitable format for intercomparison purposes.

ABSTRACT

This method for the determination of sucralose in beverages is based on high-performance thin layer chromatography (HPTLC) in combination with a reagent free derivatisation. Samples of carbonated and still beverages are diluted with methanol-water and applied directly on a NH2-HPTLC plate. The plate is developed with an acetonitrile-water mobile phase and after development directly heated at 190 ºC for 20 minutes. This procedure results in the formation of UV-light absorbing (and fluorescent) sucralose derivatives. That can be used for quantification in both modes (UV and FL).

1 SCOPE

This draft specifies a method for the determination of sucralose in beverages using HPTLC.

2 PRINCIPLE

A known quantity of beverage is spotted/sprayed without any clean-up on a NH2-HPTLC plate. After development plate is directly heated at 190 ºC for 20 minutes to obtain UV-absorbing and fluorescing sucralose derivatives. The latter are quantitatively determined by both UV absorption and fluorescence.

3 REAGENTS

Upon receipt store all vials in a refrigerator at 4 °C and allow reach room temperature before use.

3.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvents shall be of quality for HPLC analysis.

3.2 Methanol

3.3 Acetonitrile

3.4 NH2-HPTLC plates – you will be provided with eight NH2-HPTLC plates 10 x 10 cm

3.5 HPLC syringe filter 13 mm 0.45 µm Nylon – you will be supplied with 5 filters

3.6 Dilution solution – Mix 50 parts per volume of methanol (3.2) with 50 parts per volume of water (3.1)

3.7 Mobile phase – Mix 80 parts per volume of acetonitrile (3.3) with 20 parts per volume of water (3.1)

3.8 Sucralose stock solution – Stock solution with mass concentration of 107 [mg/L]. This solution will be provided for the collaborative trial.

3.9 Working standard solutions for calibration – Pipette amounts of 600 µL, 1300 µL, 2000 µL and 2800 µL of the stock solution (3.8) into different 10 mL volumetric flasks. Fill the flasks up to the mark with methanol-water (3.6) and shake. This will result in sucralose solutions with mass concentrations of: 6.4 [mg/L], 13.9 [mg/L], 21.4 [mg/L] and 30 [mg/L].

3.10 Spike recovery solutions – You will be provided with 6 vials containing spike solutions of unknown sucralose concentrations.

4 APPARATUS

Usual laboratory equipment and, in particular, the following:

4.1 Syringe of 3 mL – you will be provided with one sterile syringe for single use

4.2 Volumetric flasks of 10 mL

4.3 Spotting or spraying device – (Micro-capillary pipettes of 5 µL can be used instead)

4.4 HPTLC chamber for planar chromatography

4.5 Densitometer (UV) and/or (FL) with Data processing system

4.6 Drying oven capable of maintaining temperature of 190 ± 5 °C for at least 20 minutes should be used. Alternatively a **temperature controlled heating plate** can be used provided the heating surface is equally hot at all locations (thick metal plate).

5 PROCEDURE

5.1 Dilution

Dilute 1000 µL of sucralose test solutions “Sample 01”, “Sample 02”, “Sample 03”, “Sample 05”, “Sample 06”, “Sample 07” and spike solutions B, C, D, F to exactly 10.00 mL (e.g. in a 10 mL volumetric flask) with methanol-water [50+50] and shake well to mix thoroughly.

Dilute 2000 µL of test solutions “Sample 04”, “Sample 08” and spike solutions A and E to exactly 10.00 mL (e.g. in a 10 mL volumetric flask) with methanol-water [50+50] and shake well for complete mixing.

Shake well turbid solutions (“Sample 02” and “Sample 06”) and after dilution prior to application filter through 0.45 µm Nylon HPLC syringe filter.

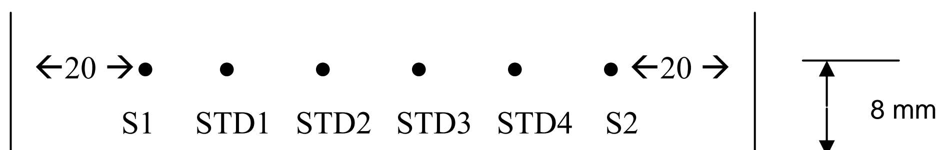
All solutions can directly be used for spotting on the HPTLC plate and determination of sucralose, no further cleanup is required.

5.2 Application

On a 10 x 10 cm NH2-HPTLC plate apply (by spotting or spraying) each 5 µL of the diluted solutions in the following manner:

Keep a distance of 20 mm from left and right for the outer spots and of 8 mm from the bottom edge for the application line.

You will need to spot/spray two test solutions (S1 and S2) and four working standard solutions for calibration (3.9), resulting in 6 spots per plate. Alternatively band wise application can be used (e.g. 7 mm-bands).



5.3 Preparation of a calibration graph

Prepare calibration graph by applying 5 µL of the four standard solutions proposed in 3.9 on each HPTLC plate. Plot the peak height values of the sucralose calibration solutions (3.9) against the concentration of sucralose in [mg/L] in the sample.

5.4 Development

After the solvent has evaporated, develop the plate in either a horizontal or a standard TLC-chamber for 7 cm with the mobile phase (acetonitrile-water [8+2]).

5.5 Derivatisation

For derivatisation, put the developed plate immediately into a drying oven at 190°C for 20 min., alternatively a temperature controlled heating plate can be used.

5.6 Evaluation

After heating for 20 minutes, allow the plate reach room temperature. If needed, you can examine the plate first under UV-light (long wavelength filter). Sucralose appears as yellow fluorescent spot.

Scan the plate in the densitometer for UV-absorption at 254 nm, with a slit width of about 120% of the diameter of the spot (for band wise application 80%, e.g. 5 mm slit). Scan also for fluorescence (FL) at 366 nm with the same slit width.

5.7 Calculation

Quantitative determination of sucralose is carried out by integration both in UV and FL mode of the peak height. Determine the content of sucralose in the test material, in mg/L, via the given formula:

$$\text{Sucralose } [mg/L] = c[mg/L] \times \frac{V1}{V2}$$

Where:

c = concentration in the applied solution calculated from linear regression

V1 = volume of diluted sample solution [10 mL]

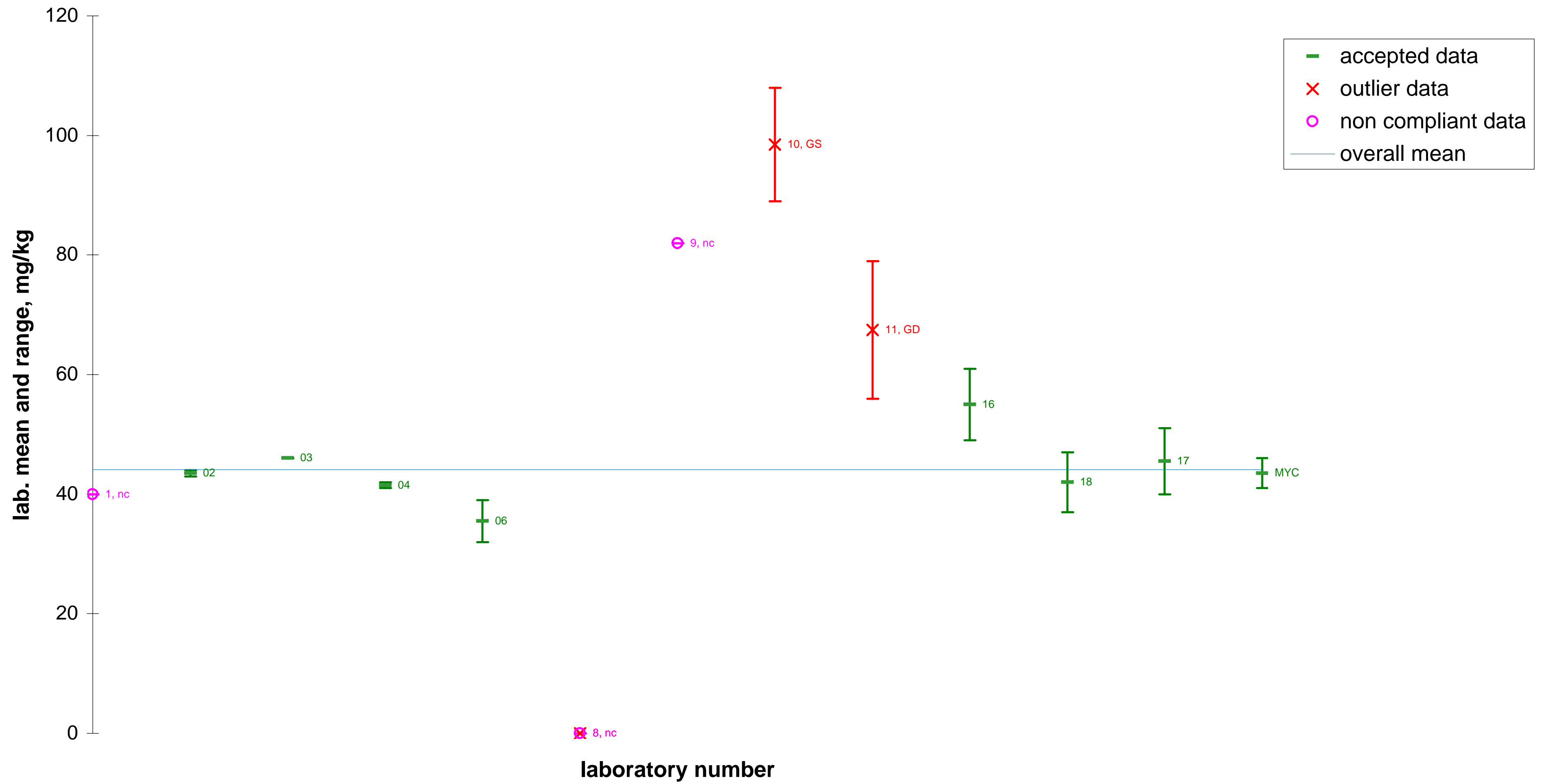
V2 = volume of sample taken for dilution [mL]

6 REPORTING

For reporting please use the attached reporting sheet (one per each HPTLC plate) and also report any comments on the procedure you think are worth mentioning.

Annex IIa

Mean & Range Plots for UV-determination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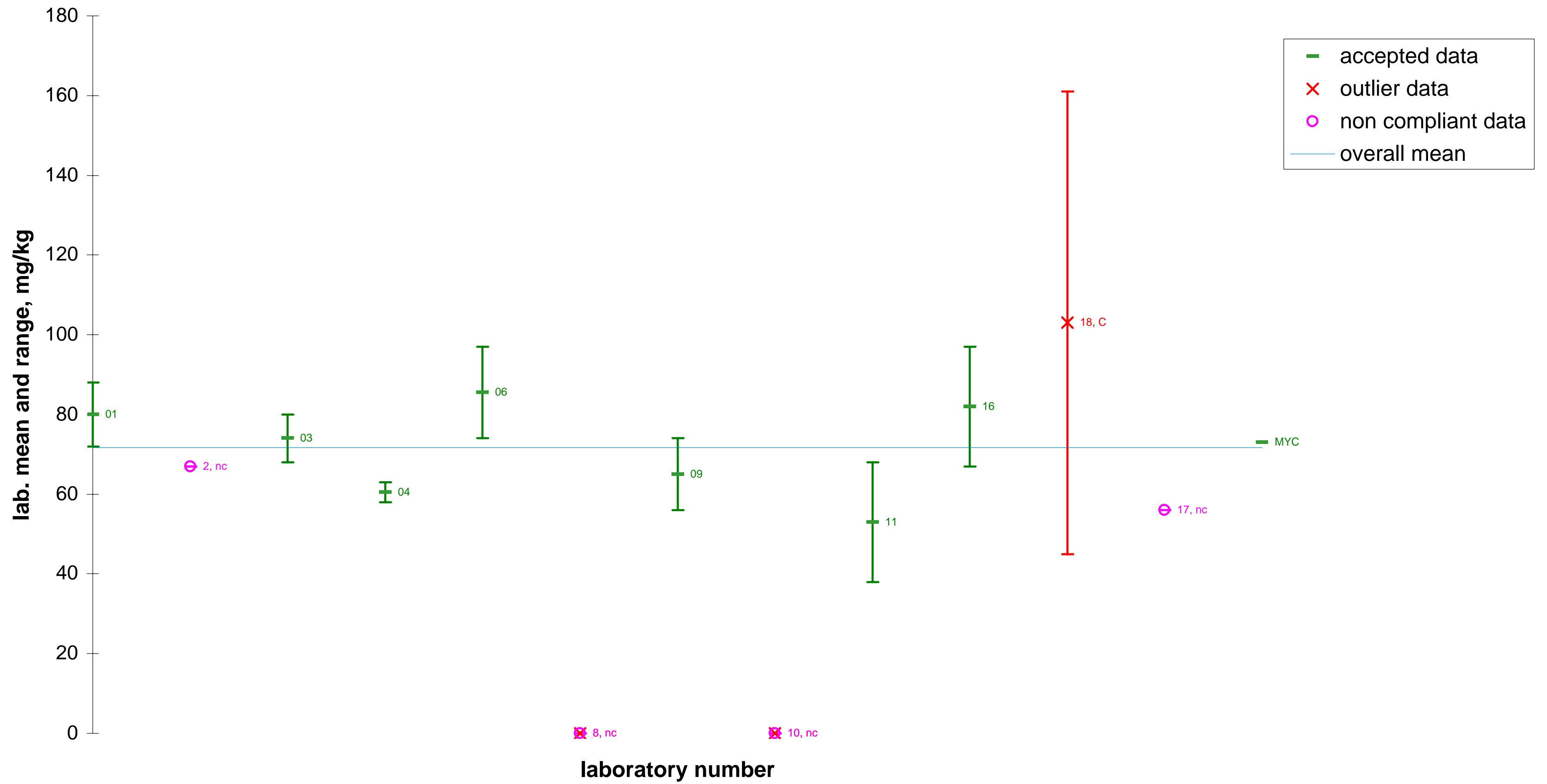
lab. mean and range, mg/kg
120
100
80
60
40
20
0
laboratory number
accepted data
outlier data
non compliant data
overall mean
1, nc
02
03
04
06
8, nc
9, nc
10, GS
11, GD
16
18
17
MYC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lab. mean and range, mg/kg
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60
40
20
0
laboratory number
accepted data
outlier data
non compliant data
overall mean
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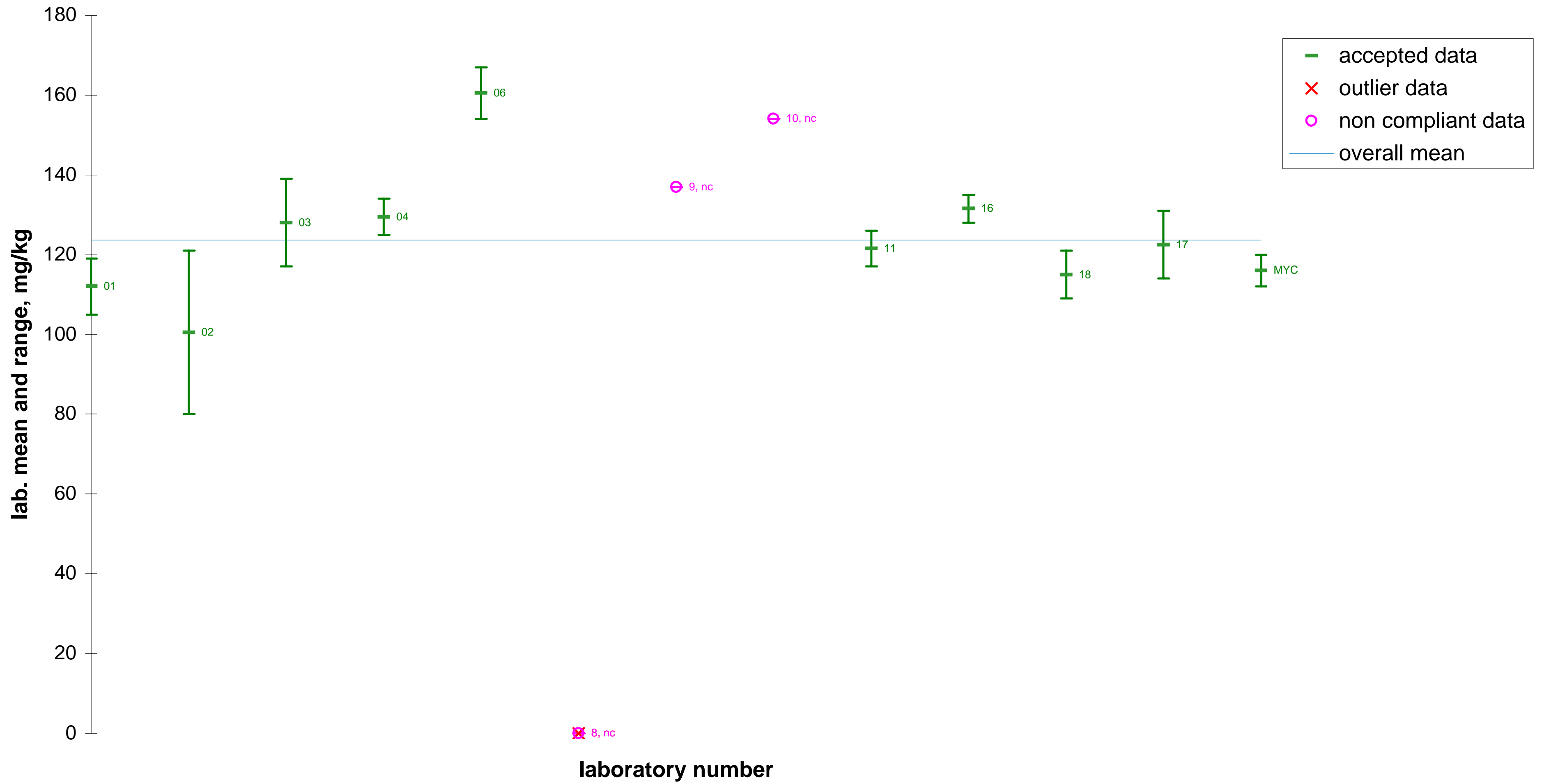
03
04
06
8, nc
09
10, nc
11
16
18, C
17, nc
MYC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lab. mean and range, mg/kg
laboratory number
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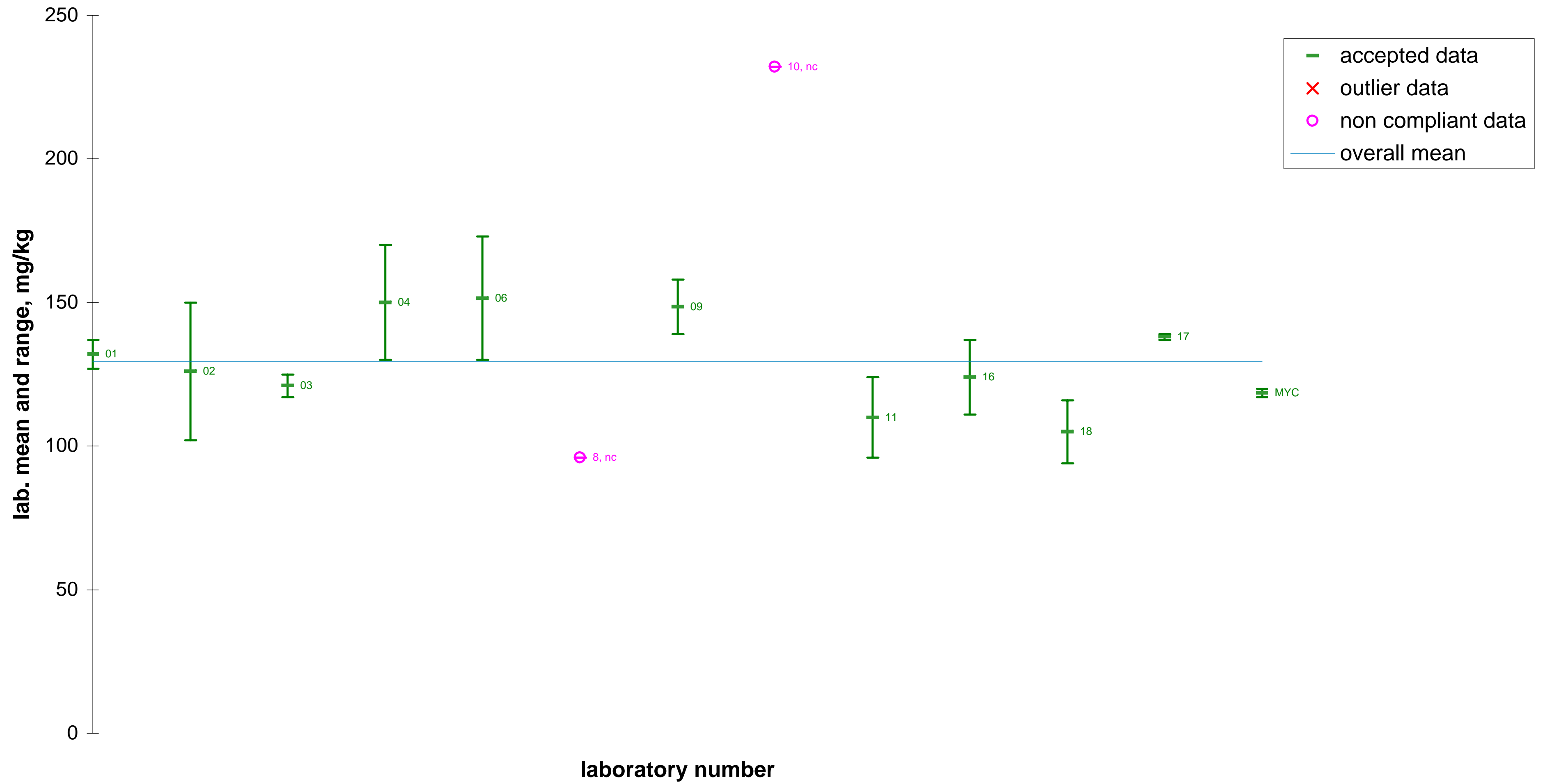
160
140
120
100
80
60
40
20
0
01
02
03
04
06
8, nc
9, nc
10, nc
11
16
18
17
MYC
accepted data
outlier data
non compliant data
overall mean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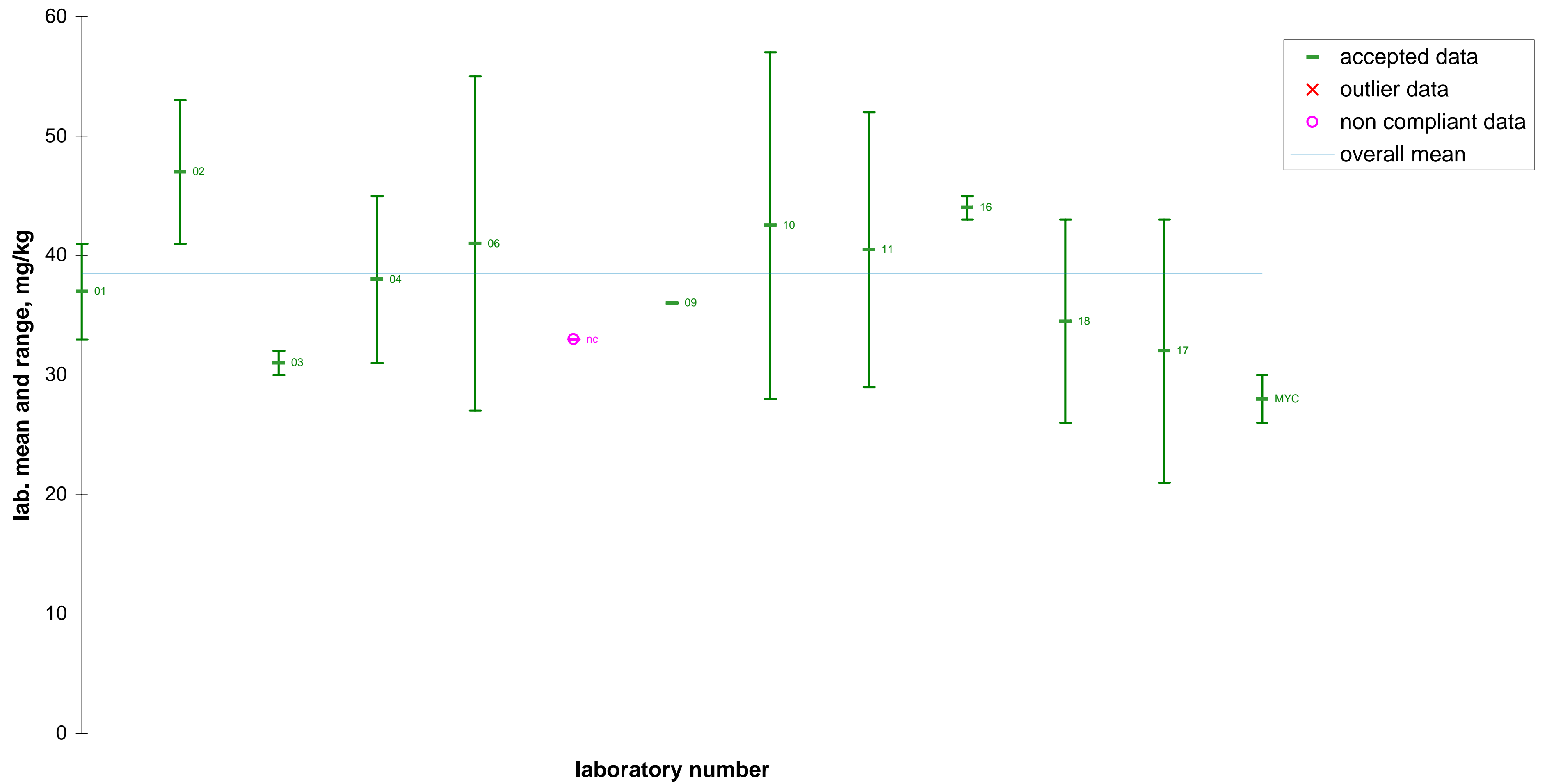
lab. mean and range, mg/kg
laboratory number
250
200
150
100
50
0
accepted data
outlier data
non compliant data
overall mean
01
02
03
04
06
8, nc
09
10, nc
11
16
18
17
MYC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lab. mean and range, mg/kg
laboratory number
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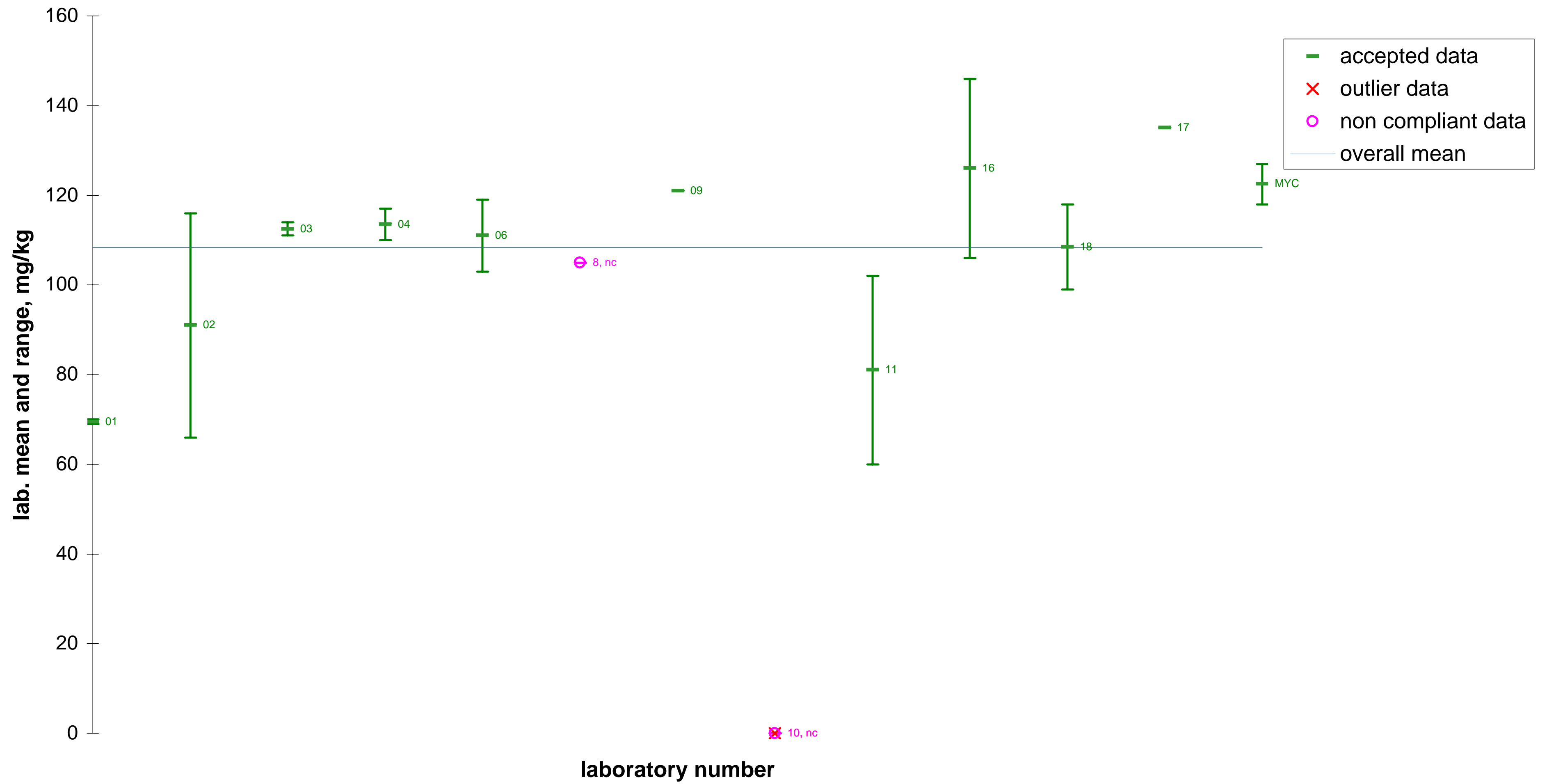
50
40
30
20
10
0
01
02
03
04
06
nc
09
10
11
16
18
17
MYC
accepted data
outlier data
non compliant data
overall mean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140
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100
80
60
40
20
0
lab. mean and range, mg/kg
laboratory number
01
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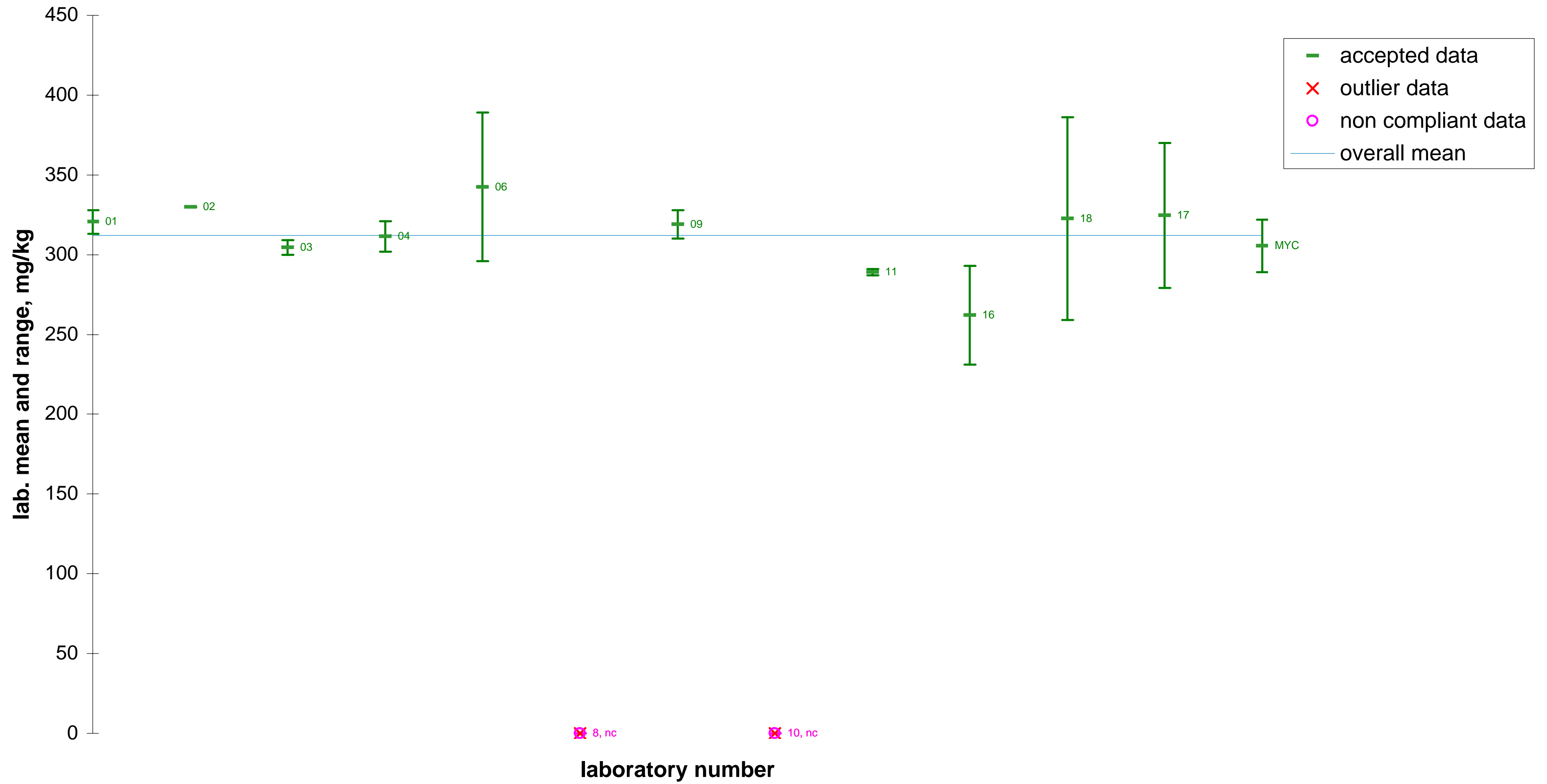
8, nc
09
10, nc
11
16
17
18
MYC
accepted data
outlier data
non compliant data
overall mean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lab. mean and range, mg/kg
laboratory number
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400
350
300
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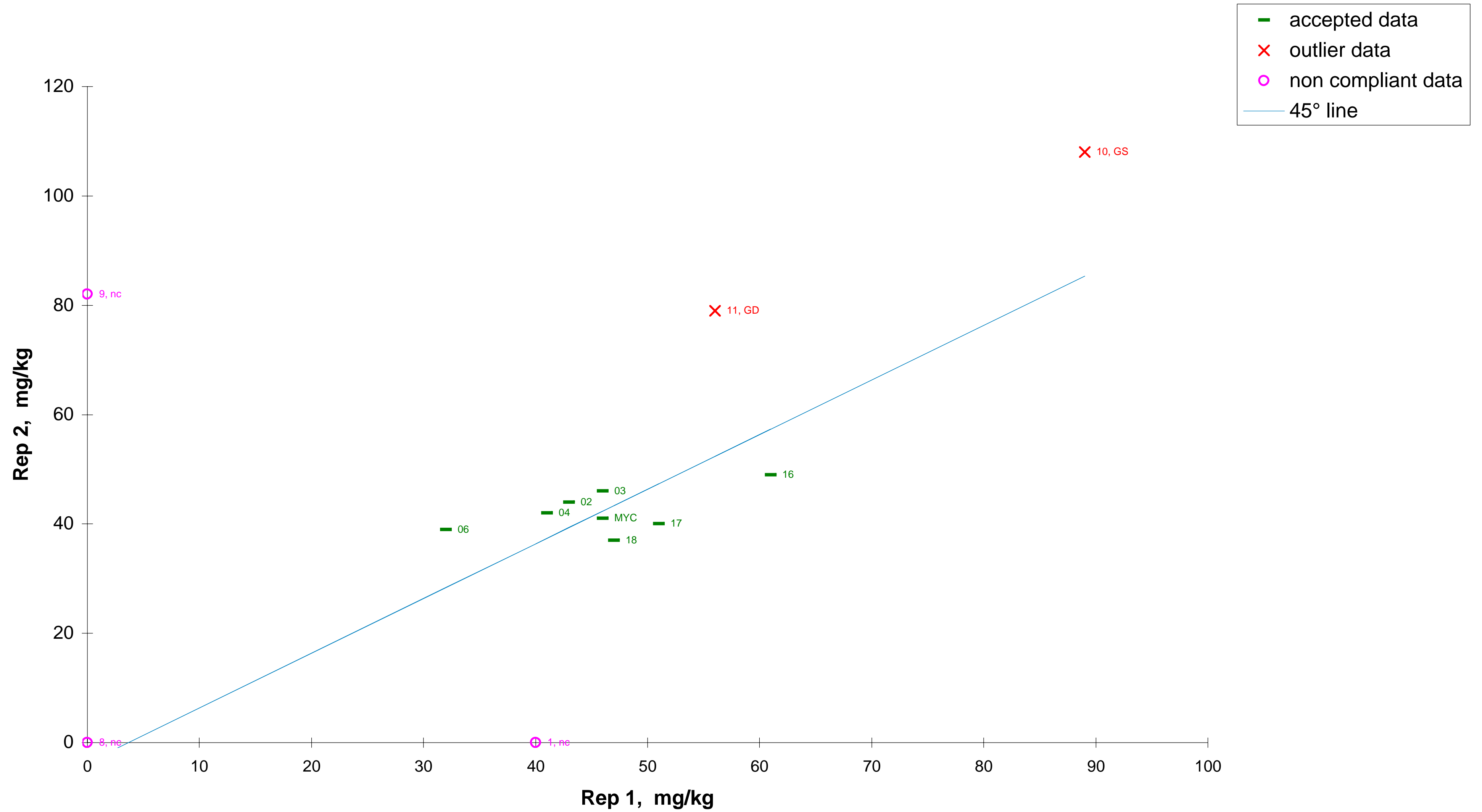
100
50
0
01
02
03
04
06
09
11
16
18
17
MYC
8, nc
10, nc
accepted data
outlier data
non compliant data
overall mean

Annex IIb

Youden Plots
for UV-determination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outlier data
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10, GS
11, GD
9, nc
8, nc
1, nc
16
03
02
04
MYC
17
18
06
120
100
80
60
40
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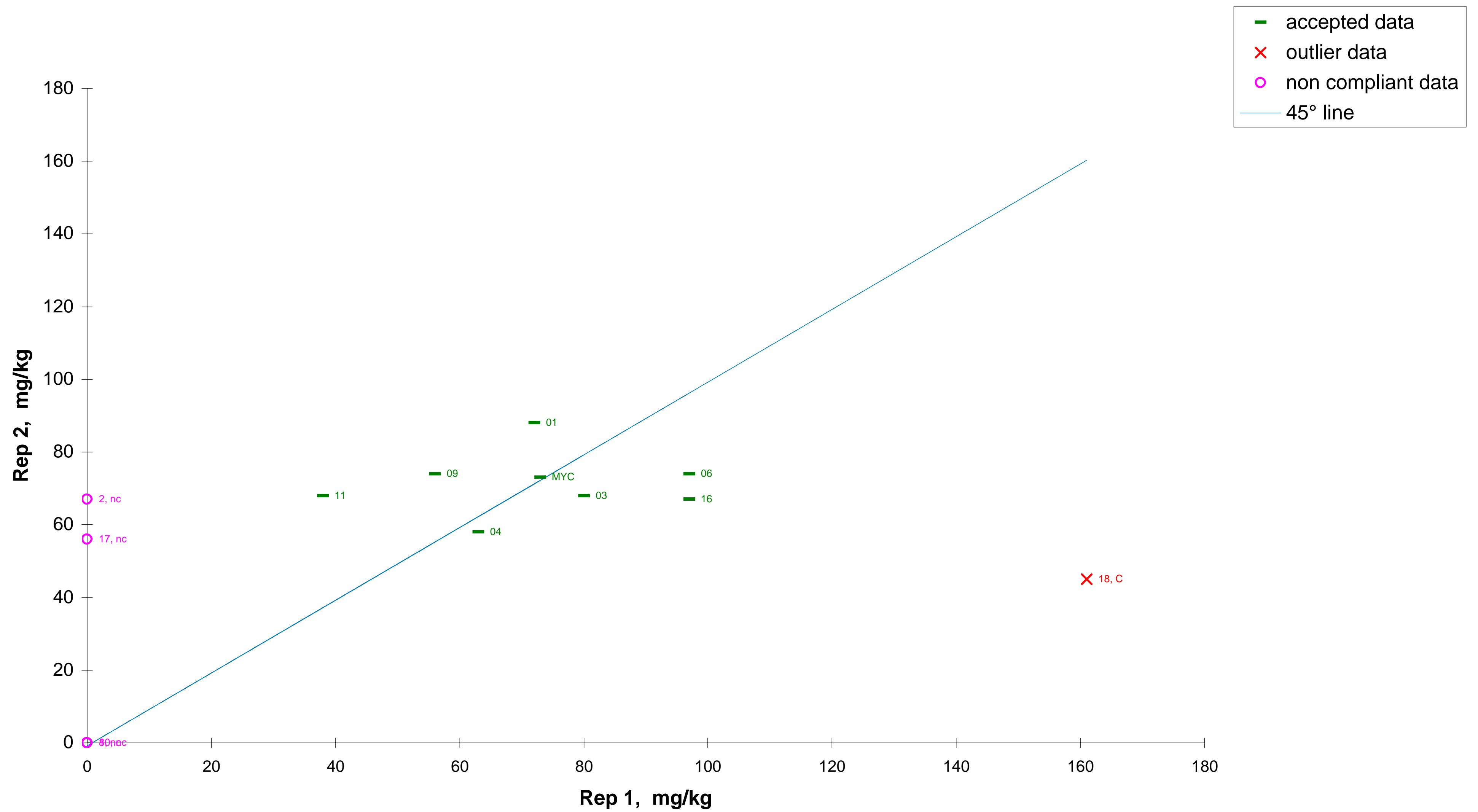
0
0
10
20
30
40
50
60
70
80
90
100
Rep 2, mg/kg
Rep 1, mg/kg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accepted data
outlier data
non compliant data
45° line
Rep 2, mg/kg
Rep 1, mg/kg
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20
40
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80
100
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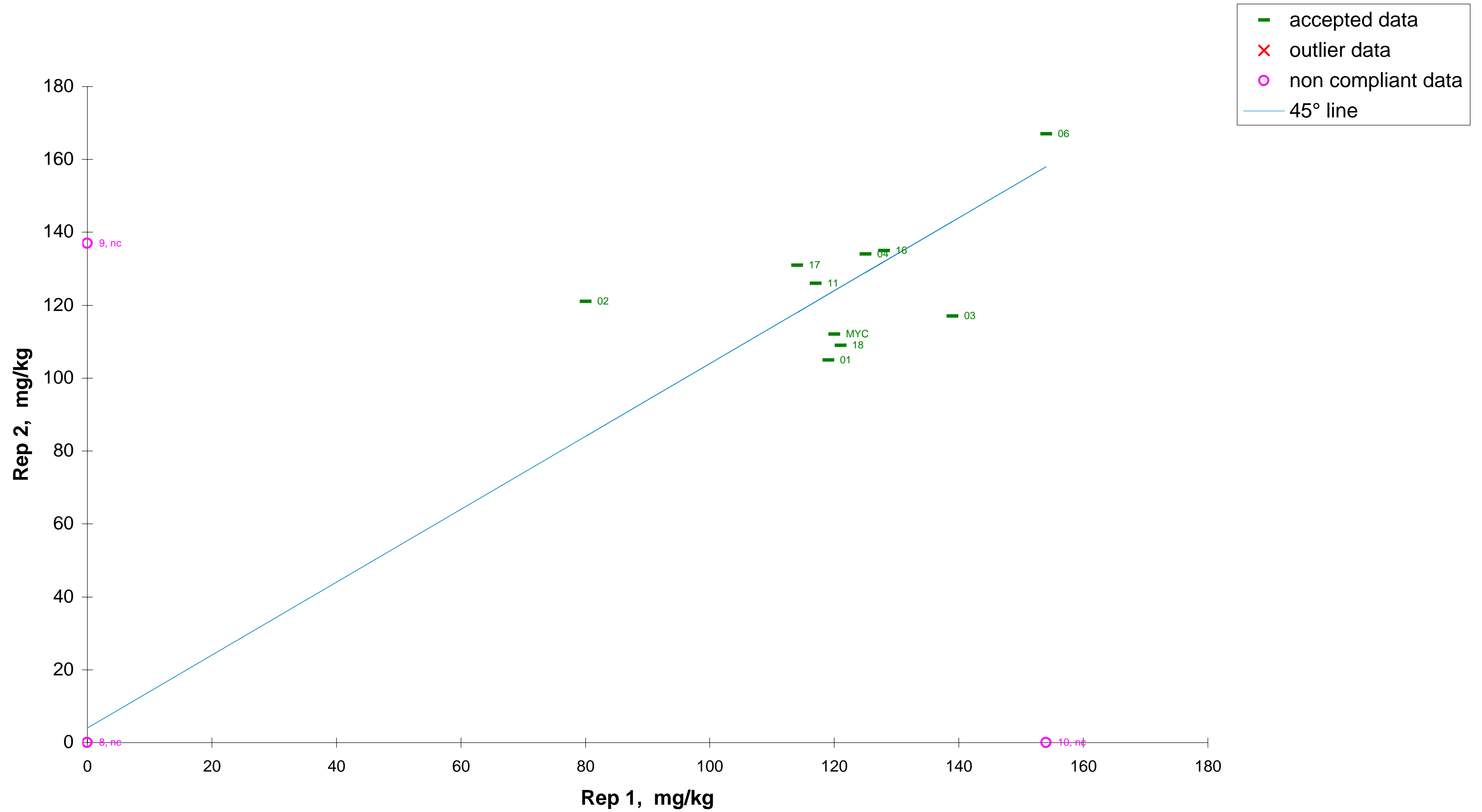
160
180
01
09
MYC
06
11
03
16
04
2, nc
17, nc
18, C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outlier data
non compliant data
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Rep 2, mg/kg
Rep 1, mg/kg
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160
140
120
100
80
60
40
20
0
20
40
60
80
100
120
140
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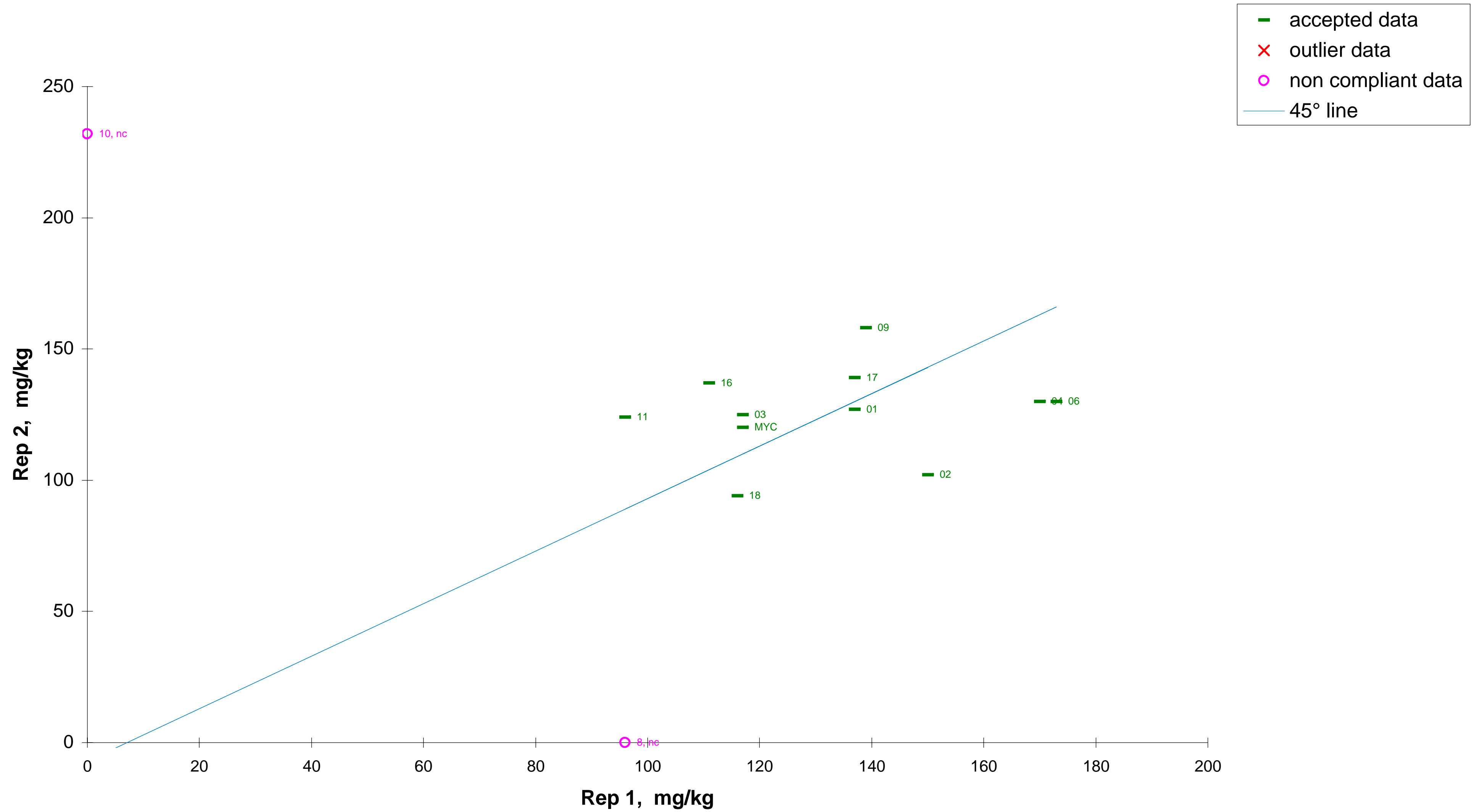
180
06
9, nc
17
04
16
11
02
03
MYC
18
01
8, nc
10, nc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accepted data
outlier data
non compliant data
45° line
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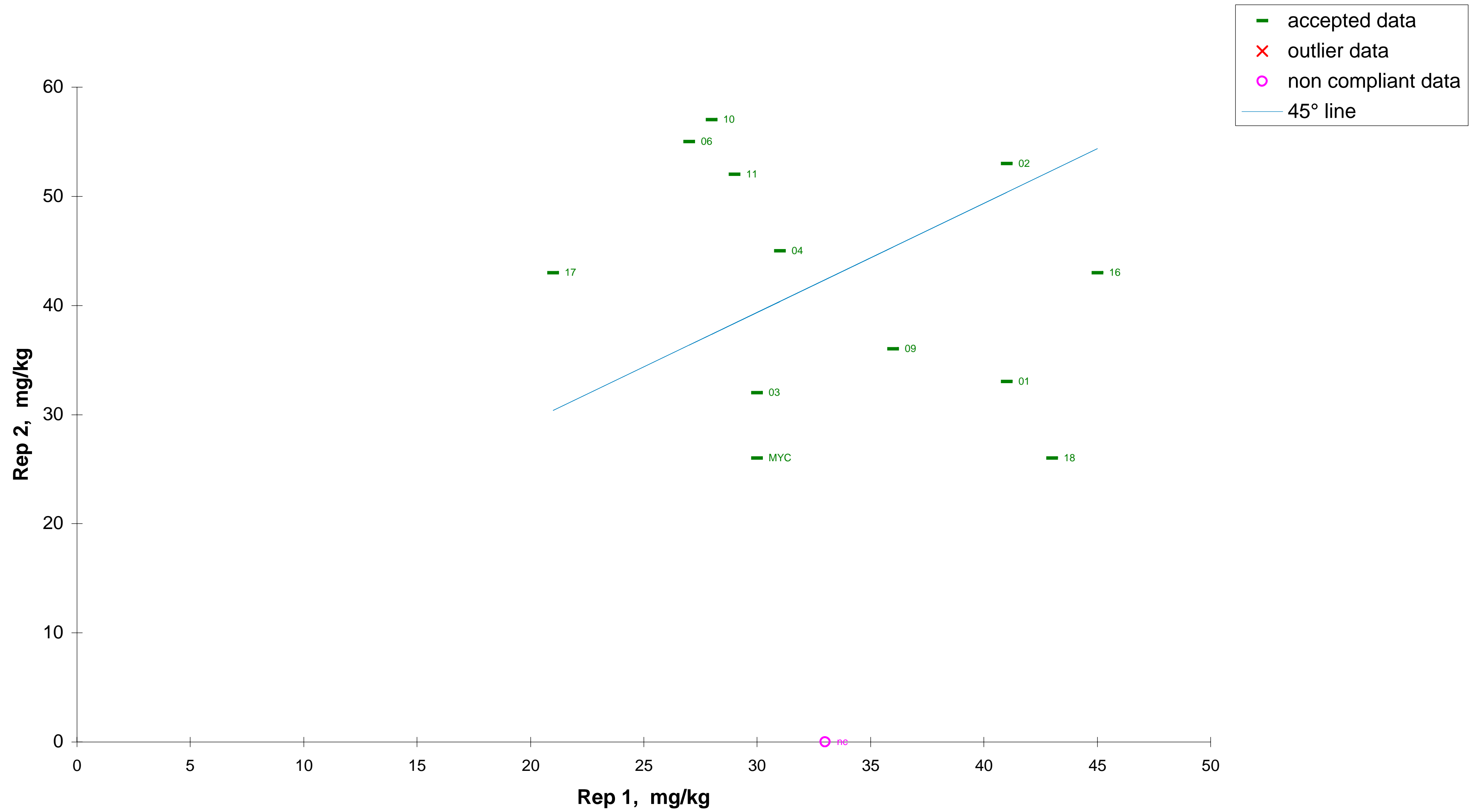
200
150
100
50
0
Rep 2, mg/kg
0
20
40
60
80
100
120
140
160
180
200
Rep 1, mg/kg
10, nc
8, nc
09
16
17
11
03
MYC
01
04
06
02
18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accepted data
outlier data
non compliant data
45° line
Rep 2, mg/kg
Rep 1, mg/kg
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50
40
30
20
10
0
0
5
10
15
20
25
30
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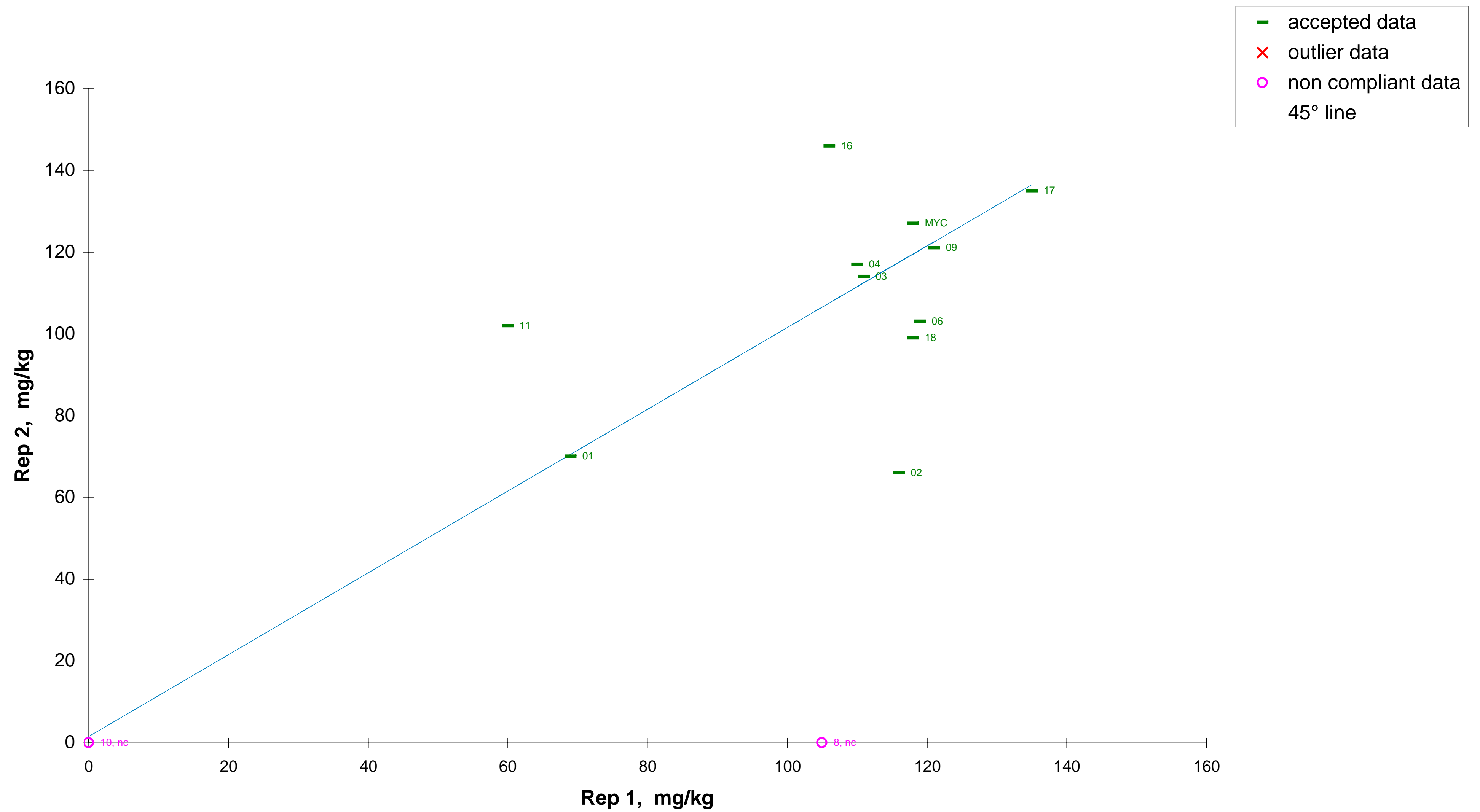
40
45
50
10
06
11
02
04
17
16
09
01
03
MYC
18
nc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Rep 1, mg/kg
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140
120
100
80
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40
20
0
0
20
40
60
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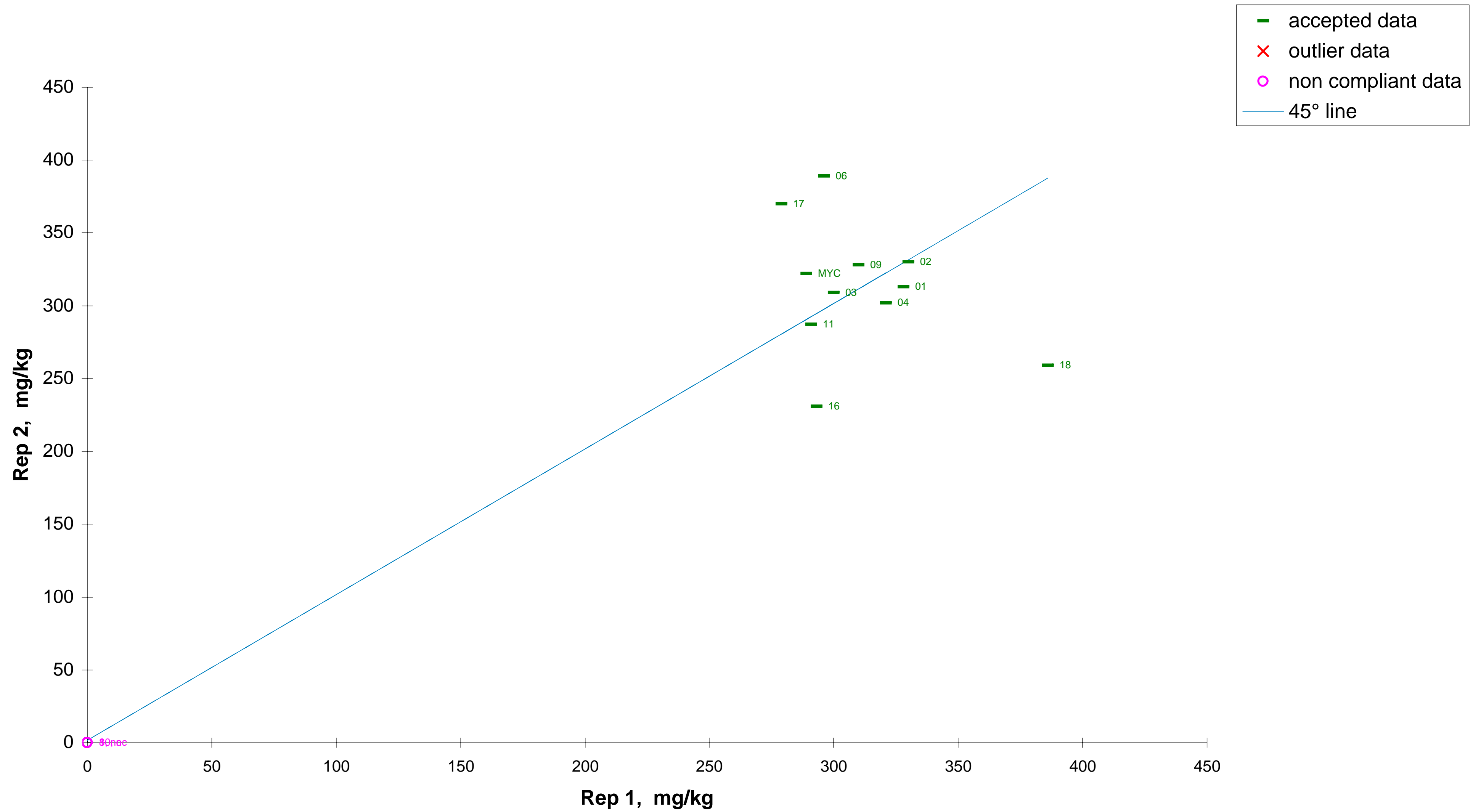
16
17
MYC
09
04
03
11
06
18
01
02
10, nc
8, nc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accepted data
outlier data
non compliant data
45° line
Rep 2, mg/kg
Rep 1, mg/kg
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50
100
150
200
250
300
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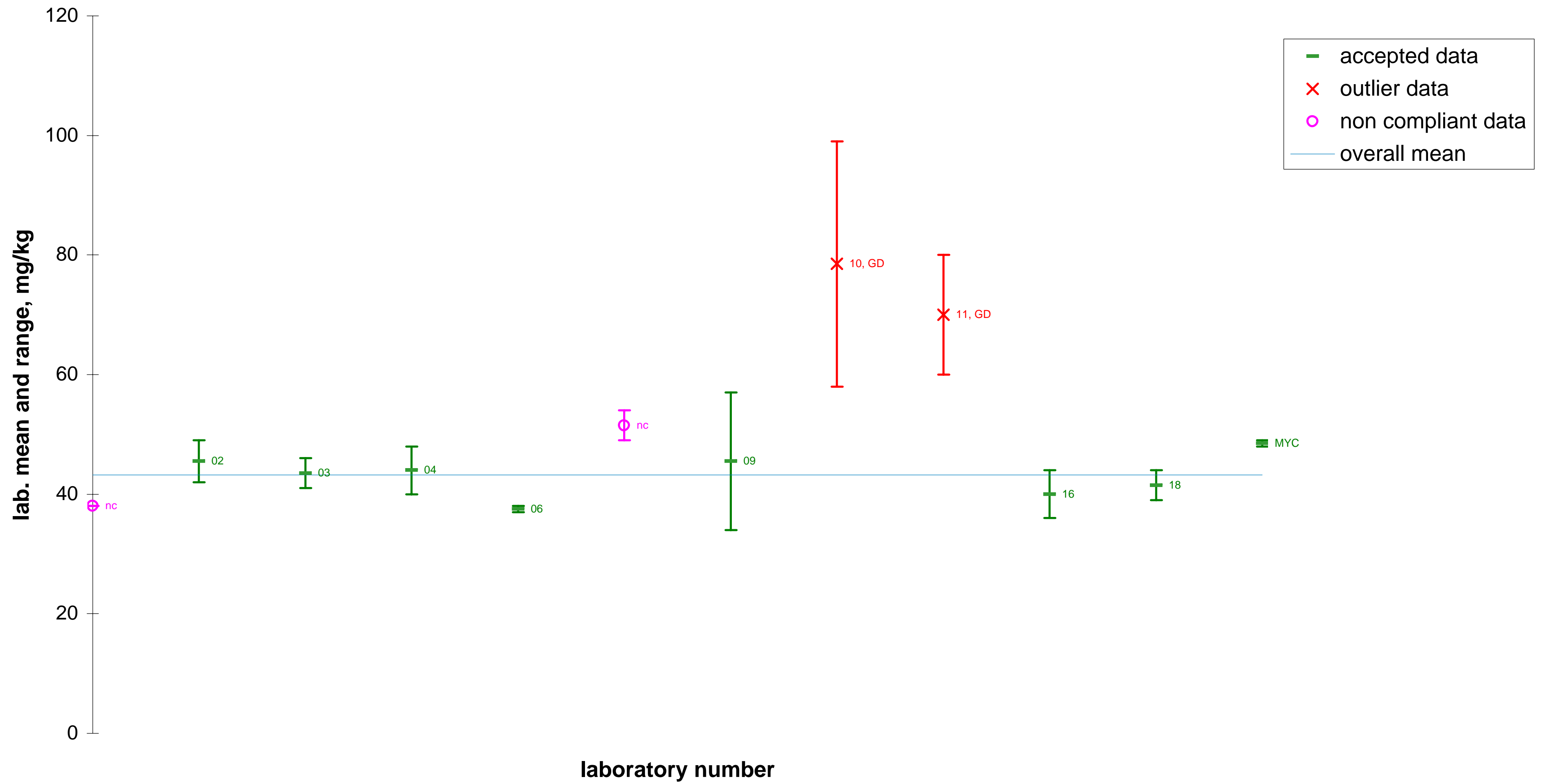
450
06
17
MYC
09
02
01
03
04
11
18
16

Annex IIIa

Mean & Range Plots
for FL-determination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laboratory number
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100
80
60
40
20
0
accepted data
outlier data
non compliant data
overall mean
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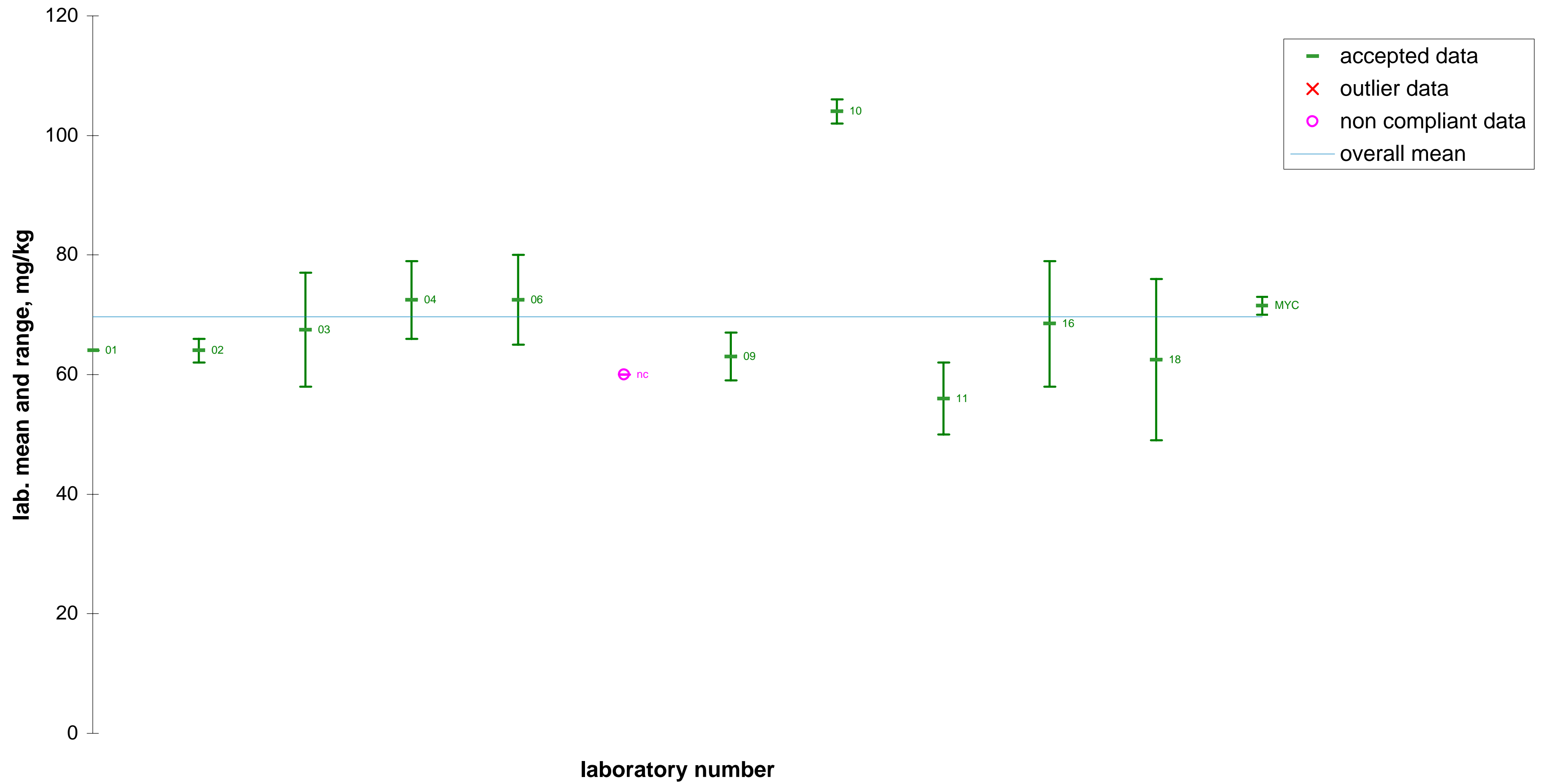
02
03
04
06
nc
09
10, GD
11, GD
16
18
MYC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lab. mean and range, mg/kg
laboratory number
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100
80
60
40
20
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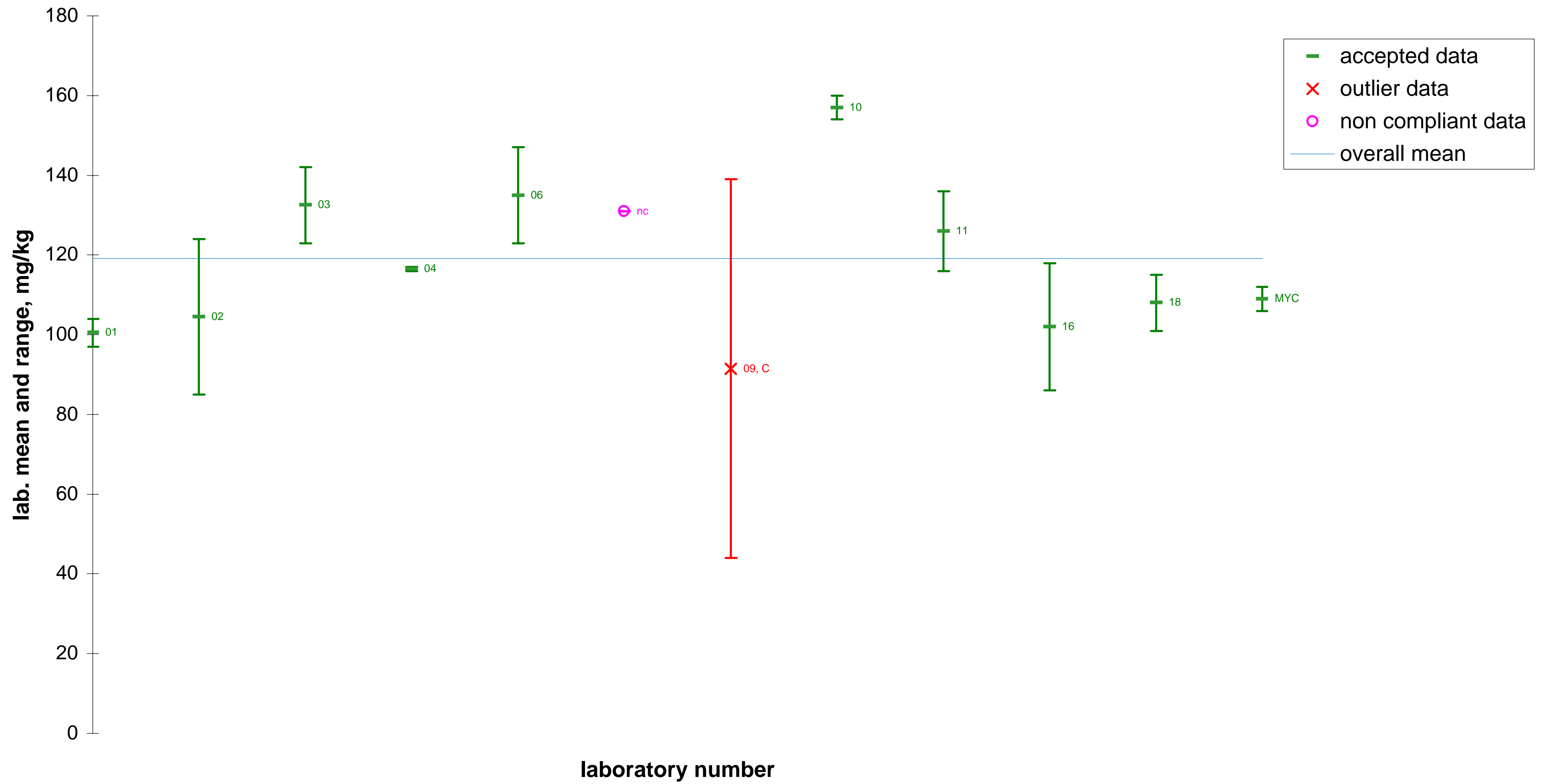
04
06
nc
09
10
11
16
18
MYC
accepted data
outlier data
non compliant data
overall mean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lab. mean and range, mg/kg
laboratory number
180
160
140
120
100
80
60
40
20
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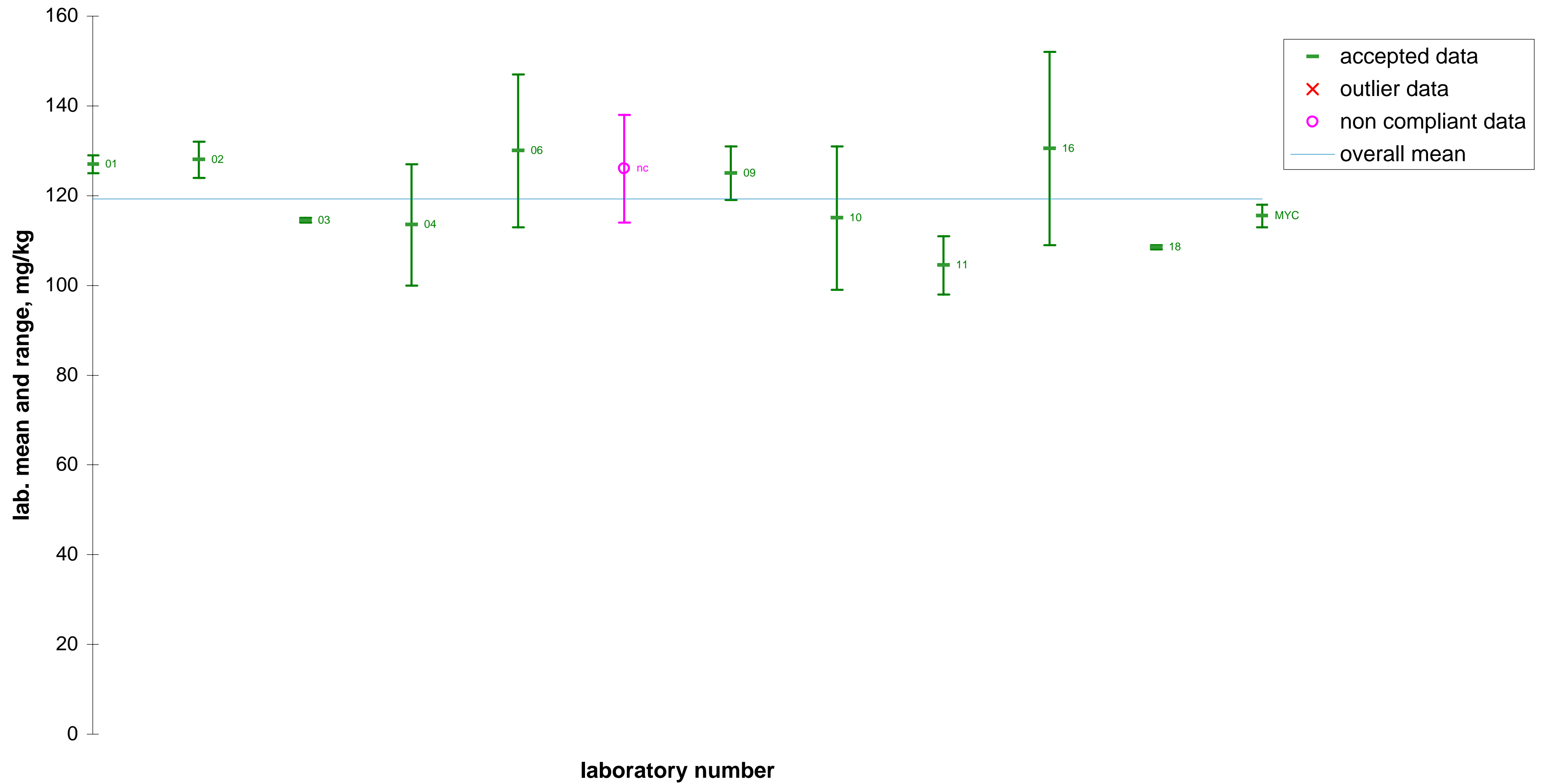
03
04
06
nc
09, C
10
11
16
18
MYC
accepted data
outlier data
non compliant data
overall mean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140
120
100
80
60
40
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nc
09
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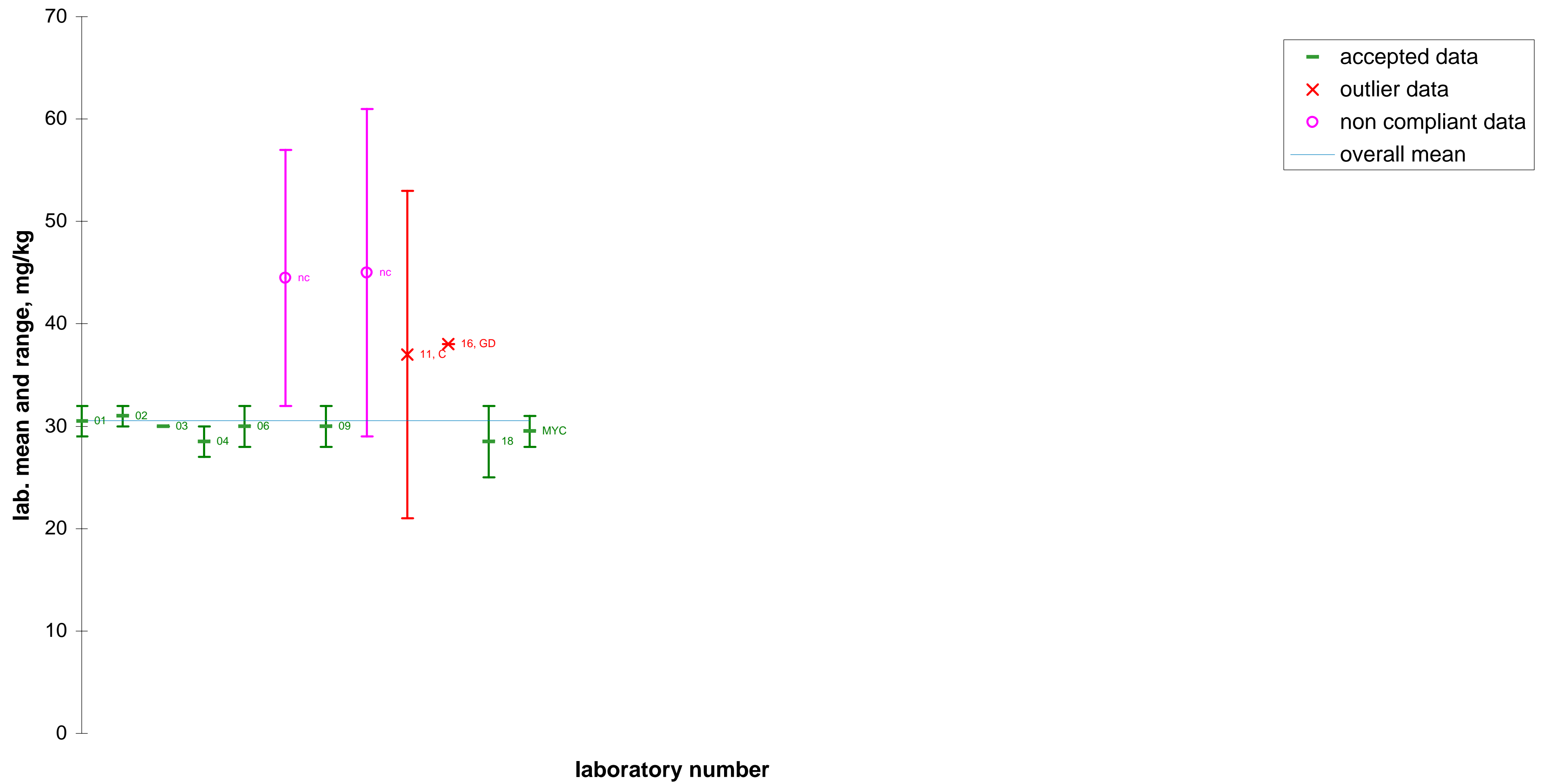
11
16
18
MYC
accepted data
outlier data
non compliant data
overall mean
laboratory number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50
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30
20
10
0
01
02
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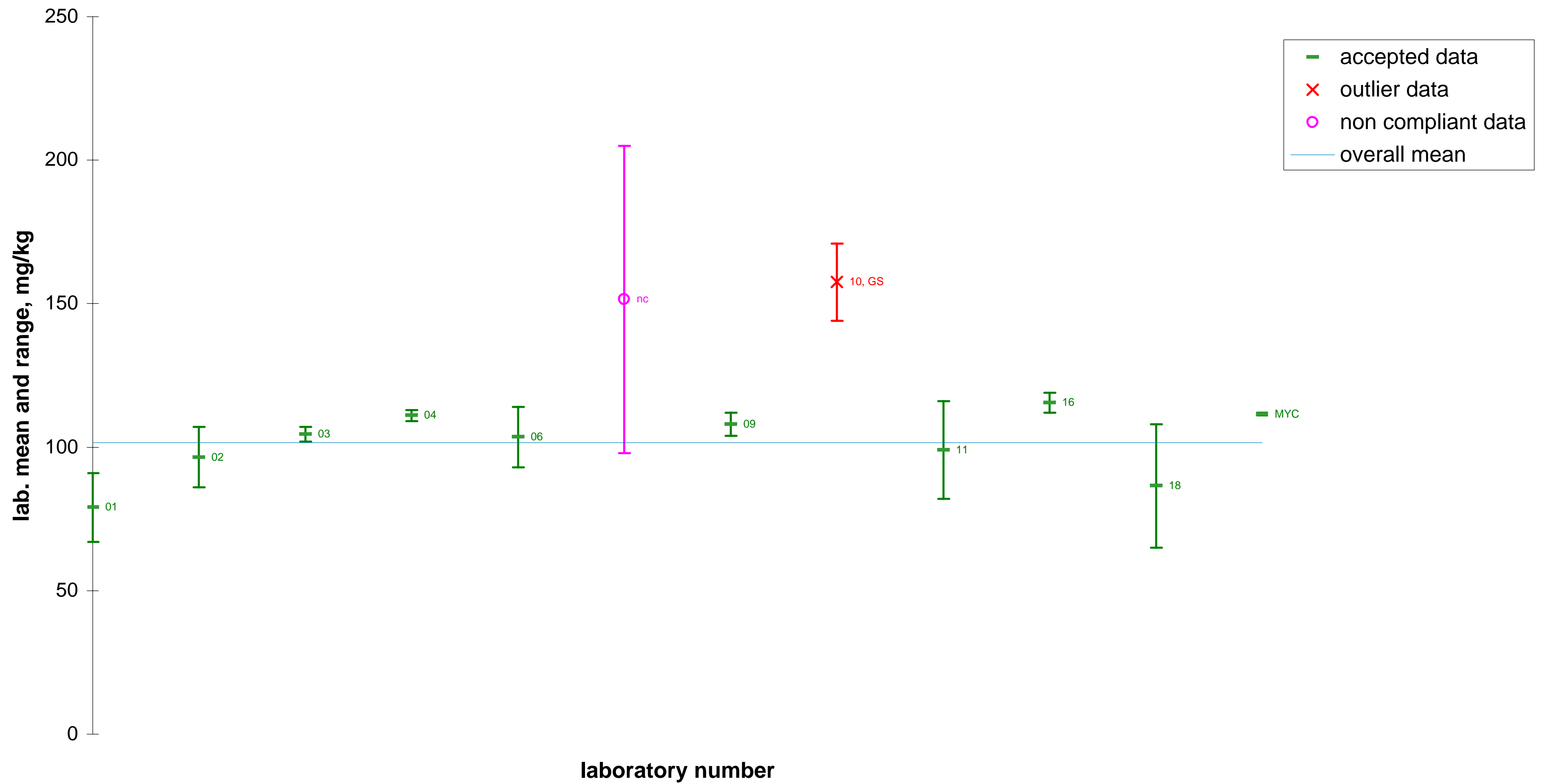
09
nc
11, C
16, GD
18
MYC
laboratory number
accepted data
outlier data
non compliant data
overall mean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lab. mean and range, mg/kg
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200
150
100
50
0
laboratory number
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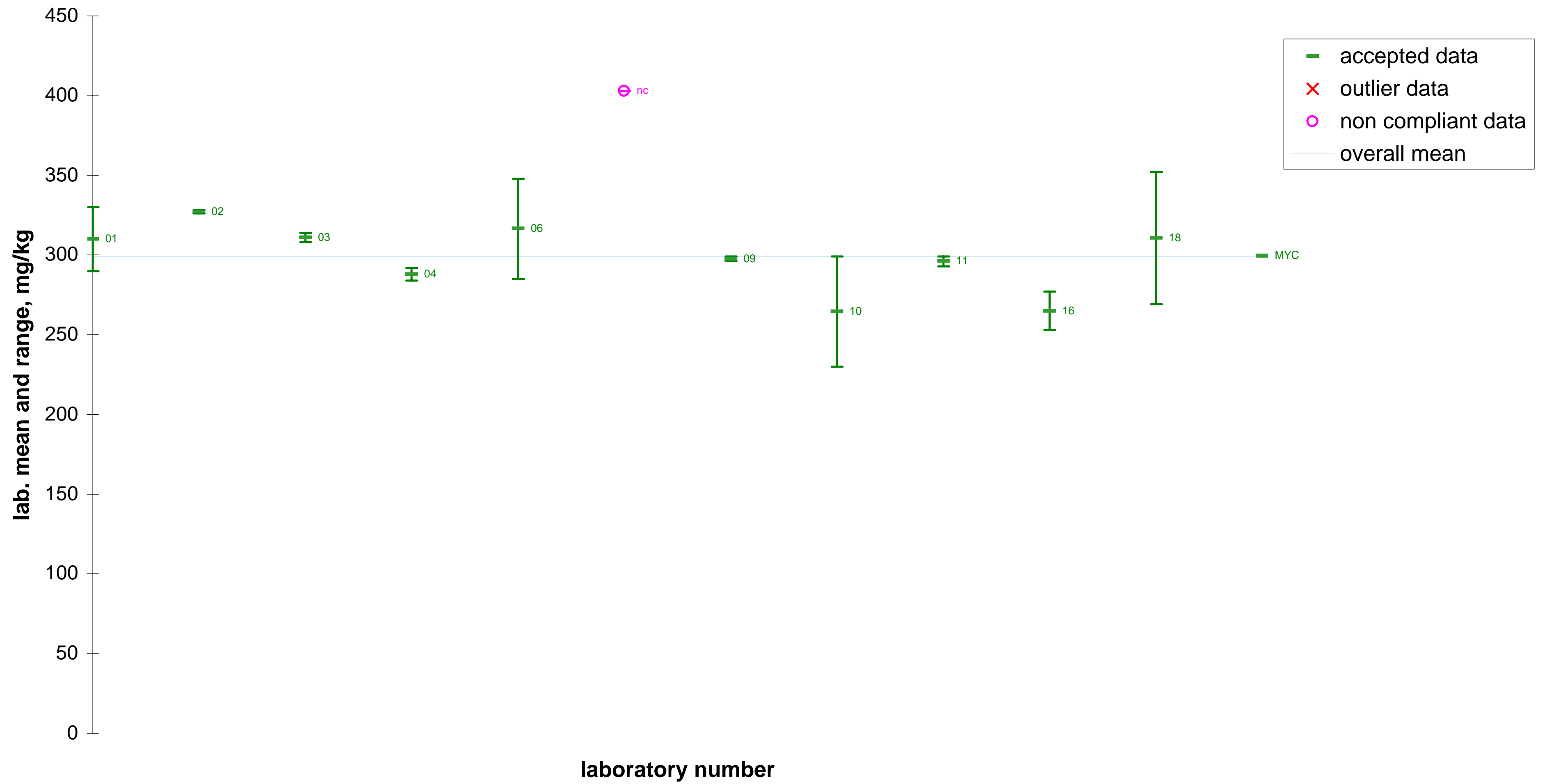
outlier data
non compliant data
overall mean
01
02
03
04
06
nc
09
10, GS
11
16
18
MYC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lab. mean and range, mg/kg
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100
50
0
laboratory number
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03
04
06
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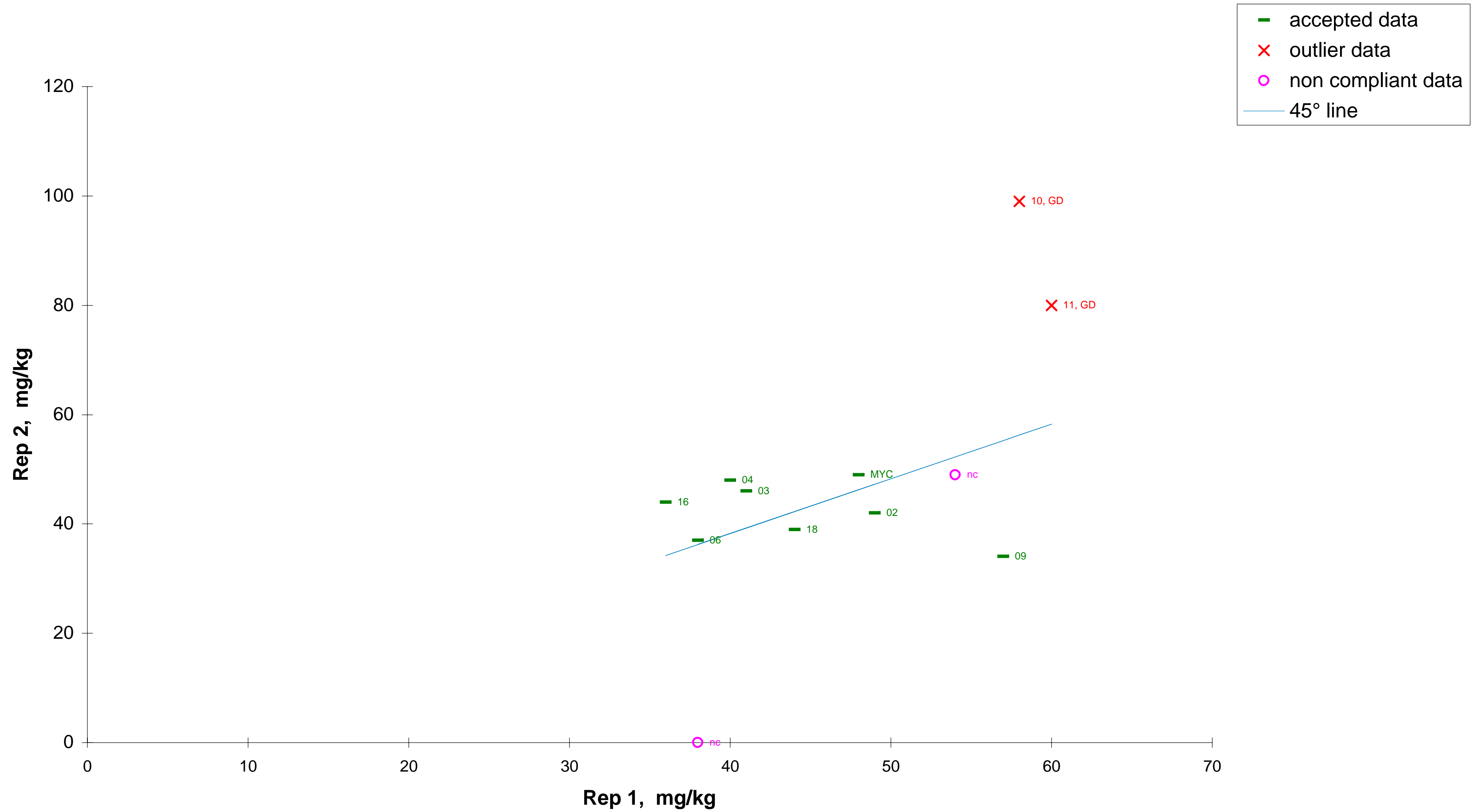
18
MYC
accepted data
outlier data
non compliant data
overall mean

Annex IIIb

Youden Plots
for FL-determination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outlier data
non compliant data
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Rep 2, mg/kg
Rep 1, mg/kg
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100
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60
40
20
0
0
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60
70
10, GD
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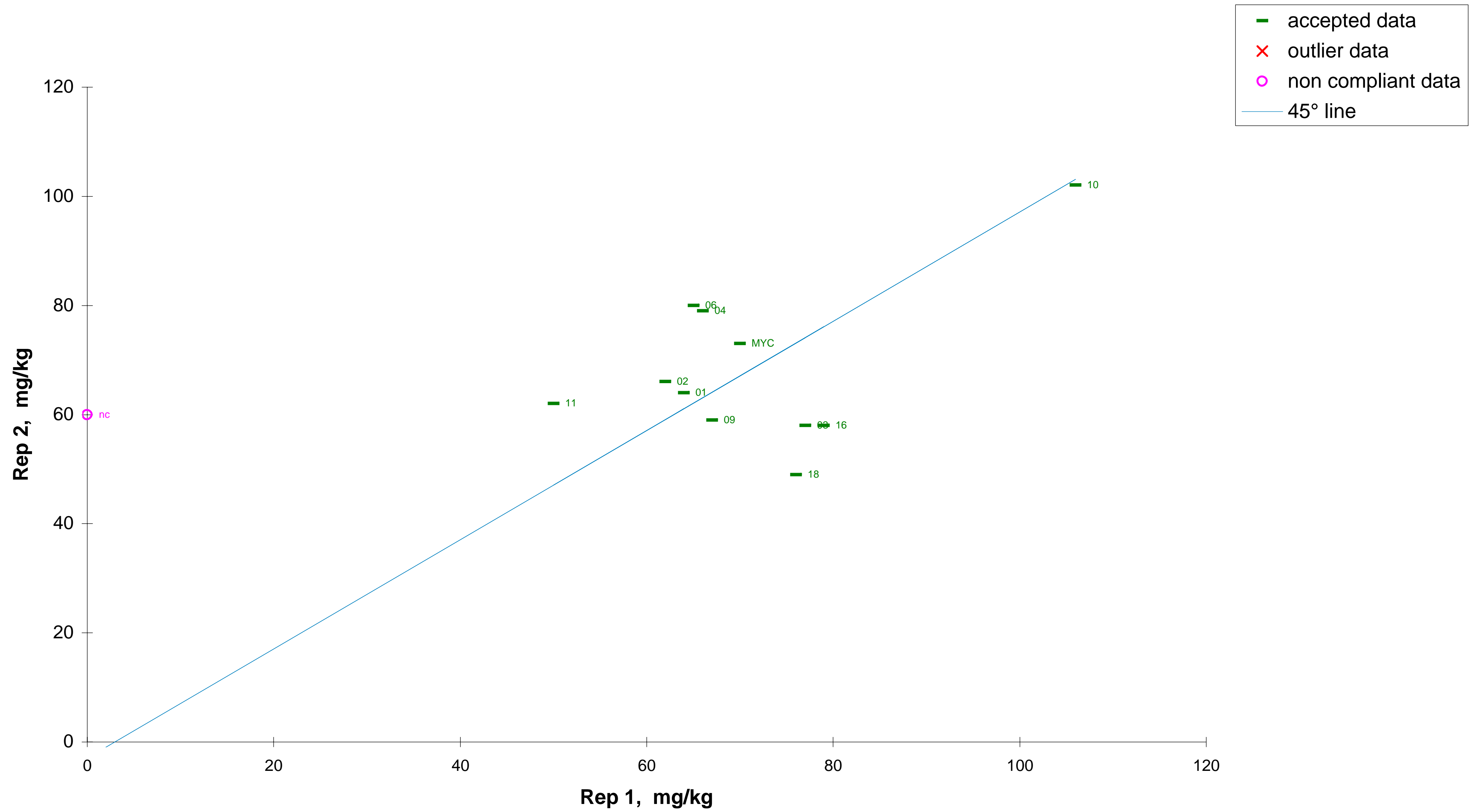
16
06
MYC
nc
02
18
09
nc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non compliant data
45° line
Rep 2, mg/kg
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06
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MYC
09
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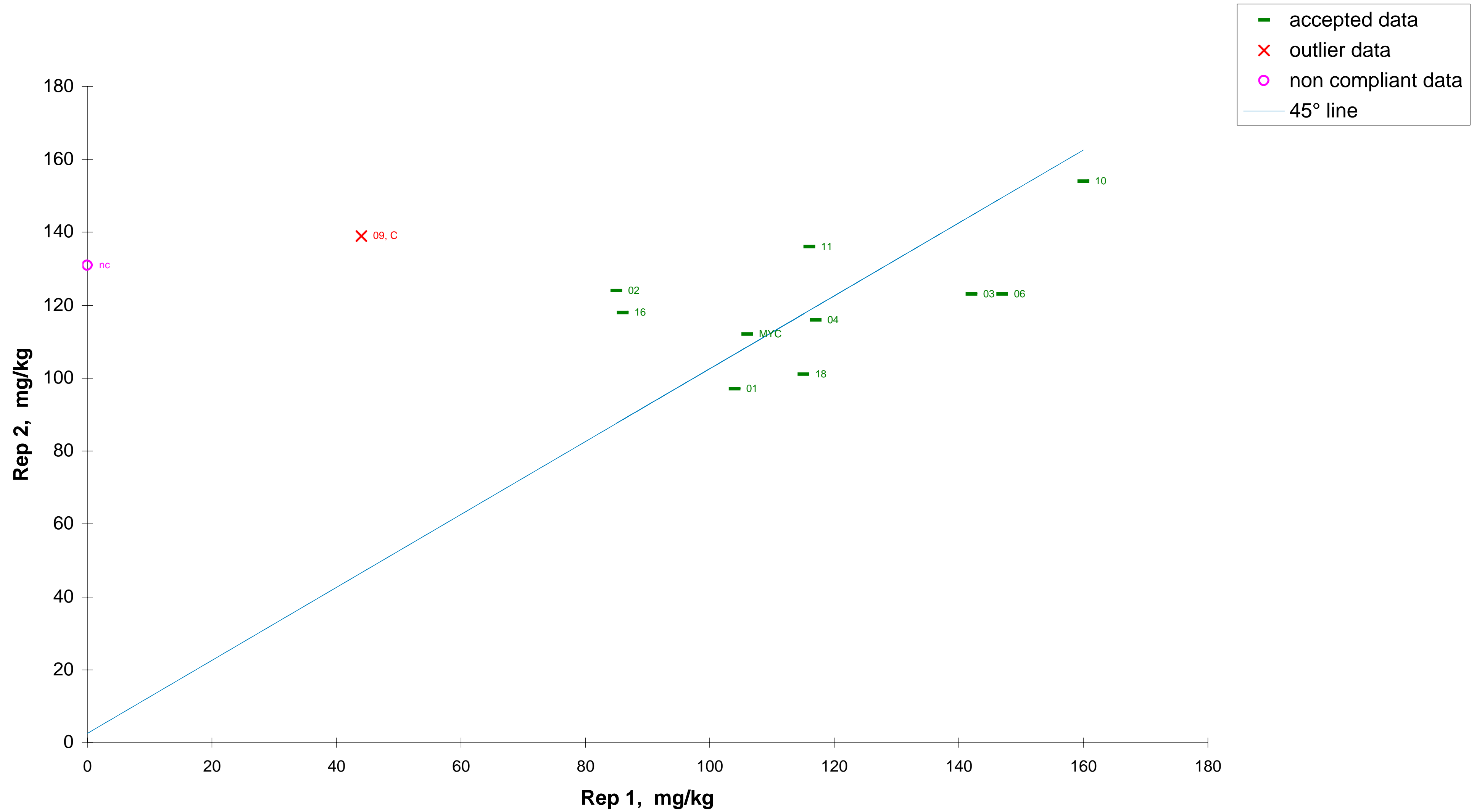
10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45° line
Rep 2, mg/kg
Rep 1, mg/kg
0
20
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160
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09, C
nc
02
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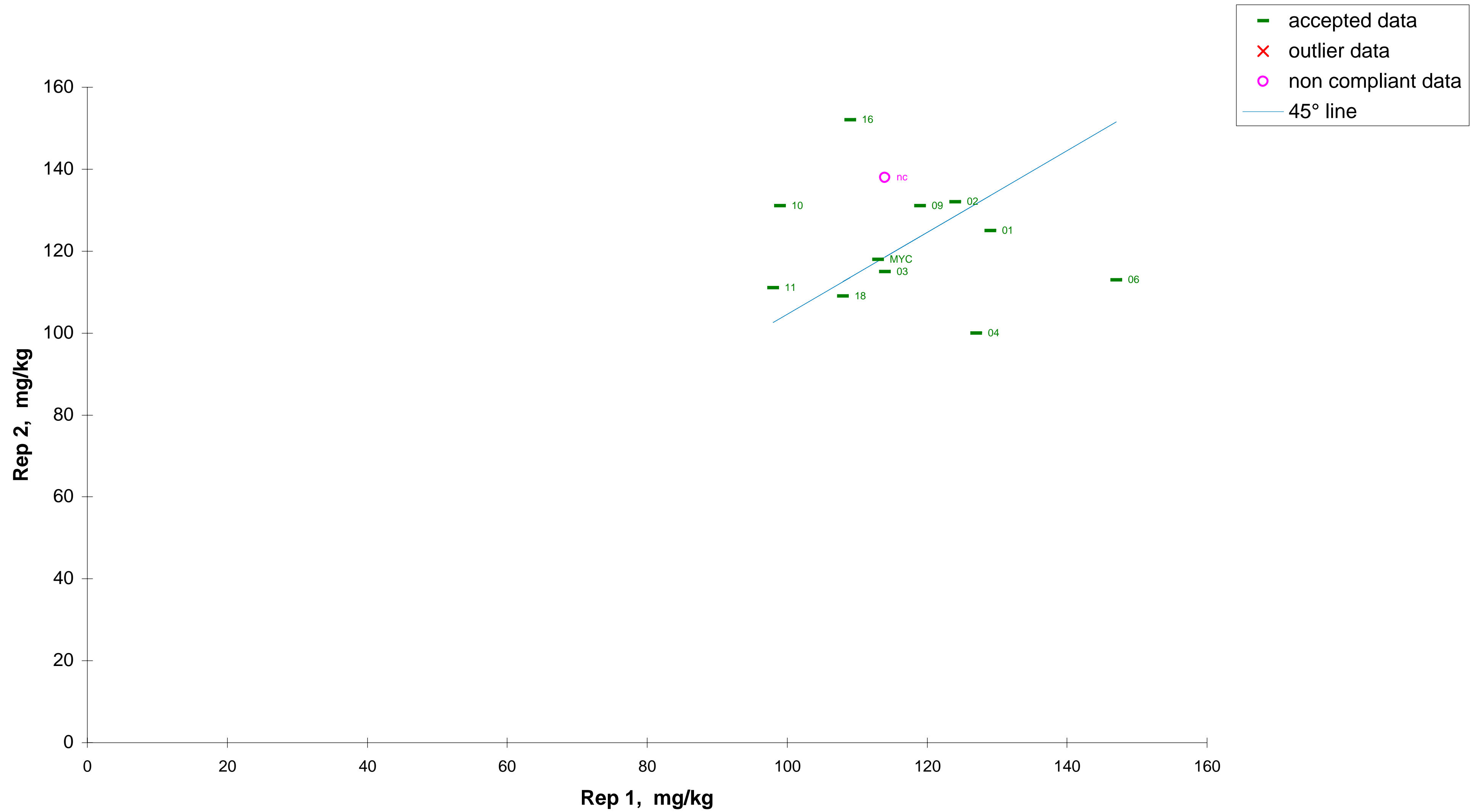
11
04
MYC
18
01
03
06
10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outlier data
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45° line
Rep 2, mg/kg
Rep 1, mg/kg
160
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02
01
MYC
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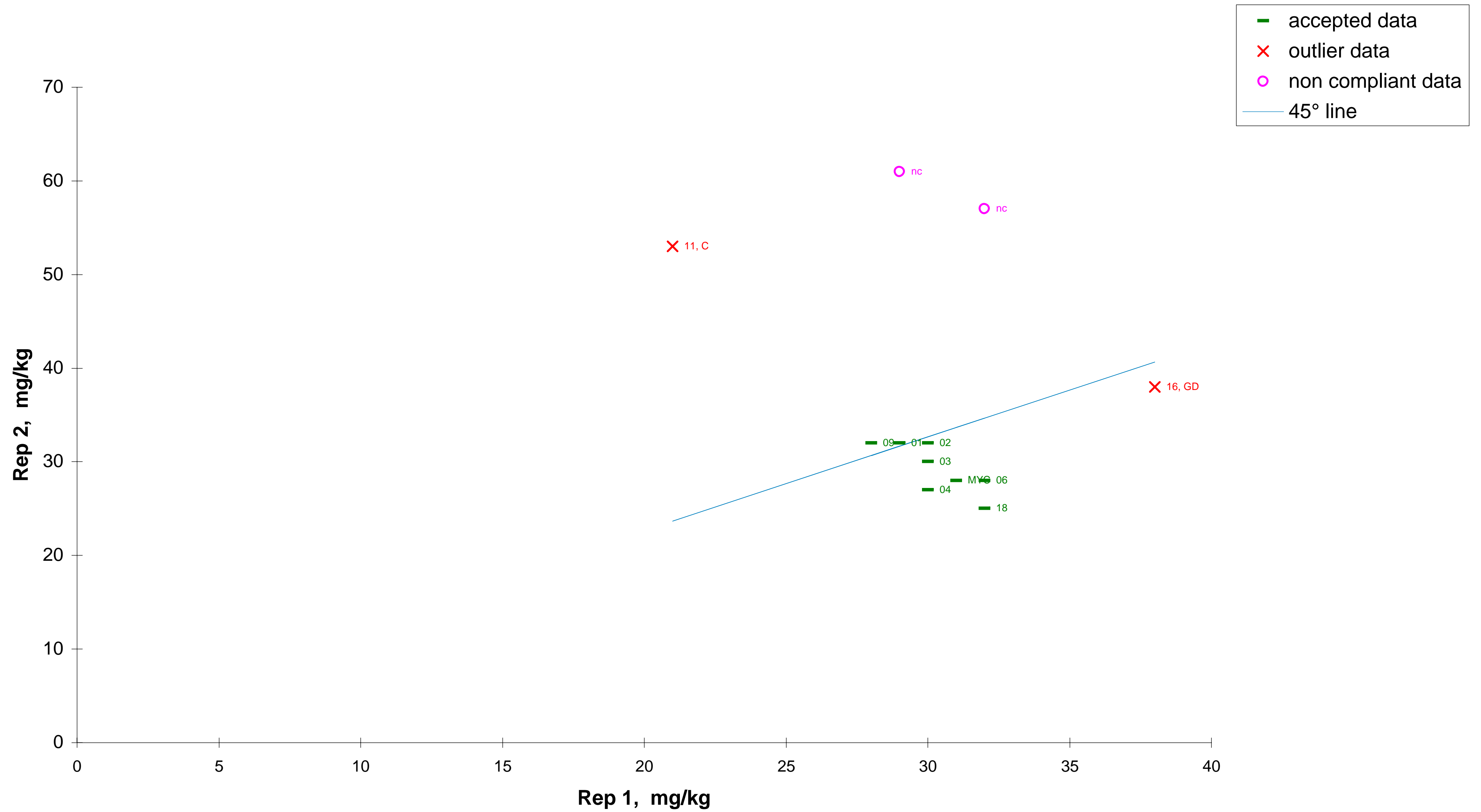
06
04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Rep 2, mg/kg
Rep 1, mg/kg
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nc
11, C
16, GD
09
01
02
03
MYC
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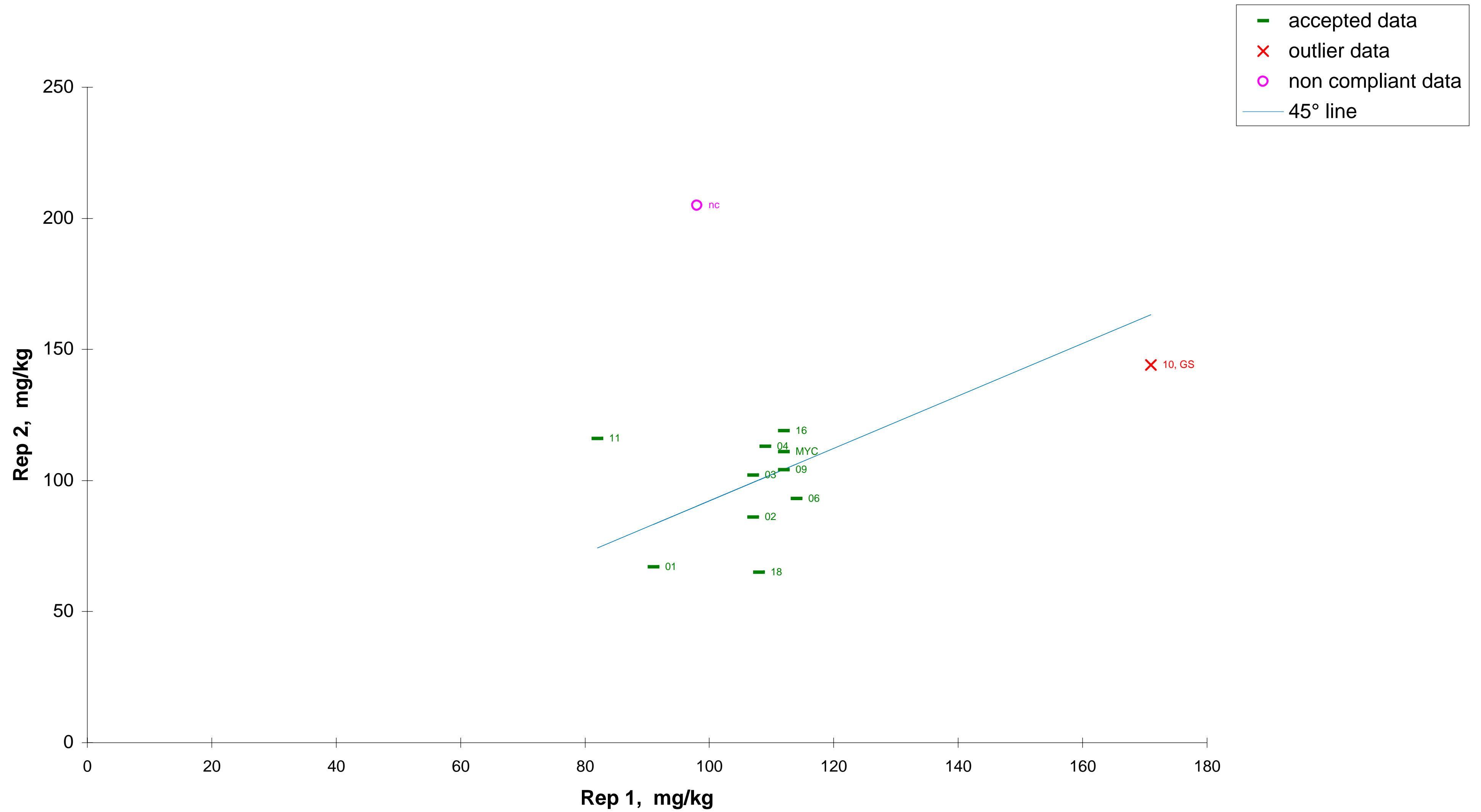
0 : blind replicates
accepted data
outlier data
non compliant data
45° line
Rep 2, mg/kg
Rep 1, mg/kg
250
200
150
100
50
0
0
20
40
60
80
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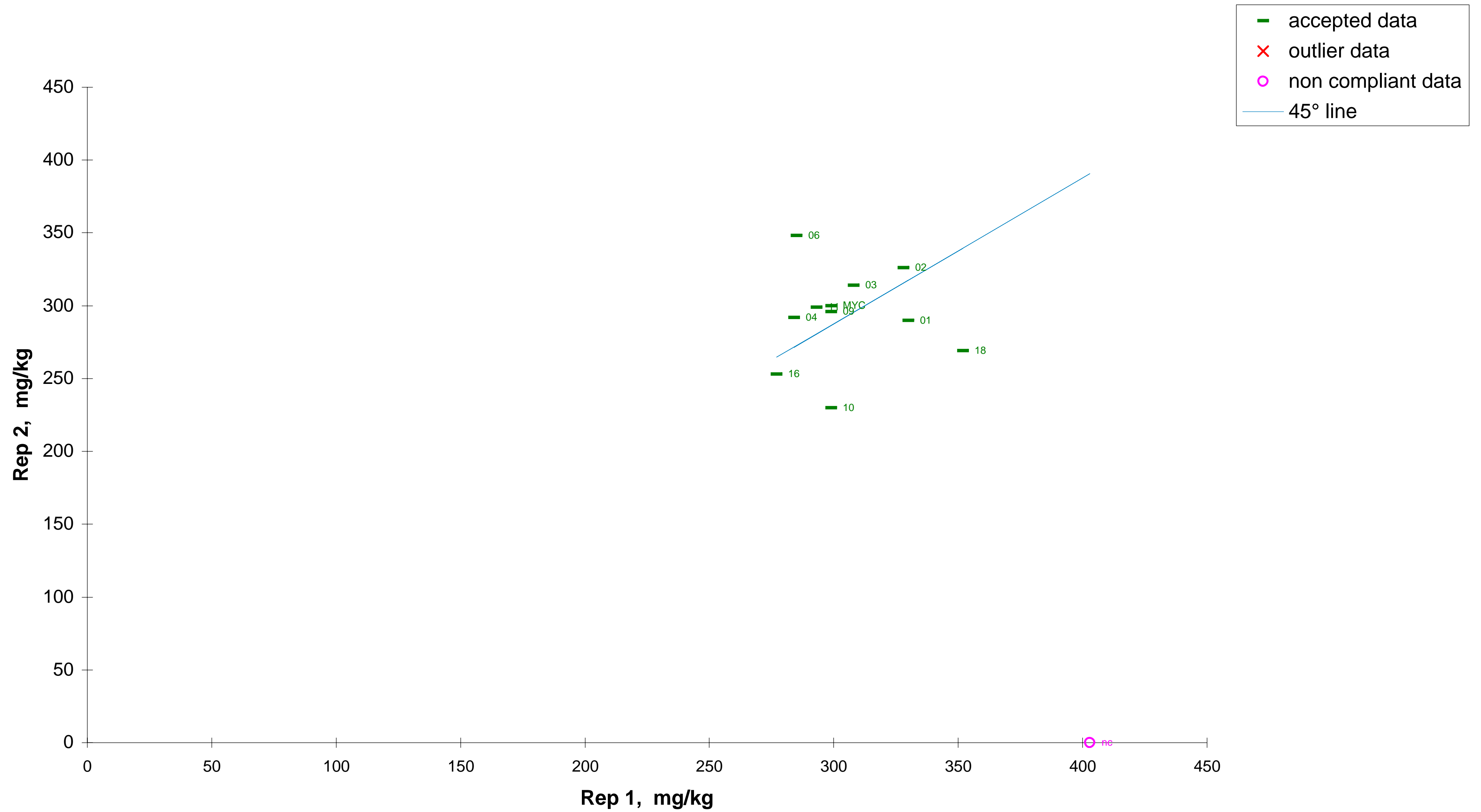
140
160
180
nc
10, GS
16
11
04
MYC
09
03
06
02
01
18


0 : blind replicates
accepted data
outlier data
non compliant data
45° line
Rep 2, mg/kg
Rep 1, mg/kg
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
400
450
06
02
03
MYC
09
04
01
18
16
10
nc

European Commission

EUR 23056 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Validation of an Analytical Method to Determine the Content of Sucralose in Beverages
Author(s): I. Doncheva and J. Stroka
Luxembourg: Office for Official Publications of the European Communities
2007 – 62 pp. – 21 x 29,7 cm
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Abstract

An inter-laboratory comparison was carried out to evaluate the performance characteristics of a method for the determination of sucralose in beverages, which was developed at the JRC-IRMM. The method is based on high-performance thin layer chromatography (HPTLC), and reagent-free derivatisation followed by ultraviolet/fluorescence detection. It was tested for the determination of Sucralose ($C_{12}H_{19}Cl_3O_8$; (2R,3R,4R,5S,6R)-2-[(2R,3S,4S,5S)-2,5-bis(chloromethyl)-3,4-dihydroxy-oxolan-2-yl]oxy-5-chloro-6-hydroxymethyl)oxane-3,4-diol; CAS No: 56038-13-2) in carbonated and still alcoholic and non-alcoholic beverages at proposed European regulatory limits according to Directive 2003/115/EC ([i]). Precise determination of Sucralose levels in some of the matrices for which European legislative limits apply, required a robust and reliable analytical method. HPTLC employing reagent-free derivatisation offered such a reliable but simple, fast, cost-effective and environment friendly method (very limited quantities of organic solvents methanol and acetonitrile were used). Separation of Sucralose was performed by direct application of samples (diluted, degassed and/or filtered, if necessary) on amino-bonded silica gel HPTLC plates without prior cleanup and development with acetonitrile:water. The sweetener was determined after heating of the developed plate to 190°C and quantified both in ultraviolet absorption and fluorescence measurement mode. Beverages spiked with sucralose as well as beverages taken from the market and labelled to contain sucralose, were sent to 14 laboratories in five different countries following IUPAC guidelines. A sample that did not contain measureable amounts of sucralose was spiked at levels of 30.5 mg/L, 100.7 mg/L and 299 mg/L. Recoveries ranged from 104 – 125 % with an average of 112 % for ultraviolet detection and from 98 – 101 % with an average of 100 % for fluorescent detection. Based on results for spiked samples (blind duplicates at three levels), as well as samples containing Sucralose (blind duplicates at three levels and one split level), the relative standard deviation for repeatability (RSD_r) ranged from 10 – 31 % for ultraviolet detection and from 9 – 16 % for fluorescence detection. The relative standard deviation for reproducibility (RSD_R) ranged from 14 – 31 % for ultraviolet detection and from 9 – 21 % for fluorescence detection. The limit of quantification on the basis of 10x the baseline noise was 6 mg/L and response was linear in the range between 30 – 150 ng/spot. The method is therefore considered suitable for the determination of Sucralose in beverages at the proposed European legislative limits.

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